

**1-04**  
**18 February 2004**

## **FINAL ASSESSMENT REPORT**

### **APPLICATION A454**

#### ***BACILLUS CEREUS* LIMITS IN INFANT FORMULA**

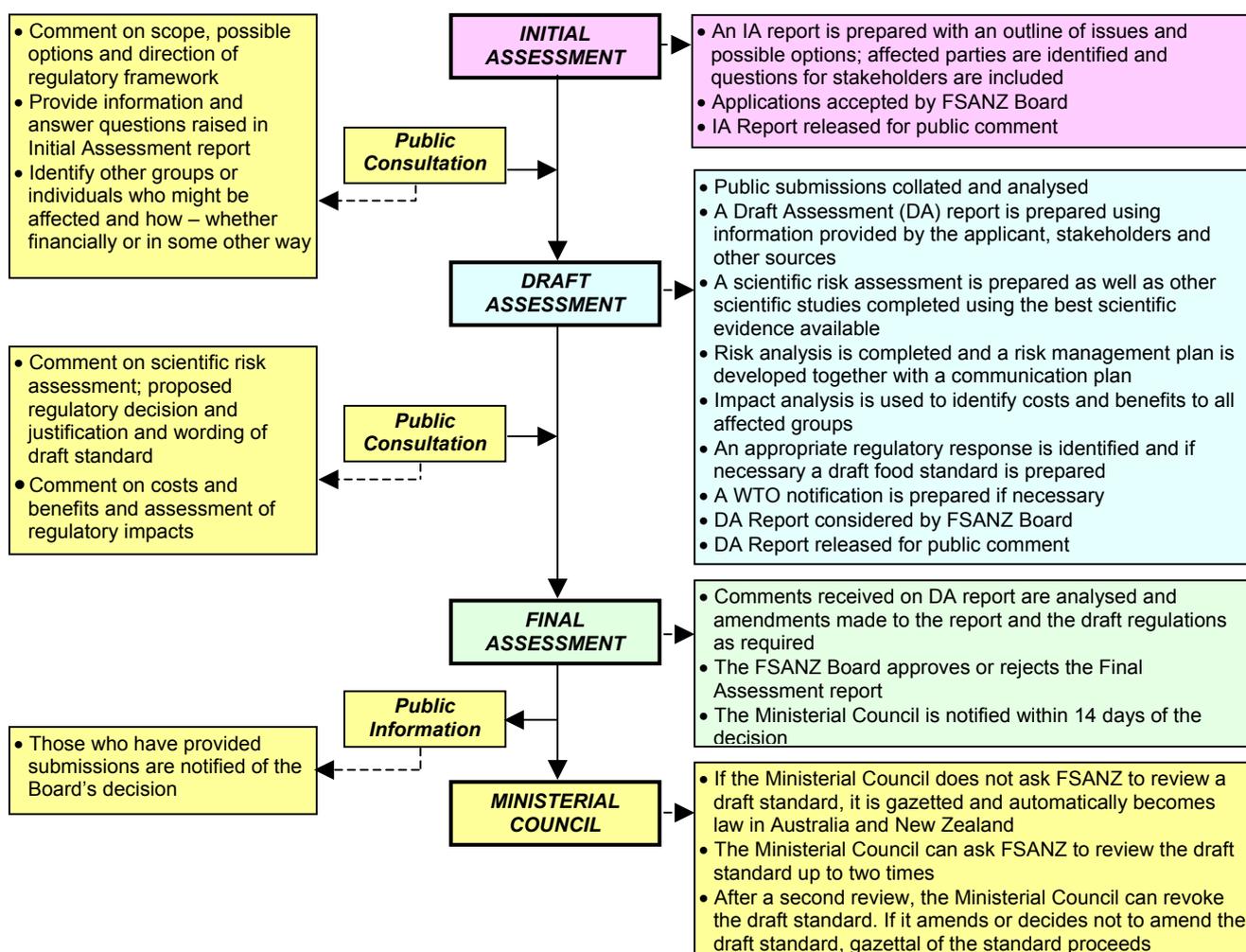
## FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



## **Final Assessment Stage**

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

## **Further Information**

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Assessment reports are available for viewing and downloading from the FSANZ website [www.foodstandards.gov.au](http://www.foodstandards.gov.au) or alternatively paper copies of reports can be requested from FSANZ's Information Officer at [info@foodstandards.gov.au](mailto:info@foodstandards.gov.au) including other general enquiries and requests for information.

