



**RISK PROFILE:
BACILLUS SPP. IN RICE**

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

Dr Rob Lake
Dr Andrew Hudson
Peter Cressey

February 2004

Client Report
FW0319

RISK PROFILE:
BACILLUS SPP. IN RICE

Professor Ian Shaw
Food Safety Programme Manager

Dr Rob Lake
Project Leader

Dr Tecklok Wong
Peer Reviewer

DISCLAIMER

This report or document ("the Report") is given by the Institute of Environmental Science and Research Limited ("ESR") solely for the benefit of the New Zealand Food Safety Authority ("NZFSA"), Public Health Services Providers and other Third Party Beneficiaries as defined in the Contract between ESR and the NZFSA, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

ACKNOWLEDGEMENTS

We would like to thank the following for providing information for this Risk Profile:

- Health Protection Officers at Taranaki Health and the Nelson Marlborough District Health Board;
- Environmental Health Section of the Dunedin City Council; and,
- Population and Environmental Health Group, ESR Kenepuru Science Centre.

CONTENTS

SUMMARY	1
1 INTRODUCTION	2
2 HAZARD IDENTIFICATION: THE ORGANISM.....	5
2.1 <i>Bacillus cereus</i>	5
2.1.1 The organism/toxin	5
2.1.2 Growth and survival.....	5
2.1.3 Inactivation and inhibition of growth (CCPs and Hurdles)	6
2.1.4 Sources	6
2.2 Spore Formation.....	7
2.3 Toxins	7
2.3.1 Diarrhoeal toxin	7
2.3.2 Emetic toxin	8
2.3.3 Toxin production by other species of <i>Bacillus</i>	8
3 HAZARD IDENTIFICATION: THE FOOD	10
3.1 Relevant Characteristics of the Food: Rice.....	10
3.1.1 Behaviour of spores in rice	10
3.1.2 Toxin production by <i>Bacillus cereus</i> in rice	11
3.2 The Food Supply in New Zealand	11
3.2.1 Imported food.....	11
3.3 Rice Processing.....	12
4 HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS.....	13
4.1 <i>Bacillus</i> Intoxication	13
4.1.1 Diarrhoeal syndrome.....	13
4.1.2 Emetic syndrome.....	13
4.2 Mortality.....	14
4.3 Dose-response	14
5 EXPOSURE ASSESSMENT	15
5.1 The Hazard in the New Zealand Food Supply: <i>Bacillus</i> spp. in Rice.....	15
5.2 Food Consumption: Rice	15
5.3 Qualitative Estimate of Exposure	18
5.3.1 Number of servings and serving sizes.....	18
5.3.2 Frequency of contamination.....	19
5.3.3 Predicted contamination level at retail.....	19
5.3.4 Growth rate during storage and most likely storage time	19
5.3.5 Heat treatment	19
5.3.6 Exposure summary.....	19
5.4 Overseas Context	20
5.4.1 <i>Bacillus</i> in rice	20
6 RISK CHARACTERISATION.....	23
6.1 Adverse Health Effects in New Zealand	23
6.1.1 Outbreaks	23

6.1.2	<i>Bacillus</i> in rice – information from the Ministry of Health’s suspect foodborne illness investigation programme	24
6.1.3	Illnesses linked to <i>Bacillus</i> transmitted by rice in New Zealand	24
6.1.4	Clinical consequences of <i>Bacillus</i> infection	24
6.2	Adverse Health Effects Overseas	25
6.2.1	Contributions to outbreaks and incidents.....	25
6.2.2	Implicated foods.....	25
6.2.3	Case control studies	27
6.2.4	Risk assessments and other activity overseas	27
6.2.5	Secondary transmission.....	27
6.3	Qualitative Estimate of Risk	27
6.4	Risk Categorisation.....	27
6.5	Summary.....	28
7	RISK MANAGEMENT INFORMATION.....	29
7.1	Control Measures	29
7.2	Industry Sector in New Zealand.....	29
7.3	Economic Costs	29
8	CONCLUSIONS	30
8.1	Description of Risks to New Zealand Consumers	30
8.1.1	Risks associated with rice	30
8.1.2	Risks associated with other foods	30
8.1.3	Quantitative risk assessment	30
8.2	Commentary on Risk Management Options.....	30
8.3	Data Gaps.....	30
9	REFERENCES	32
	APPENDIX 1: CATEGORIES FOR RISK PROFILES.....	37

LIST OF TABLES

Table 1:	Kinetic data for growth of vegetative cells of <i>Bacillus cereus</i> (Penna <i>et al.</i> , 2002; McElroy <i>et al.</i> , 2000)	11
Table 2:	Cooked grain rice – percentage of respondents consuming	16
Table 3:	Cooked grain rice – median consumption by consumers (g/day)	16
Table 4:	Cooked grain rice – 95 th percentile consumption by consumers (g/day)	17
Table 5:	Cooked grain rice – mean consumption by persons (g/day)	17
Table 6:	Prevalence of <i>Bacillus</i> spp. in ready-to-eat rice	20
Table 7:	Quantitative data for <i>Bacillus</i> in rice	20
Table 8:	Reported outbreak data for <i>Bacillus cereus</i> in New Zealand.....	23
Table 9:	Contribution of <i>Bacillus</i> to foodborne disease.....	25
Table 10:	Outbreaks of <i>Bacillus</i> food poisoning involving rice overseas.....	26

LIST OF FIGURES

Figure 1:	Risk Management Framework	2
-----------	---------------------------------	---

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, ranking of a particular food safety issue.

This Risk Profile concerns *B. cereus* in rice, as this food has often been associated with food poisoning outbreaks where *B. cereus* has been identified as the causative agent. *B. cereus* bacteria may produce either or both of diarrhoeal and emetic toxins. The disease caused is generally considered to be mild and of short duration.

Illness caused by *Bacillus cereus* is not a notifiable disease in New Zealand. Therefore the only data concerning illness will derive from outbreaks or investigations of specific incidents. *B. cereus* is responsible for 1.2 – 4.5% of outbreaks reported in New Zealand.

The limited data from reported outbreaks indicate that rice or rice dishes are reasonably common vehicles for the small proportion of outbreaks attributed to *B. cereus* or other *Bacillus* species. Takeaways, often Chinese-style or Indian-style, are premises frequently cited as a source of the implicated food. This suggests that, as in other countries, a small proportion of rice is not handled in a safe manner, allowing the regeneration and growth of spores. This is supported by the results of a survey in Dunedin, where 2/46 (4%) of samples had unsatisfactory levels of *B. cereus*.

It is likely that a proportion, probably a small proportion, of New Zealand takeaway and restaurant operators are handling rice inappropriately, as shown by the Dunedin survey. Education to correct unsuitable practices should address a relatively easy problem to fix, although there may be language barriers to overcome.

Some data gaps exist that could be addressed by:

- A broader (nationwide) survey of *B. cereus* in rice from takeaway or restaurant premises to confirm the proportion of rice from these sources that contain unacceptable levels of *B. cereus*.
- Establishment of assays to detect the *B. cereus* emetic toxin to facilitate food poisoning investigations.
- Adoption of non-targeted microbiological methods to include other *Bacillus* species which are also capable of producing toxins.

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

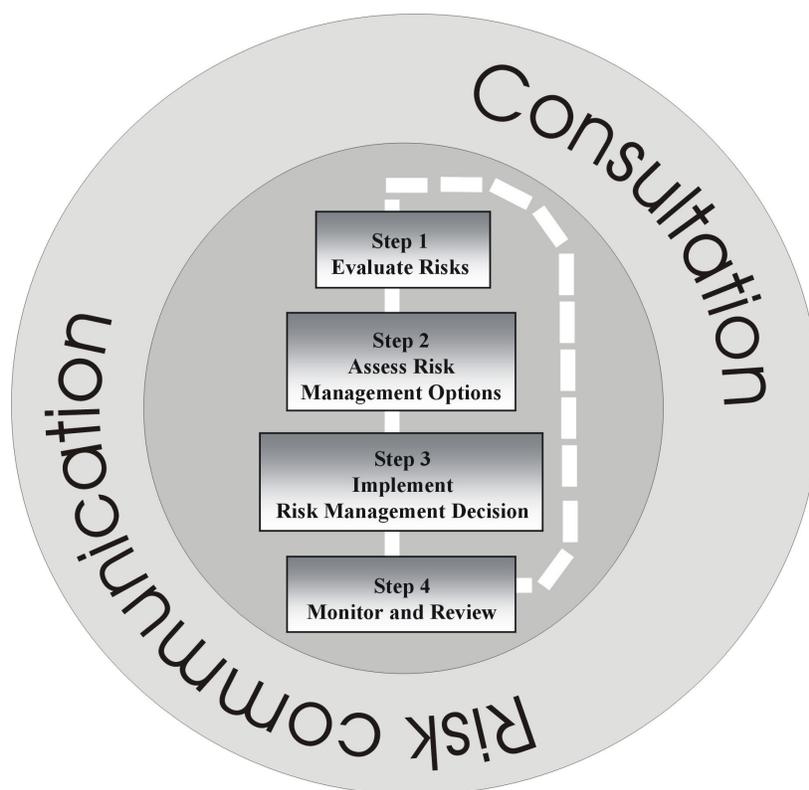


Figure reproduced from “Food Administration in New Zealand. A risk management framework for food safety” (Ministry of Health/Ministry of Agriculture and Forestry, 2000).

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 *Bacillus* spp. in rice. This organism causes both a diarrhoeal and an emetic syndrome, as well as being a spoilage-causing organism. While the diarrhoeal syndrome is mostly associated with proteinaceous foods, the emetic syndrome is mostly associated with farinaceous foods, including cooked rice (Notermans and Batt, 1998).

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 data sheets prepared by ESR under a contract for the Ministry of Health. The data sheets are intended for use by regional public health units.