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## Executive Summary and Statement of Reasons

### Background

Anchor Products Limited, now Fonterra Co-operative Group Limited, a New Zealand infant formula manufacturer, has made an application (A454) to FSANZ to vary the microbiological limit for *Bacillus cereus* (*B. cereus*) in infant formula products currently specified in Standard 1.6.1 – Microbiological Limits for Food of the *Australia New Zealand Food Standards Code* (the Code). This Application to amend the limit is made on the basis that the current standard is too restrictive and cannot be complied with consistently under good manufacturing and hygienic practice.

*B. cereus* spores can occur in milk at very low levels. Processing of the milk into powder to be used in infant formula will not eliminate the spores present. The levels of *B. cereus* in raw milk are higher when supplementary feeding of dairy cattle with silage is required, and as a consequence the level of spores in the dried milk powder may result in the final infant formula exceeding the current limit of 10 cfu per gram in Standard 1.6.1. The Applicant proposed that a sampling plan in which the lower limit would be achievable and safe. In addition, the Applicant emphasised that the limit set should also consider the limit of enumeration of the analytical method to be used.

In addition to the microbiological limits for infant formula in Standard 1.6.1, Division 2 of Standard 1.1A.1 - Transitional Standard for Infant Formula Products also specifies microbiological limits for infant formula (Standard R7 of the former Australian *Food Standards Code*). The *B. cereus* limits in this standard are more lenient than those contained in Standard 1.6.1 or proposed by the applicant. Standard 1.1A.1 will operate as an alternative standard until 20 June 2004.

The current microbiological risk assessment concluded that:

- Low numbers of *B. cereus* are present in infant formula due to contamination of raw milk from the farm environment. There is good evidence to suggest that there are seasonal increases in the level of *B. cereus* spores present in milk due to, for example, supplementary feeding of dairy cattle with silage, such that the level of spores in the dried milk powder will result in the final infant formula exceeding the current limit of 10 cfu per gram in Standard 1.6.1. This is a world-wide occurrence. Spores of *B. cereus* contaminate milk via contamination of the udder from faeces, soil, grass and silage. The levels of *B. cereus* in raw milk are seasonally dependent with highest levels occurring in spring and summer.
- Illness resulting from contamination of food with *B. cereus* is generally mild. *B. cereus* can produce diarrhoea and vomiting following consumption of food containing  $10^5$  cells/spores or greater. However, the infectious dose of *B. cereus* for infants may be lower because their immune systems are not fully developed and infants are more susceptible to bacterial infections than healthy adults and older children.

- Although there is very little epidemiological evidence linking *B. cereus* to illness in infants, diarrhoea is a significant cause of ill health and death among infants and children in developed countries. As diarrhoea is usually only a mild illness, people do not always seek medical attention, and therefore it is often unreported. In addition if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* does not require notification to health authorities. Therefore it is possible that some infants become ill from *B. cereus* when the bacteria is present in sufficient numbers to provide an infectious dose.
- Infant formula is designed as a human milk substitute for infants and as such may represent the sole source of nutrition in some infants, i.e. the frequency of consumption is very high, especially for infants under the age of 6 months.
- Surveys have shown that some consumers prepare infant formula incorrectly with warm tap water, leave the bottle at room temperature for more than 2 hours, and store prepared warm formula in insulated carriers when going out.

The risk assessment models the growth of *B. cereus* under various preparation and storage scenarios and concludes that:

- Powdered infant formula containing up to 100 cfu/g of *B. cereus* and reconstituted using the following practices, would not expose infants to an infectious dose of *B. cereus*:
  - Formula reconstituted with cooled boiled water (25°C) and stored for 24 hours at 4°C.
  - Formula reconstituted with cooled boiled water and stored for 24 hours at 10°C.
- Formula reconstituted from powder with levels of 1000 cfu/g and then stored at 10°C for 24 hours may pose a risk to infants.
- The following practices are considered unsafe as they were shown to result in rapid growth of *B. cereus* to levels that could result in illness, in reconstituted formula during storage:
  - Storing formula reconstituted with warm tap water (37°C) or boiling water (80°C).
  - Storing formula at room temperature.
  - Storing warm formula in insulated carriers.

## Draft Assessment

Based on the findings of the microbiological risk assessment an amendment to Standard 1.6.1 for a regulatory limit of 100 cfu/g was proposed. This proposed regulatory option is consistent with the application, can be effectively implemented by industry and is consistent with international obligations. At this level, infants will not be exposed to levels of *B. cereus* that result in illness when formula is prepared and stored according to NHMRC and Ministry of Health infant feeding guidelines<sup>1</sup>.

<sup>1</sup> Ministry of Health (2000) Food and Nutrition guidelines for Healthy Infants and Toddlers (Aged 0 – 2 years) – A Background Paper. New Zealand  
[www.moh.govt.nz/moh.nsf/0/d755f603abd677cb4c25667100062841?OpenDocument/](http://www.moh.govt.nz/moh.nsf/0/d755f603abd677cb4c25667100062841?OpenDocument/)

## Public Consultation

All six of the submissions received at Draft Assessment, supported a change to amend the limit for *B. cereus* in infant formula in Standard 1.6.1. Of these, four submissions found acceptable, the preferred option presented at Draft Assessment (Option 4 for a regulatory limit of 100 cfu/g). Two submissions supported Option 2 (Retention of the Transitional Standard 1.1A.1).

## Final Assessment

Following public consultation, no changes have been made to the preferred regulatory option at the Draft Assessment. While two submissions supported the adoption of the Transitional Standard 1.1A.1 (Option 2), the risk assessment concluded that levels of *B. cereus* greater than 100 cfu/g may not provide adequate public health and safety for infants. Levels of 1000 cfu/g of *B. cereus* may pose a risk to infants when infant formula is stored for 24 hours under poorly regulated refrigeration temperatures of 10°C or more. A regulatory limit of 100 cfu/g is therefore recommended as:

- infant formula may be the sole source of nutrition for infants;
- infants have increased susceptibility to illness compared to the general population; and
- *B. cereus* is capable of rapid growth under conditions of time and temperature abuse.

## Statement of Reasons

FSANZ recommends an amendment to Standard 1.6.1 – Microbiological Limits for Food for *B. cereus* in infant formula products for the following reasons:

- The proposed amendment (n=5, c=0, m= 100) will protect the health of infants from potential illness resulting from the consumption of infant formula contaminated with *B. cereus*. The proposed amendment to the Code is therefore consistent with the section 10 objectives of the FSANZ Act, in particular, the protection of public health and safety.
- The current microbiological Standard 1.6.1 (n=5, c=2, m=10, M=100) is considered too restrictive and cannot be complied with consistently by industry under good manufacturing and hygienic practice.
- The current Transitional Standard 1.1A.1 (n=5, c=1, m=10<sup>2</sup>, M=10<sup>3</sup>) may not provide adequate public health and safety for infants.

The risk assessment undertaken as part of A454 modelled the growth of *B. cereus* under various preparation and storage scenarios and concluded that:

- Powdered infant formula containing up to 100 cfu/g of *B. cereus*, and reconstituted using the following practices, would not expose infants to an infectious dose of *B. cereus*:

- Formula reconstituted with cooled boiled water (25°C) and stored for 24 hours at 4°C.
- Formula reconstituted with cooled boiled water and stored for 24 hours at 10°C.
- Formula reconstituted from powder with levels of 1000 cfu/g and then stored at 10°C for 24 hours may pose a risk to infants.
- The following practices are considered unsafe as they were shown to result in rapid growth of *B. cereus* to levels that result in illness, in reconstituted formula during storage:
  - Storing formula reconstituted with warm tap water (37°C) or boiling water (80°C).
  - Storing formula at room temperature.
  - Storing warm formula in insulated carriers.

The proposed regulatory measure provides a comprehensive level of public health and safety protection for infants and can be effectively implemented by industry.

## 1. Introduction

The existing microbiological limits for *B. cereus* in infant formula were gazetted in December 2000 in Standard 1.6.1 – Microbiological Limits for Food of the Code. Standard 1.6.1 lists the maximum permissible levels of food-borne microorganisms that pose a risk to human health in nominated foods or classes of foods.

In addition to the microbiological limits for infant formula products in Standard 1.6.1, Division 2 of Standard 1.1A.1 - Transitional Standard for Infant Formula Products also specifies microbiological limits for infant formula (Standard R7 of the former Australian *Food Standards Code*). The *B. cereus* limits in this standard are more lenient than those contained in Standard 1.6.1. Standard 1.1A.1 will operate as an alternative standard until 20 June 2004.