2.1 *Bacillus cereus*

2.1.1 The organism/toxin

Bacillus cereus is a spore-forming organism that occurs naturally in most foods. It causes two different forms of food poisoning: an emetic illness and a diarrhoeal illness. The emetic illness is mediated by a highly stable toxin that survives high temperatures and exposure to trypsin, pepsin and pH extremes. The diarrhoeal illness is mediated by a heat- and acid-labile enterotoxin.

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

2.1.2 Growth and survival

Growth:

Temperature: Optimum 30-37°C. Some strains can grow up to 55°C while others can grow as low as 4-5°C. Many isolates from dairy products are able to grow at low temperatures.

pH: The minimum pH for growth is 4.3, maximum pH around 9.3. Growth is inhibited in the presence of 0.1% acetic acid (pH 5.1).

Atmosphere: Growth is best in the presence of oxygen. Grows well anaerobically. Toxin production is lower under anaerobic conditions.

Water activity: Minimum range of water activity (a_w) for vegetative growth is 0.912-0.950.

Survival:

Temperature: *Vegetative cells* are readily killed by heat but spores are moderately heat resistant. Heat resistance is increased in high-fat and oily foods (in soybean oil, the D time at 121°C is 30 minutes). Higher heat resistances also occur in foods with low water activity. *Spores* are more resistant to dry heat than moist heat. *Emetic toxins* are extremely resistant to heat (can survive 90 minutes at 126°C). *Diarrhoeal toxins* are inactivated at 56°C in 5 minutes.

pH: *B. cereus* organisms die suddenly in yoghurt when the pH reaches 4.5. Emetic toxin survives extremes of pH (2-11).

Water Activity: Spores survive for long periods in dried foods.

2.1.3 Inactivation and inhibition of growth (CCPs and Hurdles)

Temperature: For spores: $D_{85} = 33.8-106$ minutes. D_{95} ranged from 1.5-36.2 minutes in distilled water and 1.8-19.1 minutes in milk. Considerable variation was observed between different strains.

pH: Inactivated by 0.1 M acetic, formic and lactic acids in nutrient broth.

Water activity: 7.5% NaCl inhibits growth.

Preservatives: Growth is inhibited by 0.26% sorbic acid at pH 5.5 and 0.39% potassium sorbate at pH 6.6. The addition of 0.2% calcium propionate prevents germination of *B. cereus* in bread. Nisin is commonly used to inhibit germination and spore growth in processed cheese, dairy desserts, canned foods, cured meats and high moisture baked products such as crumpets and pikelets. A level of 3.75 µg/g of nisin in crumpet batter was effective. Other antimicrobials which have an effect on *B. cereus* include benzoate, sorbate, ethylenediaminetetraacetic acid (EDTA) and polyphosphates. Preserving foods in modified atmospheres has been shown to control the growth of *B. cereus*. Preservatives can be applied at reduced levels to inhibit the growth of *B. cereus* when used in combination (hurdle effect).

Radiation: Spores are more resistant to radiation than vegetative cells. Spores are more sensitive to heat after preirradiation at 4kGy before heating at 90°C.

2.1.4 Sources

Human: Humans are not a significant source of food contamination by *B. cereus*. This organism already exists on many foods and can therefore be transiently carried in the intestine of healthy humans (0-43%).

Animal: Animals can carry *B. cereus* on parts of their body. May occasionally cause mastitis in cows.

Food: Raw foods of plant origin are the major source of *B. cereus*. The widespread distribution of the organism, the ability of spores to survive dried storage and the thermal resistance of spores, means that ready-to-eat foods may contain *B. cereus* and will require control measures to prevent growth, especially after cooking has eliminated competing flora. Isolates producing emetic toxin grow well in rice dishes and other starchy foods, whereas those producing diarrhoeal toxin grow in a wide variety of foods from vegetables and salads to meat and casseroles. *B. cereus* is also associated with dairy products. Numerous dried herbs and spices, and dehydrated foods have been shown to contain *B. cereus*. Its ability to form spores allows survival through all stages of food processing, other than retorting. Fermented bean curd has been shown to contain high levels of *Bacillus cereus* (Wong, 1997).

Environment: *B. cereus* is widely distributed in nature and can be found in soil, dust, air, water and decaying matter.

Transmission Routes: Ingestion of contaminated food.

2.2 Spore Formation

Spores represent a metabolically dormant form of the organism derived from vegetative cells. Spore formation is generally induced by restriction in availability in one or more nutrients, or else a slowing of growth of cells. It also appears that spore production comprises part of the population of a growing culture (Setlow and Johnson, 1997).

Spores are more resistant to environmental challenges and control measures than vegetative cells. Such challenges include freezing, drying, pressure, radiation, ultraviolet light, chemicals, and heat. Reactivation of spores can be initiated by low pH, a number of chemicals (especially nutrients), and most commonly, sublethal heat. As *B. cereus* is widespread in nature and survives extended storage in dried food products, it is not practical to eliminate low numbers of spores from foods. Instead, controls are directed at preventing germination of spores, and preventing multiplication to form large populations of the organism. To achieve this, cooked foods should be rapidly and efficiently cooled, and thoroughly reheated before serving (Setlow and Johnson, 1997).

2.3 Toxins

B. cereus may produce two distinct toxins, responsible for the diarrhoeal and emetic syndromes. The majority of *B. cereus* strains appear to be capable of producing either diarrhoeal or emetic toxin, and a significant number (36% in one report) of isolates produce both (Beattie and Williams, 1999; Rusul and Yaacob, 1995).

Foods involved in diarrhoeal outbreaks are quite varied, ranging from vegetables and salads to meat dishes and casseroles. In contrast, emetic type outbreaks are usually associated with rice in some form, or else other starchy foods such as macaroni and cheese, or vanilla slices (Johnson, 1984).

2.3.1 Diarrhoeal toxin

The diarrhoeal type of food poisoning is caused by enterotoxins produced during vegetative growth of *B. cereus* in the small intestine (Granum, 1997). The toxin can be preformed in foods, e.g. bean curd (Wong, 1997), but it is unlikely this source of toxin would cause illness. One reason is that the enterotoxin is degraded in the gastrointestinal tract. The other reason is that the number of cells required to produce significant amounts of preformed toxin in food is much higher than the actual number of cells required to cause illness, and such high numbers of cells would make the food unacceptable for consumption. This suggests that there may be an 'optimum' level of food contamination – sufficiently high to result in infection of the small intestine and subsequent intoxication, but not so high that the food is unacceptable for consumption. Counts in foods associated with food poisonings have varied from 200 to 10^9 organisms/g (Granum and Lund, 1997).

It appears that *B. cereus* strains may produce either or both of at least two different three-component protein enterotoxins, although the characteristics of these are not fully understood. A three-component haemolysin (HBL) consisting of three proteins: B, L₁ and L₂ has been characterised. This has enterotoxin activity and has been suggested to be a primary virulence factor (Granum and Lund, 1997). Oxoid has developed a reverse passive latex agglutination assay for L₂.

A non-haemolytic three-component enterotoxin (NHE) has also been characterised as comprising proteins of size 39, 45 and 105 kDa. An immunoassay (Tecra) has been produced for the 45 kDa component.

Enterotoxin activity is labile. It can be inactivated by heat at 56°C for 5 minutes, is unstable at pH beyond the range 4-11 (i.e. will be degraded by stomach acidity), and is sensitive to proteolytic enzymes (Jensen and Moir, 1997).

2.3.2 Emetic toxin

The emetic toxin from *B. cereus* was characterised in 1995 as a dodecadepsipeptide named cereulide (Agata *et al.*, 1995). This circular molecule consists of three repeats of a four amino acid sequence, and is closely related to the potassium ionophore valinomycin. Cereulide is believed to be enzymatically synthesised rather than being a gene product. The toxin is resistant to heat, proteolysis and pH, but is not antigenic (Granum and Lund, 1997). The isolation and characterisation was achieved after the discovery that the toxin causes vacuolation of Hep-2 cells and this property forms the basis of an assay to detect the toxin present in foods or produced from isolates (Agata *et al.*, 2002).

A motility assay using boar spermatozoa has also been developed (Andersson *et al.*, 1998). The cereulide disrupts the outer membrane of mitochondria causing them to swell and this disrupts spermatozoa motility.

Emetic toxin is produced optimally at 30°C and only becomes detectable when approximately 10⁶ organisms /ml are present. An analysis of 107 incidents indicated that the numbers of organisms involved in emetic disease vary from 10³ to 5 x 10¹⁰ organisms/g with median values around 10⁷ organisms/g (Notermans and Batt, 1998). The emetic toxin is heat stable (e.g. 126°C for 90 minutes) and will survive frying, as well as being stable in a pH range of 2-11 (Johnson, 1984).

A recently developed HPLC-MS detection method for the emetic toxin has been used to explore the production characteristics of some *B. cereus* isolates (Hägglom *et al.*, 2002). Cereulide production commenced at the end of logarithmic growth, but was independent of sporulation. Cereulide production at temperatures at or below 8°C or at 40°C was minimal.

2.3.3 Toxin production by other species of *Bacillus*

There is a popular misconception that only the species *B. cereus* is of public health concern in terms of foodborne disease. However a few other species are capable of causing foodborne disease, and prominent among these is *B. subtilis* (Jensen and Moir, 1997). In fact, Nichols *et al.* (1999) identified *B. subtilis* more frequently than *B. cereus* (41% vs 23%) in cooked rice samples containing *Bacillus* spp at $\geq 10^3$ /g. A similar finding (20 *B. subtilis*, 4 *B. cereus* isolates) was reported by Little *et al.* (2002) who also isolated *B. licheniformis* (3 isolates) and *B. pumilus* (1 isolate) from 28 samples of heavily contaminated cooked rice samples. *B. thuringiensis* has been reported as causing food poisoning when fed to volunteers (Granum and Lund, 1997). This species has also been implicated in one outbreak investigation, although the isolation of Norwalk-like virus from two of the outbreak cases makes the

assignment of a causative pathogen questionable (Jackson *et al.*, 1995). Isolates of *B. circulans*, *B. laterosporus/cereus*, *B. lentus*, *B. licheniformis*, *B. mycoides*, *B. subtilis*, and *B. thuringiensis* have been shown to produce toxins (Beattie and Williams, 1999).

B. cereus serotypes 1, 3, 4, 5 and 8 are commonly associated with emetic food poisoning, while 2, 6, 8, 9 and 12 are associated with diarrhoeal outbreaks (Johnson, 1984). There are pronounced differences in toxin production by different *B. cereus* isolates (Beattie and Williams, 1999). Serotypes 1 and 5 were isolated from cooked rice in the UK (Little *et al.*, 2002).

Hassan and Nabbut (1996) showed that isolates from rice produced lower levels of toxin than isolates from diarrhoeal faeces. Notermans and Batt (1998) reported on a study where 31% of randomly selected *B. cereus* isolates were shown to produce emetic toxin, but the level of toxin production varied considerably. They concluded that, until more information comes to light, all types of *B. cereus* should be considered as potential pathogens.

In a survey of isolates from 16 rice samples purchased in Hong Kong most of the isolates were able to produce diarrhoeal toxin, but none was able to produce the emetic toxin. This was considered surprising, as cooked rice is normally associated with the emetic disease rather than the diarrhoeal form (Lee *et al.*, 1995). This study showed that isolation of *B. cereus* as part of a food poisoning outbreak investigation also requires demonstration of enterotoxigenic type before causation can be conclusively demonstrated.

3 HAZARD IDENTIFICATION: THE FOOD

3.1 Relevant Characteristics of the Food: Rice

Rice is of near neutral pH and is principally made up of carbohydrate (79%). Rice also contains protein (6-7%) and fat (1-2%) as well as vitamins and minerals. It therefore represents an excellent growth medium for bacteria. Such growth can only occur when rice is processed by boiling, as dry rice has a water activity beneath that supporting growth of microorganisms.

3.1.1 Behaviour of spores in rice

Spores of *B. cereus* can survive well in dried rice products. Storage under cool, dry conditions resulted in no loss of viability of spores in rice based weaning cereal over 48 weeks of storage, although storage under conditions of warm temperature (45°C) slightly higher a_w (0.78) resulted in some loss of viability from week 16 onwards (Jaquette and Beuchat, 1998).

An important characteristic of *Bacillus* is the ability of spores to survive the boiling of rice during cooking prior to their germination and toxin production. Gilbert *et al.* (1974) found some heterogeneity in D times in aqueous suspension at 100°C for *B. cereus* spores with D_{95} ranging from 5.0 to 36 minutes and D_{100} ranging from 1.2 to 7.5 minutes. Other work reported a D time of approximately 3.5 minutes for spores in rice at 97.8°C (Penna and Moraes, 2002). Using an approximate cooking time for rice of perhaps 20 minutes at close to 100°C, a minimum D kill of approximately 2.6 (a reduction of between 100 and 1000 fold) will occur depending on strain and cooking temperature. The population of spores originally present on the dry rice grains will therefore be reduced, but not necessarily by a very large factor.

B. cereus has been shown to grow in rehydrated rice to numbers around $10^7/g$ within 24 hours of incubation at 26°C (Harmon and Kautter, 1991), and $10^9/g$ at 32°C (Shelef and Liang, 1982). Rice inoculated with low numbers of spores (140 or 680 per gram) was boiled for 20 minutes and then growth rates of *Bacillus cereus* determined for periods of up to 72 hours (Gilbert *et al.*, 1974). No significant growth of spores at 4 or 10°C was observed, but growth did occur at 22°C, and was most rapid at 30-37°C. At room temperature (22°C) numbers reached 10^7 - $10^8/g$ after 33 hour incubation. Storage of rice for longer periods, even at low temperatures, will eventually allow growth of large numbers of *B. cereus*. At 8°C *B. cereus* grew from around $10^4/g$ to $10^8/g$ in 10 days (Ultee *et al.*, 2000).

Kinetic data for growth of vegetative cells in cooked rice are shown in Table 1.

Table 1: Kinetic data for growth of vegetative cells of *Bacillus cereus* (Penna *et al.*, 2002; McElroy *et al.*, 2000)

Temperature (°C)	Lag time (hours)	Generation time (minutes)
10	120.0	327.7
15	9.1	192.0
20	6.7	138.0
25	8.0	59.0
30	2.1	48.0
33	2.5	42.3

Growth in rice-based weaning cereal was shown over a temperature range of 8-21°C (Jaquette and Beauchat, 1998), but not in rice stored at 10°C over a three day period (Bryan *et al.*, 1981).

3.1.2 Toxin production by *Bacillus cereus* in rice

The growth of *B. cereus* and concomitant production of emetic toxin in boiled rice has been shown (Agata *et al.*, 2002). Most of the toxin production occurred when the stationary phase of bacterial growth was reached. In boiled and fried rice as well as rice gruel *B. cereus* reached numbers in excess of 10^8 /g after incubation at 30°C for 24 hours. Detectable toxin was produced in all three foods, but the level attained in fried and boiled rice was eight times as high as that measured in the rice gruel.

There is some speculation that food components may be required for toxin production. However, it is clear from the data available that if such compounds are required then cooked rice possesses them.

Competition is unlikely to be a factor in the growth of *B. cereus* or *B. subtilis* on rice as a large proportion of vegetative cells of other bacteria present will have been inactivated during boiling.

3.2 The Food Supply in New Zealand

Rice is not grown as a commercial crop in New Zealand.

3.2.1 Imported food

In the year ending September 2001 approximately 30,000 tonnes of grain rice were imported into New Zealand. Rice is imported into New Zealand principally from Australia (70% of total imports), but this material may originate in other countries. Significant amounts (greater than 1,000 tonnes) are also imported from Thailand (16%), Pakistan (5%), Brazil (5%), India (2%), the USA (0.8%) and Italy (0.3%). (Information obtained from Statistics New Zealand).

3.3 Rice Processing

Rice is harvested before full maturity to maintain quality, allowed to dry and then may be milled to remove husks. Milling results in heating so that the food is rendered practically free of vegetative microorganisms.

Rice may be consumed as:

- brown rice - whole rice grains with only the husk removed;
- white rice – brown rice milled or polished to remove the bran and germ layers;
- rice noodles – extruded spaghetti-like product made from cooked rice dough or a fettucine-like product from sheets of steamed rice dough;
- puffed rice – produced by short-time, high temperature treatment of rough rice (13 to 17 percent moisture), commonly used in breakfast cereals; and,
- rice crackers or wafers - produced from rice flour in an analogous manner to production of wheaten crackers from wheaten flour; and,
- ground rice flour.

4 HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS

B. cereus-associated foodborne illness occurs as two distinct syndromes: emetic and diarrhoeal.

The diarrhoeal enterotoxin disrupts the membrane of epithelial cells, but the mechanism is not understood (Notermans and Batt, 1998). The emetic toxin is believed to bind to receptors associated with the vagus nerve, which runs from the brain to various parts of the chest, including the throat and stomach. Stimulation of the nerve leads to vomiting (Notermans and Batt, 1998).

4.1 *Bacillus* Intoxication

4.1.1 Diarrhoeal syndrome

Incubation: 10-12 hours.

Symptoms: Diarrhoeal symptoms include abdominal pain, watery diarrhoea and occasional nausea (similar to *C. perfringens*). Recovery is rapid, usually within 12-24 hours.

Condition: Gastroenteritis.

People Affected: All people are believed to be susceptible to infection and subsequent intoxication but the intensity of symptoms may vary between individuals.

Long Term Effects: None

Treatment: Usually no treatment is given. Fluids may be administered when diarrhoea and vomiting are severe.

4.1.2 Emetic syndrome

Incubation: 1-6 hours after eating contaminated food.

Symptoms: The symptoms of the emetic syndrome include nausea and vomiting which is occasionally followed by diarrhoea (similar to *S. aureus*). Recovery is rapid, usually within 12-24 hours.

Condition: Gastroenteritis.

People Affected: All people are believed to be susceptible to intoxication and infection but the intensity of symptoms may vary between individuals.

Long Term Effects: None

Treatment: Usually no treatment is given. Fluids may be administered when diarrhoea and vomiting are severe.

4.2 Mortality

Very few fatal cases of foodborne illness involving *B. cereus* have been reported (Jensen and Moir, 1997). One case report involved liver failure attributed to emetic toxin (Mahler *et al.*, 1997). The toxin was present in spaghetti with homemade pesto. The dish had been prepared four days earlier, and although stored in the refrigerator, on several occasions it had been left at room temperature for one or more hours before being reheated in a pan

4.3 Dose-response

Counts in foods associated with food poisonings have varied from 200 to 10^9 organisms/g (Granum and Lund, 1997). The total dose reported varies but generally exceeds 10^5 viable cells or spores. Other discussion has indicated that the numbers of organisms involved in emetic disease incidents vary from 10^3 to 5×10^{10} organisms/g with median values around 10^7 organisms/g. It has been concluded that foods containing $>10^4$ *B. cereus*/g may not be safe to eat (Notermans and Batt, 1998). Differences in values for infective doses vary because of, among other things, the high variability between strains in their ability to produce toxins and the variable susceptibility of the person consuming the toxin.

Gilbert and Humphrey (1998) reported the following numbers (/g) of *B. cereus* in foods incriminated in outbreaks in the UK:

$<10^4$	1.8%
10^4 - $<10^5$	4.1%
10^5 - $<10^6$	22.0%
10^6 - $<10^7$	28.0%
10^7 - $<10^8$	21.7%
10^8 - $<10^9$	15.8%
10^9 - $<10^{10}$	4.8%
$>10^{10}$	1.8%

Notermans and Batt (1998) reproduced a dose response curve for the activity of the emetic toxin in the husk shrew (a small rodent). The dose required to produce emesis in 50% of test subjects (ED_{50}) was 12.9 $\mu\text{g}/\text{kg}$ bodyweight. The intraperitoneal ED_{50} was 9.8 $\mu\text{g}/\text{kg}$ bodyweight.