Currently manufacturers are able to comply with either Standard 1.6.1 or Standard 1.1A.1.

The options proposed at Initial Assessment in dealing with this application were to either amend the microbiological limit for *B. cereus* in infant formula in Standard 1.6.1 or to reject the application. An amendment to Standard 1.6.1 could include (A) accepting the sampling plan proposed by the applicant or (B) proposing an alternative sampling plan that would be achievable, measurable and adequate to protect public health and safety. The sampling plan specified in Standard 1.1A.1 was considered as an alternative sampling plan.

### 1.2 Nature of Application

An application (Application A454) was received from Anchor Products Limited, now Fonterra, an infant formula and nutritional powder manufacturer in New Zealand (Waitoa), to amend Standard 1.6.1 – Microbiological Limits for Food. The Application specifically proposed that the *B. cereus* limit for infant formula in Standard 1.6.1 – Microbiological Limits for Food be amended from:

$n = 5, c = 2, m = 10 \text{ cfu per gram}, M = 100 \text{ cfu}^2 \text{ per gram}$   
to:  
 $n = 5, c = 3, m = 50 \text{ cfu per gram}, M = 100 \text{ cfu per gram}$

Where:

**n** means the minimum number of sample units which must be examined from a lot of food

**c** means the maximum allowable number of defective sample units

**m** means the acceptable microbiological level in a sample unit

**M** means the level which, when exceeded in one or more samples would cause the lot to be rejected

#### 1.2.1 Justification for the Application

Application A454 argues that the limit of  $m = 10$  set for *B. cereus* in infant formula in Standard 1.6.1 cannot be complied with consistently. This is because there can be seasonal variation in the level of *B. cereus* spores in the raw milk which then survive the heat and processing conditions in manufacturing milk powder.

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2 cfu – colony forming units

Sufficient spores remain so that, at times, the *B. cereus* levels in dried milk products exceeds 10/g for a complete batch or succession of batches.

The following reasons are given by the applicant for a variation to the *B. cereus* standard for infant formula:

- The limit of  $m = 10$  per gram is not always achievable using milk solids from pasture and silage fed animals in New Zealand and elsewhere;
- A higher limit of  $m = 50$  per gram is not unsafe, with the margin of safety being about 20 000 times less than the toxic level under most product abuse models;
- The present limit of  $m = 10$  per gram is set at the limit of measurement for *B. cereus*, giving rise to the potential for interpretation misunderstandings between officials and commercial practitioners.

## **2. Regulatory Problem**

### **2.1 Current Standard**

#### *2.1.1 Transitional Standard for Infant Formula Products*

Standard 1.1A.1 - Transitional Standard for Infant Formula Products came into effect on 20 December 2002. This Standard incorporates Standard R7 – Infant Formula of the former Australian *Food Standards Code* (Division 2) and Regulation 242 of the New Zealand *Food Regulations* (Division 3). In Australia, Standard 1.1A.1 operates as a transitional alternative standard to Standard 2.9.1 – Infant Formula Products for a period of 2 years from the commencement of Standard 2.9.1 (until June 2004). During this time, infant formula products produced in or imported into Australia must comply with Division 2 of this Standard or Standard 2.9.1 of the Code.

In New Zealand, Standard 1.1A.1 also operates as a transitional alternative standard to Standard 2.9.1 for a period of two years until 20 June 2004. During this time infant formula products produced in or imported into New Zealand must comply with Division 2 or 3 of this Standard or Standard 2.9.1

Infant formula products complying with Division 2 of Standard 1.1A.1 must comply with the microbiological limits specified in Division 2. The microbiological standard for *B. cereus* in Division 2 is far more lenient than Standard 1.6.1 and specifies that infant formula powder shall have a *B. cereus* count not exceeding 100 microorganisms per gram (paragraph (4)(a)(v)) such that:

*when 5 sample units each consisting of at least 100g or more of infant formula powder are examined as detailed, the result shall be reported as 'not exceeding 100 microorganisms per gram of the food' when at least 4 of the 5 sample units have a Bacillus cereus count not exceeding 100 microorganisms per gram and the remaining sample unit has a Bacillus cereus count not exceeding 1000 microorganisms per gram.* (subclause (7)(e))

When written in a sampling plan format the standard for *B. cereus* in infant formula powder in Division 2 is:  $n = 5$ ,  $c = 1$ ,  $m = 10^2$ ,  $M = 10^3$

Infant formula products complying with Division 3 of Standard 1.1A.1 or Standard 2.9.1 must, however, comply with the microbiological limits specified in Standard 1.6.1 - Microbiological Limits for Food.