The amount of emetic toxin present in food samples which had caused food poisoning in Japan ranged from 0.01 to 1.28 $\mu\text{g}/\text{g}$ (Agata *et al.*, 2002).

A commercially available immunoassay kit for *Bacillus* diarrhoeal enterotoxin was used to investigate a number of foods which had been implicated in food poisoning incidents in Australia (Tan *et al.*, 1997). The sensitivity of the kit was of the order of 2 ng/g (of faeces). Foods which were positive for the enterotoxin were always contaminated with $\geq 10^5$ cfu/g of *B. cereus*. However, the enterotoxin was not always detected in foods when *B. cereus* was present at 10^4 - 10^5 cfu/g. Overall the results suggested that illness can result from toxin production in the gut following the ingestion of between 10^4 and 10^7 enterotoxigenic cells or spores.

5 EXPOSURE ASSESSMENT

5.1 The Hazard in the New Zealand Food Supply: *Bacillus* spp. in Rice

In 2000 the Environmental Health Section of the Dunedin City Council conducted a survey of local restaurants and takeaways examining the storage and handling of cooked rice, along with handwashing practices (Roger O'Brien, Environmental Health Project Officer, personal communication). Rice samples were taken from 46 of the 60 premises, and tested for aerobic plate count, faecal coliforms and *B. cereus*. *B. cereus* were detected in four samples, and for two samples the count exceeded the Microbiological Reference Criteria limit for cooked ready-to-eat foods of 10^3 cfu/g. Fifteen other samples had a total aerobic plate count exceeding the Microbiological Reference Criteria for cooked ready to eat foods of 10^5 cfu/g.

In approximately 50% of the premises the rice was prepared the day before it was subjected to further cooking. This is standard practice for preparing cooked rice for fried rice menus in Chinese-style premises, in order to achieve the desired physical characteristics. The potential for cross contamination and physical contamination was noted in 30% and 40% of premises respectively. Good handwashing technique was used by only 41% of those interviewed. The increased use of HACCP inspection techniques to audit actual operations by officers during inspections was recommended.

A survey of sushi conducted in Wellington and Christchurch in early 1999 evaluated manufacturing practices, as well as the bacteriological quality of 79 samples (Hough, 2000). Ten samples had levels of bacteria that were non-complying in terms of the Microbiological Reference Criteria for Foods, and one of these exceeded the guidelines for *B. cereus*. However, it was suspected that the contamination originated from the tuna and mayonnaise mixture rather than the rice component of the sample.

From 1994-1995 a project was conducted by ESR to evaluate the usefulness of testing for staphylococcal and *B. cereus* toxins in food samples and bacterial isolates derived from food poisoning incidents (Wong, 1996). The symptoms of 107 cases from 38 food poisoning incidents met the case definition, which suggested that they were caused by toxins. Two of these were attributed to *B. cereus* on the basis of isolation of the organism from implicated food samples. In each of these incidents the foods investigated were either cooked rice or dishes including rice.

5.2 Food Consumption: Rice

Rice may be consumed in the form of grain rice, rice flour, noodles, rice cakes/crackers, puffed rice (breakfast cereals), etc. Analysis of 24 hour diet recall records collected as part of the National Nutrition survey (Russell *et al.*, 1999) identified approximately 950 servings, which specifically identified rice or rice products. Food types, with the proportion of the total 'rice servings' were:

- Rice, white, brown or wild – boiled or fried 57%
- Noodles 18%
- Cereal, puffed, rice 10%
- Stews or stir-fries with rice as an identified ingredient 6%

- Pudding, rice 3%
- Crackers, rice 2%
- Wafers, rice 2%
- Salad, rice 1%
- Snack bars containing rice <1%
- Flour, rice <1%
- Pastry, rice <1%

For the current risk profile analysis will be restricted to grain rice.

Summary food consumption statistics can be expressed in terms of ‘consumer’ (just those people reporting to eat a particular food) or ‘persons’ (the whole population). Both will be presented here. The age groups used by the 1995 Australian National Nutrition Survey (Australian Bureau of Statistics, 1999) will initially be used so that an easy point of comparison can be made. These are 15-18 years, 19-24 years, 25-44 years, 45-64 years, 65 years and over. Comparisons with the Australian National Nutrition Survey was made with the category ‘rice and rice products’.

Table 2 gives the percentage of respondents, categorised by age and gender, who reported consuming cooked grain rice during the 24 hour dietary recall period.

Table 2: Cooked grain rice – percentage of respondents consuming

Age (years)	15-18	19-24	25-44	45-64	65+	Total
Male	12.8	15.2	14.1	7.7	4.0	10.4
Female	13.1	12.4	14.1	10.0	7.3	11.7
Total	13.0	13.6	14.1	8.9	6.0	11.2

In the Australian NNS the percentage of respondents eating rice and rice products were; males 14.0%, females 14.8%, all 14.4%. The Australian figures would be expected to be higher, as they would include rice noodles and other non-grain rice products.

There is a clear demographic trend in the consumption of rice and rice products by New Zealanders. These products are more likely to be consumed by those under 44 years of age.

Table 3 gives the median daily amount of rice eaten by consumers only.

Table 3: Cooked grain rice – median consumption by consumers (g/day)

Age (years)	15-18	19-24	25-44	45-64	65+	Total
Male	347	324	317	216	179	278
Female	217	253	216	194	113	216
Total	222	324	216	206	118	216

The median amounts of rice eaten by consumers are similar to those reported for the Australian NNS (1995). The Australian study reported an overall median (males and females) for respondents aged 19 and over of 195 g/day (rice and rice products), compared to an overall median of 216 g/day from the New Zealand NNS (1997). Overall figures for males and females are; males; Australia 285 g/day, New Zealand 278 g/day, females; Australia 190 g/day, New Zealand 216 g/day.

There is a clear demographic trend in the median amount of rice and rice products consumed by New Zealand consumers of rice, with lower amounts being consumed on a daily basis by older people.

Table 4 summarises the 95th percentile levels of consumption of rice for various population age-sex groups, based on 1997 NNS data. The 95th percentile consumer is a commonly used indicator of a high level consumer of a particular food.

Table 4: Cooked grain rice – 95th percentile consumption by consumers (g/day)

Age (years)	15-18	19-24	25-44	45-64	65+	Total
Male	754	598	754	648	494	666
Female	595	461	648	434	331	572
Total	672	560	652	532	400	648

The 95th percentile levels of consumption of rice generally show the same trends as other indices of rice exposure with decreasing amounts of rice being consumed with increasing age.

Table 5 gives the population level mean levels of consumption for various population age-sex groups.

Table 5: Cooked grain rice – mean consumption by persons (g/day)

Age (years)	15-18	19-24	25-44	45-64	65+	Total
Male	46	49	50	21	8	34
Female	35	35	36	22	11	28
Total	39	41	41	21	10	30

The Australian NNS reports an overall mean consumption of 40 g/day of rice and rice products. These figures appear consistent with New Zealand’s geographical position and culture. The World Health Organization (WHO), as part of their GEMS/Food (GEMS – Global Environmental Monitoring System) have formulated five ‘regional diets’ – diets considered to be typical for particularly geographical groupings (GEMS/Food, 1998). The rice consumption figures in the five regional diets are:

- European 11.8 g/day
- Latin American 86.5 g/day
- African 103.4 g/day
- Far Eastern 279.3 g/day

- Middle Eastern 48.8 g/day

GEMS/Food diets relate to uncooked rice while figures from the NNS refer to cooked rice. Assuming that rice absorbs approximately twice its weight of water during cooking, the New Zealand levels of consumption are consistent with the European regional diet. Rice consumption in New Zealand and Australia is likely to be influenced by our largely European heritage and by our proximity to Asia (Far Eastern regional diet). According to figures in FAO Food Balance Sheets (<http://apps.fao.org/>), New Zealand consumption of rice is higher than the European average, but is generally low by world standards.

While the 24 hour diet recall records in the New Zealand National Nutrition Survey provide information on the frequency and amount of rice consumption, they do not provide information on whether foods are cooked and eaten in the home, eaten outside the home (e.g. restaurants), or prepared outside the home for home consumption (e.g. takeaways). This information was not considered important in the design of the survey and the survey was not designed to capture the necessary information (Winsome Parnell, University of Otago, personal communication).

5.3 Qualitative Estimate of Exposure

5.3.1 Number of servings and serving sizes

Rice is a moderately commonly consumed food in New Zealand with approximately 20% of the population consuming rice on any given day. For cooked grain rice this figure is lower, at just over 10% of the population. By comparison, wheat and wheat products are consumed by virtually the entire population on any given day.

From the NNS, 518 individual dietary records were deemed to represent consumption of a serving of cooked grain rice. Using a total survey population of 4636 and a total New Zealand population (Census 2001) of 3,737,490:

$$\begin{aligned} \text{Annual number of servings (total population)} &= 518 \times 3,737,490 / 4636 \times 365 \\ &= 1.52 \times 10^8 \text{ servings} \end{aligned}$$

A FSANZ analysis of data from the 1997 National Nutrition Survey (Russell *et al.*, 1999) reported a mean daily intake for consumers only of 50 g/day (uncooked basis). This will correspond to an 'as consumed' weight of approximately 150 g/day. This is likely to represent a single serving of rice. Analysis of cooked grain servings from the 24 hour diet recall component of the 1997 National Nutrition Survey gave the following percentile serving sizes:

Percentile	Serving size (g)
50	216
75	357
95	648
99	995

5.3.2 Frequency of contamination

Studies have shown a wide range for the contamination of cooked rice with *B. cereus*. For example, values reported between 10 and 90% positive seem not uncommon.

5.3.3 Predicted contamination level at retail

In studies overseas the levels of *B. cereus* in rice at retail are usually very low, with around 90% of samples containing $<10^2$ /g.

5.3.4 Growth rate during storage and most likely storage time

Storage of cooked rice is not likely to be for more than 24 hours, but extended storage is likely to allow “drying off” to produce rice of the expected quality for fried rice preparation. Storage at room temperature for one day gives the potential for high numbers of *B. cereus* to be reached, and for toxin to be formed.

5.3.5 Heat treatment

Normal boiling of rice will result in a reduction of spore numbers to some extent, but not their elimination. Heat treatment of rice after toxin has been formed will not result in the destruction of emetic toxin. Heat treatment is likely to inactivate the diarrhoeal toxin, however, consumption of preformed diarrhoeal toxin does not appear to be a likely route of intoxication (see section 2.3.1).

5.3.6 Exposure summary

Rice products are consumed by a moderate proportion of the population (approximately 20%) on a daily basis, although the proportion eating cooked grain rice is closer to 10%. There is a clear trend towards higher consumption by younger New Zealanders, and it is likely that this trend will become more pronounced. It is also likely that Asian ethnic groups will have a higher consumption than the general population.

The survey in Dunedin indicated that 5-10% of rice samples from restaurants and takeaways presented a risk of food poisoning by *B. cereus*. This proportion is similar to those from surveys in the United Kingdom (see Section 5.4). However, it is unknown what proportion of rice consumed by New Zealanders is from restaurants and takeaways, as opposed to home preparation and consumption. Home preparation appears less likely to result in toxin formation.

Exposure to *B. cereus* from consumption of rice is likely to be a common event, but consumption of rice containing high levels of *B. cereus* and significant levels of toxin is likely to be a much rarer event.

5.4 Overseas Context

5.4.1 *Bacillus* in rice

Unlike other risk profiles that have been produced most of the data available concerning the occurrence of *B. cereus* in rice is quantitative, presumably because it has been known for some time that a large number of cells needs to be present for sufficient toxin to be formed to cause disease. The data in Tables 6 and 7 show that both the prevalence and numbers of the pathogen in rice are highly variable. The majority of samples contain low levels of *Bacillus* spp. ($<10^2$ cfu/g), while a small proportion of samples contain much higher numbers of cells, up to 10^7 cfu/g.

Table 6: Prevalence of *Bacillus* spp. in ready-to-eat rice

Country	Samples tested	Number (%) positive	Reference
USA	Ready-to-eat rice	11/12 (91.7)	Harmon and Kautter, 1991
NS	Boiled rice	(10-93)	Notermans and Batt, 1998
NS	Fried rice	(12-86)	Notermans and Batt, 1998
NS	Rice dishes	(3-40)	Notermans and Batt, 1998

NS=not stated

Table 7: Quantitative data for *Bacillus* in rice

Country	Samples tested	Counts	Reference
Hong Kong	Raw rice	0 (31.3%) 0-5x10 ² /g (56.3%) 3x10 ² -10 ³ /g (6.3%) 10 ⁴ -2x10 ⁵ /g (6.3%)	Lee <i>et al.</i> , 1995
India	Plain cooked rice	0/10 (0%)	Varadaraj <i>et al.</i> , 1992
India	Idii (rice containing snack food)	Min 3.3 log ₁₀ , mean 3.5 log ₁₀ , max 3.7 log ₁₀ /g (<i>Bacillus</i> spp.)	Varadaraj <i>et al.</i> , 1992
India	Bisibele bhath (rice containing snack food)	Min 2.6 log ₁₀ , mean 4.2 log ₁₀ , max 5.8 log ₁₀ /g (<i>Bacillus</i> spp.)	Varadaraj <i>et al.</i> , 1992
India	Curd rice	Min 3.1 log ₁₀ , mean 3.2 log ₁₀ , max 3.7 log ₁₀ /g (<i>Bacillus</i> spp.)	Varadaraj <i>et al.</i> , 1992
Lebanon	Raw rice (assumed)	34/50 (68%) positive <0.04/g (assumed for negative) 16/50 (32%) >0.04/g <10 ² /g 31/50 (62%) >10 ³ /g <10 ⁴ /g 3/50 (6%)	Hassan and Nabbut, 1996
Netherlands	Raw rice	40-100% positive, range 10 ² -10 ³ /g	Notermans and Batt, 1998
Netherlands	Boiled rice	10-93% positive, range 10 ¹ -10 ⁷ /g	Notermans and Batt, 1998

Country	Samples tested	Counts	Reference
Netherlands	Fried rice	12-86% positive, range 10^1 - 10^5 /g	Notermans and Batt, 1998
Netherlands	Rice dishes	3-40% positive, range 10^1 - 10^5 /g	Notermans and Batt, 1997
United Kingdom	Pre-cooked rice	For <i>B. cereus</i> : $<10^2$ /g 1,782 (93.9%) 10^2 - $<10^3$ /g 44 (2.2%) 10^3 - $<10^4$ /g 33 (1.7%) 10^4 - $<10^5$ /g 17 (0.9%) 10^5 - $<10^6$ /g 15 (0.8%) 10^6 - $<10^7$ /g 5 (0.3%) $\geq 10^7$ /g 1 (0.1%)	Nichols <i>et al.</i> , 1999
United Kingdom	Pre-cooked rice	For <i>Bacillus</i> spp. $<10^2$ /g 1,630 (82.0%) 10^2 - $<10^3$ /g 61 (3.1%) 10^3 - $<10^4$ /g 65 (3.3%) 10^4 - $<10^5$ /g 69 (3.5%) 10^5 - $<10^6$ /g 47 (2.4%) 10^6 - $<10^7$ /g 20 (1.0%) $\geq 10^7$ /g 5 (0.3%)	Nichols <i>et al.</i> , 1999
United Kingdom	Point of sale rice	For <i>B. cereus</i> : $<10^2$ /g 1,943 (98.5%) 10^2 - $<10^3$ /g 15 (0.8%) 10^3 - $<10^4$ /g 5 (0.3%) 10^4 - $<10^5$ /g 5 (0.3%) 10^5 - $<10^6$ /g 4 (0.2%)	Nichols <i>et al.</i> , 1999
United Kingdom	Point of sale rice	For <i>Bacillus</i> spp. $<10^2$ /g 1,854 (94.0%) 10^2 - $<10^3$ /g 40 (2.0%) 10^3 - $<10^4$ /g 33 (1.7%) 10^4 - $<10^5$ /g 20 (1.0%) 10^5 - $<10^6$ /g 10 (0.5%) 10^6 - $<10^7$ /g 11 (0.6%) $\geq 10^7$ /g 4 (0.2%)	Nichols <i>et al.</i> , 1999
United Kingdom	Cooked rice	For <i>Bacillus</i> spp. $< 10^5$ /g 492 (96.9%) $\geq 10^5$ /g 16 (3.1%)	Little <i>et al.</i> , 2002
USA	Raw polished rice	100% positive, numbers ranged from 10/g to 10^2 /g	Bryan <i>et al.</i> , 1981
USA	Boiled rice after hot storage	2/5 (40%) positive, both <10 /g	Bryan <i>et al.</i> , 1981
USA	Boiled rice after cold storage	5/13 (38.5%) positive, 3 <10 /g, 1 10^1 /g, 1 $>10^1$ $<10^2$ /g	Bryan <i>et al.</i> , 1981

Country	Samples tested	Counts	Reference
USA	Boiled rice after cooling at room temperature	13/14 (92.9%) positive, 10 <10/g, 2 10 ¹ /g, 1 10 ² -10 ³ /g	Bryan <i>et al.</i> , 1981
USA	Fired rice after hot storage	5/8 (92.9%) positive, 5 <10/g	Bryan <i>et al.</i> , 1981
USA	Fried rice after cold storage	4/6 (75.0%) positive, 1 <10/g, 2 >10 ¹ <10 ² /g, 1 10 ² -10 ³ /g	Bryan <i>et al.</i> , 1981
USA	Fried rice after cooling at room temperature	12/14 (85.7%) positive, 9 <10/g, 1 >10 ¹ <10 ² /g, 2 10 ² -10 ³ /g	Bryan <i>et al.</i> , 1981

The investigation of rice cooking practices in Chinese restaurants in the United States (Bryan *et al.*, 1981) found that, in addition to *B. cereus* being present in all samples of raw rice tested, contamination could also occur after cooking, particularly from inadequately cleaned storage pans or from spatulas or spoons used to mix the rice during frying or to transfer cooked rice to pans.

The large study of cooked rice from restaurants and takeaway premises in the UK (Nichols *et al.*, 1999) found that the majority of point-of-sale cooked rice samples (94%) were of acceptable microbiological quality. Of the remainder, 1% were of unacceptable quality (*Bacillus* spp., *B. cereus* >10⁵ cfu/g; *E. coli* >10⁴ cfu/g) indicating a potential risk to health. The prevalence of these bacterial species was significantly greater in pre-cooked stored rice than in point-of-sale cooked rice. Rice from Indian-style premises was of poorer microbiological quality than that from other premises.

Similar findings came from the further survey of cooked rice from takeaways and sandwich bars in the UK in 2001 (Little *et al.*, 2002). Problems occur with the practice of preparing boiled rice in bulk in advance. The rice may then be kept at room temperature before reheating; refrigeration of freshly boiled rice apparently leads to a poorer finished product and hence there is resistance to the practice. In this survey 87% of samples were of satisfactory or acceptable microbiological quality. The remainder were unsatisfactory (10⁴ - <10⁵) or unacceptable (>10⁵) due to the presence of *B. cereus* or other pathogenic *Bacillus* species.

This survey also collected information on the businesses producing the food. Significantly more samples of pilau rice were of unsatisfactory microbiological quality compared to other types of rice. Significantly more samples of rice from Indian-style takeaways were of unsatisfactory quality compared to samples from Chinese-style takeaways. Both these observations may be due to the addition of spices which may be contaminated with *Bacillus* spores.

Smaller businesses were more likely to have samples classed as unsatisfactory or unacceptable than larger businesses (based on the UK Local Authority Inspector's Consumer at Risk scores). Significantly more unsatisfactory and unacceptable cooked rice samples were collected from takeaways where the manager had received no food hygiene training, and there were indications from sandwich samples collected during the same survey that the presence of a hazard analysis system in the premise was associated with higher microbiological quality.