### 2.1.2 Standard 1.6.1 – Microbiological Limits for Food

Standard 1.6.1 lists the maximum permissible levels of food-borne microorganisms that pose a risk to human health in nominated foods or classes of foods. The sampling plan included in this standard for *B. cereus* in infant formula products and powdered infant formula products with added lactic acid producing cultures, (collectively referred to in this paper as ‘powdered infant formula products’), specifies an acceptable microbiological level in a sample unit ( $m$ ) of 10 cfu per gram and a failing level ( $M$ ) of  $10^2$  cfu per gram:

| Food                             | Microorganism             | n | c | m  | M      |
|----------------------------------|---------------------------|---|---|----|--------|
| Powdered infant formula products | <i>Bacillus cereus</i> /g | 5 | 2 | 10 | $10^2$ |

(Extract from Standard 1.6.1, Schedule)

Where:

**n** means the minimum number of sample units which must be examined from a lot of food

**c** means the maximum allowable number of defective sample units

**m** means the acceptable microbiological level in a sample unit

**M** means the level, when exceeded in one or more samples would cause the lot to be rejected

## 2.3 International Regulations

The Codex *Code of Hygienic Practice for Foods for Infants and Children* (CAC/RCP 21-1979) contains advisory microbiological specifications for infant formula, which includes mesophilic aerobic bacteria, coliforms and *Salmonella*. Limits for *B. cereus* are not included.

Current USA Food and Drug Administration (FDA) regulations have set a microbiological limit for *B. cereus* in infant formula of not more than 1000 per gram<sup>3</sup>. However, the USA FDA has more recently proposed to tighten this regulation to not more than 100 per gram<sup>4</sup>. In Canada<sup>5</sup>, the Health Protection Branch has recommended microbiological guidelines for *B. cereus* in powdered infant formula of  $n=10$ ,  $c=1$ ,  $m=10^2$ ,  $M=10^4$ . Within the European Union, the Netherlands and Portugal have set a regulatory limit of 100 cfu/g and Hungary and Poland have an agreed limit of 100 cfu/g<sup>6</sup>. Switzerland has a regulatory limit of 1000 cfu/g whereas Iran and Denmark have a regulatory limit for *B. cereus* to be absent in 0.01 per gram (approximately equivalent to less than 100 per gram).<sup>7</sup>

<sup>3</sup> Regulation 54 FR 3783

<sup>4</sup> Proposed Sec. 106.55(c) requires that powdered infant formula be tested for *B. cereus* when the APC exceeds 100 cfu/g and establishes an M value for *B. cereus* of 100 MPN/g or 100 CFU/g.

<sup>5</sup> The Canadian microbiological limits for *B. cereus* in infant formula can be found in the ‘Standards and Guidelines for the Microbiological Safety of Foods’, published by the Health Product and Food Branch Health Canada in January 2003.

<sup>6</sup> As supplied by Fonterra Co-operative Group

<sup>7</sup> As supplied by Fonterra Co-operative Group

## **2.4 Difficulties with compliance and risks to public health and safety under the current arrangements.**

Contamination of food with *B. cereus* generally results in a relatively mild illness. However, consumption of food with large numbers of *B. cereus* can produce diarrhoea and vomiting. Although there is no epidemiological evidence linking *B. cereus* illness with infants, diarrhoea is a significant cause of ill health among infants.

The current microbiological Standard 1.6.1 is considered too restrictive and the applicant claims that it cannot be complied with consistently by industry under good manufacturing and hygienic practice.

## **3. Objective**

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

In considering this Application, the key objective is the protection of infant health by appropriate regulatory measures that are effective in mitigating the risks of *B. cereus* infection from infant formula.

In considering a Standard, FSANZ has also needed to balance public health issues with that of industry being able to effectively implement any proposed regulatory measure.

## 4. Background

### 4.1 Historical Background

Microbiological criteria for infant formula were incorporated into Standard R7 of the former Australian *Food Standards Code* in July 1988. Microbiological criteria for infant formula included limits for standard plate count, coliforms, *Salmonella*, coagulase positive *Staphylococci*, and *B. cereus*. Standard 1.6.1 of the Code, has retained limits for all of these organisms.