6 RISK CHARACTERISATION

6.1 Adverse Health Effects in New Zealand

Illness caused by *B. cereus* is not a notifiable disease in New Zealand. Therefore the only data concerning illness will derive from outbreaks or investigations of specific incidents.

It should be noted that it is thought that foodborne disease caused by pathogenic *Bacillus* spp. is highly underreported due to the short duration of both forms of the disease (Granum, 1997) and the technical difficulty of detecting the emetic toxin from foods or isolates.

An estimate of the annual number of cases of illness in New Zealand attributable to toxins produced by *Clostridium perfringens*, *Bacillus* spp., or *Staphylococcus aureus* in foods put the figure at 15,256 cases (414 per 100,000), including 5375 visits to a GP plus 51 hospitalised cases (Lake *et al.*, 2000). This estimate was based on NZHIS data for hospitalisations, plus rates of disease in the community from a study in the United Kingdom (Wheeler *et al.*, 1999). All cases of this type of illness were considered to be foodborne; secondary transmission is uncommon. No breakdown into illnesses caused by individual bacteria was performed.

6.1.1 Outbreaks

Outbreaks attributed to *B. cereus* cause only a small proportion of the total outbreaks in each year.

Table 8: Reported outbreak data for *Bacillus cereus* in New Zealand

Year	Outbreaks*	Cases**	Reference
1997	2/97 (2.1%)	6/1209 (0.5%)	ESR, 1998
1998	6/207 (2.9%)	21/1552 (1.4%)	Naing <i>et al.</i> , 1999
1999	16/352 (4.5%)	45/2302 (2.0%)	Perks <i>et al.</i> , 2000
2000	12/273 (4.4%)	66/1903 (3.5%)	Lopez <i>et al.</i> , 2001
2001	6/369 (1.6%)	21/2095 (1.0%)	Thornley, 2002
2002	4/337 (1.2%)	16/2890 (0.6%)	Boxall and Ortega, 2003

* Totals are for outbreaks of enteric disease only

** Includes both suspected and confirmed cases

Information from outbreaks attributed to *B. cereus* reported from 1998 to 2002 was reviewed in more detail to identify implicated food vehicles. During those 5 years, an implicated food was reported from 37 outbreaks. Rice dishes were implicated in 7 outbreaks (and *B. cereus* was isolated from leftover food in one of these outbreaks). Takeaways were reported in 15 instances (excluding fish and chips or pizza – these types of takeaways were implicated in two and three outbreaks respectively). Chinese-style or Indian-style takeaway or restaurant premises were implicated in 12 outbreaks.

6.1.2 Bacillus in rice – information from the Ministry of Health’s suspect foodborne illness investigation programme

Information collated through the Ministry of Health’s suspect foodborne illness investigation programme contains two types of information relating particular foods to episodes of suspected foodborne illness. The food may be implicated as the cause of the illness. This may be due to the fact that it is a genuine risk factor related to the symptoms presented, or may be due to 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 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).

Details of episodes in which *Bacillus* species were implicated during the 1998/99, 1999/00, 2000/2001 and 2001/2002 years were reviewed. In this period 30 investigations resulted in good evidence to suggest that *Bacillus* species were the causative agents. Of these 30 episodes eight were strongly suggestive of rice as the food responsible.

6.1.3 Illnesses linked to Bacillus transmitted by rice in New Zealand

Health Protection Officers provided two examples of foodborne outbreaks where *B. cereus* or other *Bacillus* spp. were the suspected aetiological agents. One concerned an outbreak in Nelson where 7 from 20 diners became ill after eating at a Chinese restaurant (Matt Molloy, Nelson Marlborough District Health Board, personal communication). The rice-containing meal contained 3.8×10^2 *B. cereus* /g, and $> 5.0 \times 10^3$ *Bacillus* spp. One stool specimen contained *Bacillus* diarrhoeal enterotoxin (as well as high numbers of *Clostridium perfringens*).

The second incident arose after an ethnic fair in Taranaki that included food stalls (Maree Rohleder, Taranaki District Health Board, personal communication). An unknown number of people became sick with short incubation, short duration diarrhoea. Investigation revealed that rice at one stall was stored for extended periods without refrigeration. Symptoms implicated *B. cereus* but no samples were available for analysis.

HACCP evaluations and identification of uncontrolled Critical Control Points of the suspect foods preparation process, often confirms the source of foodborne illness (Jenny Bishop, NZFSA, personal communication).

6.1.4 Clinical consequences of Bacillus infection

The disease caused is generally considered to be mild and of short duration. However, an outbreak in Norway caused by contaminated stew resulted in three of 17 affected people being hospitalised (Notermans and Batt, 1998). A similar report from the USA reported that 10 of 11 affected people sought treatment at emergency rooms and two of these were hospitalised because of dehydration (CDC, 1986).

Mead *et al.* (1999) estimated that *B. cereus* was responsible for 0.2% of foodborne illness cases in the USA, 0.0% of the hospitalisations and 0.0% of deaths.

6.2 Adverse Health Effects Overseas

6.2.1 Contributions to outbreaks and incidents

The data summarised in Table 9 indicates the proportion of outbreaks and incidents of food poisoning attributed to *Bacillus* species in overseas countries. The data indicate that outbreaks attributed to intoxication by *B. cereus* or other *Bacillus* species make up generally a small proportion of reported incidents, and this is similar to the situation in New Zealand. However, investigation and reporting of potential incidents may be less comprehensive than for other bacterial diseases.

Table 9: Contribution of *Bacillus* to foodborne disease

Country	Incidents	Outbreaks	Year(s)	Reference
Australia, New South Wales	39% of all incidents	ND	1977-1984	Davey, 1985
Canada	1.4%	1.3%	1979	Todd, 1987
Canada	2.1%	2.2%	1980	Todd, 1987
Canada	1.1%	ND	1975-1984	Todd, 1992
Netherlands	1.5% Varied between 7.7 and 48.3% of cases where a vehicle was confirmed.	1.8%	1991-1994	Simone <i>et al.</i> , 1997
Sweden	5%	5%	1992-1997	Lindqvist <i>et al.</i> , 2000
Taiwan	ND	5.8%	1981-1989	Chiou <i>et al.</i> , 1991
UK	ND	1%	1992-1994	Djuretic <i>et al.</i> , 1996
UK	ND	1%	1995	Evans <i>et al.</i> , 1998
UK	ND	2.5%	1996	Evans <i>et al.</i> , 1998
USA	ND	2% (of known aetiology)	1973-1987	Bean and Griffin, 1990
USA	ND	0.9%	1988-1992	Bean <i>et al.</i> , 1996
USA	ND	0.5%	1993-1997	Olsen <i>et al.</i> , 2000

ND No Data supplied. Figures are for all incidents/outbreaks, including those of unknown aetiology.

6.2.2 Implicated foods

Representative details of outbreaks caused by *Bacillus cereus* are given in Table 10.

Table 10: Outbreaks of *Bacillus* food poisoning involving rice overseas

Country	Number Cases	Notes	Year	Reference
Finland	18 of 26 eating boiled rice	Typical short incubation time <i>B. cereus</i> food poisoning	1976	Raeuori <i>et al.</i> , 1976
UK	NS	Seven examples of outbreaks caused by <i>Bacillus</i> in rice.	1992-1999	Kessel <i>et al.</i> , 2001
USA	14 from 48 who ate fried rice	Attack rate 29%, children mainly involved, >10 ⁶ /g in rice, >10 ⁵ /g in child's vomit. Rice was cooked on the previous evening and cooled at room temperature before refrigeration.	1993	CDC, 1994
USA	11 from 11 contacted	Two people hospitalised. No <i>Bacillus</i> isolated from rice, but this does not exclude pre-formed toxin. <i>B. cereus</i> was isolated from meat.	1985	CDC, 1986
NS	NS	291 employees ate the meal, 87 sought medical attention, 84 as emergencies. Contaminated rice and chicken implicated.	1986	Baddour <i>et al.</i> , 1986

NS Not stated

A risk assessment of *B. cereus* and its toxins (Notermans and Batt, 1998) summarised data on outbreaks of *B. cereus* from the USA, the Netherlands, Canada and England/Wales. Chinese-style food was the most commonly identified food vehicle in the USA (12/21 from 1988-1992), the Netherlands (17/40 from 1992-1994) and Canada (17/39 from 1985-1986). In England and Wales from 1989-1991 mixed foods were the most commonly identified (27/59) followed by rice (19/59).

Simone *et al.* (1997) reported that 42.5% of Dutch outbreaks involving *B. cereus* were associated with Chinese-Indonesian-style foods. Chinese-Indonesian-style foods were the most frequently suspected vehicle, reported at twice the number of the next highest category (meat and meat products). This is possibly due to the additional spices used in the Indonesian component of this food style. However, only 4% of outbreaks in the USA with a specific food vehicle between 1973 and 1987 were attributed to Chinese-style food (Bean and Griffin, 1990). In the UK 6.3% of outbreaks with a suspect food vehicle were attributed to rice dishes, and 47.8% of these outbreaks were caused by *B. cereus* (Evans *et al.*, 1998). A similar figure of 43.8% was found in Canada for Chinese-style food (Todd, 1992).

During 1992 to 2000 in England and Wales there were 12 reported outbreaks of food poisoning attributed to enterotoxigenic *B. cereus*, in which 53 people were affected after consuming rice purchased from Chinese-style or Indian-style takeaways (Little *et al.*, 2002).

6.2.3 Case control studies

Information on case control studies was not identified.

6.2.4 Risk assessments and other activity overseas

A risk assessment of *Bacillus cereus* and its toxins in a range of foods has been published by Notermans and Batt (1998). The authors concluded that it would be advisable to carry out risk characterisation of boiled rice, fried rice, rice dishes, cream, pasteurized milk and cereals, as these foods have been reported to have contamination rates high enough to lead to an increased probability of disease in man.

6.2.5 Secondary transmission

Person-to-person transmission is not usual for this organism. However, one outbreak report described an incident where children became sick after handling coloured rice as part of a class activity (Briley *et al.*, 2001). The rice had been left to rehydrate at room temperature for 12-24 hours prior to being handled by the children. After handling the rice the children and a teacher ate lunch without washing their hands beforehand and later became sick. Another group of children and a teacher who did wash their hands did not become sick. The coloured rice contained 5.6×10^5 *Bacillus/g*. Cross contamination from the rice to the lunch via the children's hands was considered to be the cause of the outbreak.

6.3 **Qualitative Estimate of Risk**

Reported outbreaks of gastrointestinal illness attributed to *Bacillus* spp. in New Zealand are a small percentage (<5%) of all reported outbreaks and involve small numbers of cases (<4% of total cases). This is similar to reports from the US, UK and Europe. Nevertheless, within this small number of outbreaks, the food vehicles most commonly implicated are rice or rice dishes, and takeaways (often Chinese-style or Indian-style). Although there may be a predisposition by ill people to suspect such foods when episodes of illness are investigated, this suggests that these types of foods are important vehicles for illness caused by *Bacillus* spp. in New Zealand, as is the case in Europe and the US.

6.4 **Risk Categorisation**

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

There are few if any serious outcomes from intoxication by *Bacillus* spp., placing this food hazard combination in the lowest category with respect to severity (Category 3: <0.5% serious outcomes).

The estimated rate of illness (based on UK data) from the three toxin producing bacteria *Clostridium perfringens*, *Bacillus cereus* and *Staphylococcus aureus*, for New Zealand was 414 per 100,000 (Lake *et al.*, 2000). The outbreak summary reports for 1998-2001 (ESR, 1998; Naing *et al.*, 1999; Perks *et al.*, 2000; Lopez *et al.*, 2001, Thornley, 2002) indicate that reported outbreaks (and case numbers) attributed to *Clostridium perfringens* are always more frequent than those attributed to the other two bacteria. In addition, in all years (except 1999

when they were equal) outbreaks attributed to *Staphylococcus aureus* were more frequent than those attributed to *B. cereus*.

Given these indications, and the fact that rice will be the vehicle in only some of the food poisoning incidents caused by *Bacillus* spp., it is estimated that the incidence of illness caused by *Bacillus* spp. in rice is substantially less than one third of the overall estimated rate, placing this food hazard combination in the second incidence category (Category 2: 10-100 per 100,000 population).

6.5 Summary

Food/hazard combination	Severity	Incidence	Trade importance	Other considerations
<i>Bacillus</i> spp. in rice	3 (<0.5% serious outcomes)	2 (10-100 per 100,000)	None – all rice is imported.	Potential for increased risk in the future as rice consumption increases

7 RISK MANAGEMENT INFORMATION

It is generally accepted that *Bacillus* species in raw dried grains such as rice are so common that the only accepted way to avoid illness is proper cooking, holding and chilling (Briley *et al.*, 2001).

7.1 Control Measures

In the UK guidelines for ready-to-eat food have the following criteria for *B. cereus* and *B. subtilis* (Gilbert *et al.*, 2000):

Satisfactory	$<10^3/g$
Borderline	$10^3- <10^4/g$
Unsatisfactory	$10^4- <10^5/g$
Unacceptable	$\geq 10^5/g$

In an investigation of critical control points in Chinese restaurants with respect to *B. cereus* in rice, the following preventive measures were identified (Bryan *et al.*, 1981):

- Boil only small quantities of rice on several occasions during a day's operation;
- Keep boiled rice, to be held hot, in covered pans on heated ranges, so that the internal temperature of the rice does not fall below 55°C (preferably not below 60°C);
- Put boiled rice and fried rice, to be cooled, into clean, open shallow pans spreading in a layer no more than 9 cm in depth. If the rice must be held at room temperature, the holding period should not exceed 1 hour. Keep the shallow layers of rice refrigerated until the rice is reheated;
- Fry rice until the internal temperature, after thorough mixing, reaches 74°C or more.

7.2 Industry Sector in New Zealand

The restaurant industry in New Zealand is represented by the Restaurant Association of New Zealand (<http://www.restaurantnz.co.nz/background.asp>). This organisation was formerly known as the Foodservice Association of New Zealand. It includes approximately 1,400 businesses, including restaurants, cafés and takeaways.

A “Code of Practice for the development of a Food Safety Programme for a Food Service Operation” has been developed by the Association. This includes general instructions for the rapid cooling and refrigeration of food, and describes potential problems with *B. cereus* in rice in an appendix on food poisoning. Although this association may not cover all takeaway businesses in New Zealand, it may be a useful ally for further promoting safe food handling of rice.

7.3 Economic Costs

Illness caused by bacterial toxins from *Clostridium perfringens*, *Bacillus cereus* and *Staphylococcus aureus* is considered to be of relatively short duration. In the estimate of the number of cases in New Zealand (Lake *et al.*, 2000), the duration was considered to be 2 days, and (along with direct medical costs) this generated a total cost of \$3,368,000 (Scott *et al.*, 2000). This amount represented 6.1% of the total foodborne illness costs.

8 CONCLUSIONS

8.1 Description of Risks to New Zealand Consumers

8.1.1 Risks associated with rice

The limited data from outbreaks indicate that rice or rice dishes are reasonably common vehicles for the small proportion of outbreaks attributed to *B. cereus* or other *Bacillus* species. Takeaways, often Chinese-style or Indian-style, are premises frequently cited as a source of the implicated food. This suggests that, as in other countries, a small proportion of rice is not handled in a safe manner, allowing the regeneration and growth of spores. This is supported by the results of the survey in Dunedin, where 2/46 (4%) of samples had unsatisfactory levels of *B. cereus*.

Given the lack of serious longer term outcomes of *Bacillus* spp. infection or intoxication, and the low proportion of outbreaks caused by this pathogen relative to other bacteria, this suggests that this food/hazard combination is a minor food safety issue in New Zealand compared with other bacterial pathogens.

8.1.2 Risks associated with other foods

A wide variety of foods have been implicated as transmission vehicles for *B. cereus* including meat products (e.g. casseroles, sausages), dairy products (e.g. desserts, milk and icecream), and occasionally fish and pasta (Notermans and Batt, 1998). Other foods implicated in New Zealand outbreaks have included pizza and fish and chips. These foods may be at risk if dough or batter is prepared and then stored incorrectly.

8.1.3 Quantitative risk assessment

Given the low frequency of outbreaks, a quantitative risk assessment seems unnecessary.

8.2 Commentary on Risk Management Options

It is likely that a proportion, probably a small proportion, of New Zealand takeaway and restaurant operators are handling rice inappropriately, as shown by the Dunedin survey. Education to correct unsuitable practices should address a relatively easy problem to fix, although there may be language barriers to overcome.

8.3 Data Gaps

A broader (nationwide) survey of *B. cereus* in rice from takeaway or restaurant premises would assist in confirming the proportion of rice from these sources that contain unacceptable levels of *B. cereus*.

Assays are available to detect *B. cereus* and the diarrhoeal toxin. Until recently no readily applicable assay was available for the emetic toxin, but an HPLC-MS method has now been published (Hägglom *et al.*, 2002). While a number of foodborne outbreaks are suspected to have been caused by the emetic toxin there is no way of demonstrating this conclusively.

Adoption of the HPLC-MS assay by ESR would allow more accurate data to be obtained concerning the health effects of this group of organisms in New Zealand.

Methods used by the Public Health Laboratories in New Zealand are focused on *B. cereus*, whereas the information in this Risk Profile indicates that other *Bacillus* species may be just as important and perhaps more so. Adoption of non-targeted methods to include other *Bacillus* species, and further identification of those species, would also help to give a clearer picture of the health burden that this group of organisms is imposing.

9 REFERENCES

- Agata N, Ohta M, Mori M, Isobe M. (1995) A novel dodecadeptide, cerulide, is an emetic toxin of *Bacillus cereus*. FEMS Microbiology Letters; 129: 17-20.
- Agata N, Ohta M, Yokoyama K. (2002) Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. International Journal of Food Microbiology; 73: 23-27.
- Andersson MA, Mikkola R, Helin J, Andersson C, Salkinoja-Salonen M. (1998) A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. Applied and Environmental Microbiology; 64: 1338-1343.
- Australian Bureau of Statistics. (1999) National Nutrition Survey. Foods Eaten. Canberra: Australian Bureau of Statistics.
- Baddour LM, Gaia SM, Griffin R, Hudson R. (1986) A hospital cafeteria-related food-borne outbreak due to *Bacillus cereus*: unique features. Infection Control; 7: 462-465.
- Bean NH, Griffin PM. (1990) Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, trends. Journal of Food Protection; 53: 804-817.
- Bean NH, Goulding JS, Christopher L, Agnulo FJ. (1996) Surveillance of foodborne disease outbreaks - United States 1988-1992. Morbidity and Mortality Weekly Report; 45: 1-67.
- Beattie SH, Williams AG. (1999) Detection of toxigenic strains of *Bacillus cereus* and other *Bacillus* spp. with an improved cytotoxicity assay. Letters in Applied Microbiology; 28: 221-225.
- Boxall N, Ortega J. (2003) Annual summary of outbreaks in New Zealand 2002. ESR Client Report FW0326. Porirua: ESR.
- Briley RT, Teel JH, Fowler JP. (2001) Nontypical *Bacillus cereus* outbreak in a child care center. Journal of Environmental Health; 63(7): 9-11, 21.
- Bryan FL, Bartleson CA, Christopherson N. (1981) Hazard analysis, in reference to *B. cereus*, of boiled and fried rice in Cantonese style restaurants. Journal of Food Protection; 44: 500-512.
- CDC. (1986) *Bacillus cereus*-Maine. Morbidity and Mortality Weekly Report; 35: 408-410.
- CDC. (1994) *Bacillus cereus* food poisoning associated with fried rice at two child day care centers. Morbidity and Mortality Weekly Report; 43: 177-178.
- Chiou A, Chen L-H, Chen S-K. (1991) Foodborne illness in Taiwan, 1981-1989. Food Australia; 43: 70-71.
- Codex. (1999) Draft principles and guidelines for the conduct of microbiological risk assessment. Report of the thirty first session of the Codex committee on food hygiene. ALINORM 99/13A. Rome: Codex Alimentarius Commission.
-

- Davey GR. (1985) Food poisoning in New South Wales: 1977-1984. *Food Technology in Australia*; 37: 453-456.
- Djuretic T, Wall PG, Ryan MJ, Evans SH, Adak GK, Cowden JM. (1996) General outbreaks of infectious intestinal disease in England and Wales 1992 to 1994. *Communicable Disease Report*; 6: R57-R63.
- ESR. (1998) Annual surveillance summary 1997. Porirua: ESR.
- Evans HS, Madden P, Douglas C, Adak GK, O'Brien SJ, Djuretic T, Wall PG, Stanwell-Smith R. (1998) General outbreaks of infectious intestinal disease in England and Wales: 1995 and 1996. *Communicable Disease and Public Health*; 1: 165-171.
- GEMS/Food. (1998) GEMS/Food Regional Diets. Regional per capita consumption of raw and semi-processed agricultural commodities. WHO Publication WHO/FSF/FOS/98.3 <http://www.who.int/fsf/GEMS/gemsregi.pdf>
- Gilbert RJ, Humphrey TJ. (1998) Food-borne bacterial gastroenteritis. In: Hausler WJ, Sussman M (eds). *Topley and Wilson's Microbiology and Microbial Infections* 9th edn. Volume 3. Bacterial Infections. London: Arnold; 539-565.
- Gilbert RJ, Stringer MF, Peace TC. (1974) The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *Journal of Hygiene*; 73: 433-444.
- Gilbert RJ, de Louvais J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ. (2000) Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Communicable Disease and Public Health*; 3: 163-167.
- Granum PE. (1997) *Bacillus cereus*. In: Doyle MP, Beuchat LR, Montville TJ (eds). *Food microbiology fundamentals and frontiers*. Washington DC: ASM press; 327-352.
- Granum PE, Lund T. (1997) *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Letters*; 157: 223-228.
- Hägglom MM, Apetroaie C, Andersson MA, Salkinoja-Salonen, MS. (2002) Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. *Applied and Environmental Microbiology*; 68: 2479-2483.
- Harmon SM, Kautter DA. (1991) Incidence and growth potential of *Bacillus cereus* in ready-to-serve foods. *Journal of Food Protection*; 54: 372-374.
- Hassan G, Nabbut N. (1996) Prevalence and characterization of *Bacillus cereus* isolates from clinical and natural sources. *Journal of Food Protection*; 59: 193-196.
- Hough A. (2000) Critical control point management and bacteriological status of sushi in New Zealand (F13/99/3). ESR Client Report FW9946. Christchurch: ESR.

- Jackson SG, Goodbrand RB, Ahmed R, Kasatiya S. (1995) *Bacillus cereus* and *Bacillus thuringiensis* isolated in a gastroenteritis outbreak investigation. *Letters in Applied Microbiology*; 21: 103-105.
- Jacquette CB, Beuchat LR. (1998) Survival and growth of psychrotrophic *Bacillus cereus* in dry and reconstituted infant rice cereal. *Journal of Food Protection*; 61: 1629-1635.
- Jenson I, Moir CJ. (1997) *Bacillus cereus* and other *Bacillus* species. In: *Foodborne Microorganisms of Public Health Significance*. Sydney: Australian Institute of Food Science and Technology; 379-406.
- Johnson KM. (1984) *Bacillus cereus* foodborne illness-An update. *Journal of Food Protection*; 47: 145-153.
- Kessel AS, Gillespie IA, O'Brien SJ, Adak GK, Humphrey TJ, Ward LR. (2001) General outbreaks of infectious intestinal disease linked with poultry, England and Wales, 1992-1999. *Communicable Disease and Public Health*; 4: 171-177.
- Lake RJ, Baker MG, Garrett N, Scott WG, Scott HM. (2000) Estimated number of cases of foodborne infectious disease in New Zealand. *New Zealand Medical Journal*; 113: 278-281.
- Lee PK, Buswell JA, Shinagawa K. (1995) Distribution of toxigenic *Bacillus cereus* in rice samples marketed in Hong Kong. *World Journal of Microbiology and Biotechnology*; 11: 696-698.
- Lindqvist R, Andersson Y, de Jong B, Norberg P. (2000) A summary of foodborne disease incidents in Sweden, 1992 to 1997. *Journal of Food Protection*; 63: 1315-1320.
- Little CL, Barnes J, Mitchell RT. (2002) Microbiological quality of take-away cooked rice and chicken sandwiches: effectiveness of food hygiene training of the management. *Communicable Disease and Public Health*; 5: 289-298.
- Lopez L, Baker M, Kieft C. (2001) Annual summary of outbreaks in New Zealand 2000. ESR Client Report FW0151. Porirua: ESR
- Mahler H, Pasi A, Kramer JM, Schulte P, Scoging AC, Bar W, Krahenbuhl S. (1997) Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. *New England Journal of Medicine*; 336(16): 1173-1174.
- McElroy DM, Jaykus L-A, Foegeding PM. (2000) Validation and analysis of modeled predictions of growth of *Bacillus cereus* spores in boiled rice. *Journal of Food Protection*; 63: 268-272.
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. (1999) Food-related illness and death in the United States. *Emerging Infectious Diseases*; 5: 607-625.

Ministry of Health/Ministry of Agriculture and Forestry. (2000) Food Administration in New Zealand: A Risk Management Framework for Food Safety. Wellington: Joint Ministry of Health and Ministry of Agriculture and Forestry Food Harmonisation Project.

Naing T, Baker M, Galloway Y, Margolin T. (1999) Annual summary of outbreaks in New Zealand in 1998. Institute of Environmental Science and Research. Porirua: ESR.

Nichols GL, Little CL, Mithani V, de Louvois J. (1999) The microbiological quality of cooked rice from restaurants and take-away premises in the United Kingdom. *Journal of Food Protection*; 62: 877-882.

Notermans S, Batt CA. (1998) A risk assessment approach for food-borne *Bacillus cereus* and its toxins. *Journal of Applied Microbiology Symposium Supplement*; 84: 51S-61S.

Olsen SJ, MacKinnon LC, Goulding JS, Bean NH, Slutsker L. (2000) Surveillance for foodborne disease outbreaks-United States, 1993-1997. *Morbidity and Mortality Weekly Reports*; 49: 1-51.

Penna TCV, Moraes DA. (2002) The influence of nisin on the thermal resistance of *Bacillus cereus*. *Journal of Food Protection*; 65: 415-418.

Penna TCV, Moraes DA, Fajardo DN. (2002) The effect of nisin on growth kinetics from activated *Bacillus cereus* spores in cooked rice and in milk. *Journal of Food Protection*; 65: 419-422.

Perks M, Baker M, Galloway Y. (2000) Annual summary of outbreaks in New Zealand in 1999. Institute of Environmental Science and Research. Porirua: ESR.

Raevuori M, Kiutamo T, Niskanen T, Salminen K. (1976) An outbreak of *Bacillus cereus* food-poisoning in Finland associated with boiled rice. *Journal of Hygiene*; 76: 319-327.

Russell DG, Parnell WR, Wilson NC *et al.* (1999) NZ Food: NZ People. Key results of the 1997 National Nutrition Survey. Wellington: Ministry of Health.

Rusul G, Yaacob NH. (1995) Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. *International Journal of Food Microbiology*; 25: 131-139.

Scott WG, Scott HM, Lake RJ, Baker MG. (2000) Economic cost to New Zealand of foodborne infectious disease. *New Zealand Medical Journal*; 113: 281-284.

Setlow P, Johnson EA. (1997) Spores and their significance. In: Doyle MP, Beuchat LR, Montville TJ (eds). *Food Microbiology: Fundamentals and Frontiers*. Washington, DC: American Society for Microbiology; 30-65.

Shelef LA, Liang P. (1982) Antibacterial effects of butylated hydroanisole (BHA) against *Bacillus* spp. *Journal of Food Science*; 47: 796-799.

Simone E, Goosen M, Notermans SHW, Borgdorff MW. (1997) Investigation of foodborne disease by food inspection services in the Netherlands, 1991-1994. *Journal of Food Protection*; 60: 442-446.

Tan A, Heaton S, Farr L, Bates J. (1997) The use of *Bacillus* diarrhoeal enterotoxin (BDE) detection using an ELISA technique in the confirmation of the aetiology of *Bacillus*-mediated diarrhoea. *Journal of Applied Microbiology*; 82: 677-682.

Thornley C. (2002) Annual summary of outbreaks in New Zealand 2001. Porirua: ESR

Todd ECD. (1987) Foodborne and waterborne disease in Canada-1980 annual summary. *Journal of Food Protection*; 50: 420-428.

Todd ECD. (1992) Foodborne disease in Canada-a 10-year summary from 1975 to 1984. *Journal of Food Protection*; 55: 123-132.

Ultee A, Slump RA, Steging G, Smid EJ. (2000) Antimicrobial activity of cavacrol toward *Bacillus cereus* on rice. *Journal of Food Protection*; 63: 620-624.

Varadaraj MC, Keshava N, Devi N, Dwarakanath CT, Manjrekar SP. (1992) Occurrence of *Bacillus cereus* and other *Bacillus* species in Indian snack and lunch foods and their ability to grow in a rice preparation. *Journal of Food Science and Technology*; 29: 344-347.

Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ, Roderick PJ. (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. *British Medical Journal*; 318: 1046-1050.

Wong TL. (1996) Staphylococcal and *Bacillus cereus* enterotoxins in food-borne intoxications. ESR Client Report FW96/33. Christchurch: ESR.

Wong TL. (1997) Report on fermented bean curd. ESR Client Report FW96/54. Christchurch: ESR.

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