The regulatory limit for *B. cereus* became more restrictive following Proposal P178 – Review of Microbiological Standards, which was gazetted in December 2000. During this review the decision was made to lower the *B. cereus* levels by members of the expert committee following the publication of an article by Notermans and Batt<sup>8</sup> which looked at *B. cereus* from a risk assessment perspective. Some basic modelling at this time on reconstituted formula predicted that the limits in the then Standard R7 of the former Australian *Food Standards Code* might not provide adequate protection for infants.

Infant formula is considered a high-risk food, due to the greater susceptibility of infants to illness compared to the general population. Infants have an underdeveloped immune system, a severe response to enteric pathogens, and increased mortality. In general, infants are considered vulnerable to food-borne illness because of their underdeveloped immune systems and infant formula may represent their sole source of nutrition.

### 4.2 Compliance with and enforcement of Standard 1.6.1

Sampling plans are used for determining when a lot or consignment of food should be accepted or rejected based on the possible risk posed to human health. Sampling plans presented in Standard 1.6.1 of the Code are in the format devised by the International Commission on Microbiological Specifications for Foods (ICMSF). They indicate:

- the number of samples to be tested (n);
- the level of micro-organisms considered to be acceptable (m);
- the level of micro-organisms considered to be unacceptable (M)
- the number of samples which should conform to these limits (c)

Sampling plans are statistically based and provide a uniform basis for acceptance of a lot against defined criteria. The two widely accepted types of sampling plans defined by the ICMSF are the 2-class and 3-class plans. A 2-class sampling plan is used primarily for pathogens where a presence/absence test is to be used. Only one level of acceptance is provided – ‘m’. A 3-class plan is used where enumeration is required and the acceptance of the lot is based on a spread in distribution, such that two limits are given, ‘m’ and ‘M’. In a 3-class plan, the number of samples allowed to exceed the lower limit is denoted by ‘c’. No samples are allowed to exceed ‘M’. In general, ‘m’ is the level that is acceptable and attainable using good manufacturing practice (GMP) and ‘M’ is the limit above which there is an unacceptable level of contamination caused by poor hygienic practice. Application A454 presents data that suggests that the ‘m’ level currently set for *B. cereus* in infant formula in Standard 1.6.1 is not consistently attainable using GMP.

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<sup>8</sup> Notermans, S., and C.A. Batt (1998) A risk assessment approach for Food-borne *Bacillus cereus* and its toxins. *J. Applied Microbiology* 84: 51s-61s

The ICMSF publication *Microorganisms in Foods 7, Microbiological Testing in Food Safety Management* (2002)<sup>9</sup>, provides detailed discussion on choosing a suitable sampling plan when establishing microbiological criteria, based on the severity of the hazard and whether it is appropriate for the particular food.

## 5. Relevant Issues

### 5.1 *B. cereus* in raw milk

*B. cereus* organisms from the farm environment can easily find their way into raw milk. Spores of *B. cereus* contaminate milk via faecal contamination of the udder. High numbers of *Bacillus* spores in raw milk have been associated with high spore numbers in fresh cow faeces<sup>10</sup>. *B. cereus* is also spread from soil and grass to the udders of cows and into raw milk<sup>11</sup>.

Another source of contamination is poorly made or stored silage. Silage is the term used for forage with a high moisture content preserved by fermentation and is used in seasons when fresh forage is unavailable. Silage is preserved through rapid pH reduction and maintenance of anaerobic conditions. Proliferation of *Bacillus* spp. (*B. cereus*, *B. lentus*, *B. firmus*, *B. sphaericus*, *B. licheniformis*, and *B. polymyxa*) usually occurs during the later stages of aerobic spoilage of silage. *B. cereus* levels up to 10<sup>7</sup> cfu/g have been reported in or on bedding and up to 10<sup>5</sup> cfu/g in silage<sup>12</sup>.

*B. cereus* contamination has also been sourced from raw milk from soil via dirty teats. The levels of *B. cereus* in different feeds (silage, hay and concentrates), cow dung and milking equipment was found to be very low compared to the levels found in soil. The spore content of the soil varied from <50 to 380,000/g<sup>13</sup>.

The levels of *B. cereus* in raw milk are seasonally dependent with highest levels occurring in spring and summer<sup>14</sup>. In Australia seasonal conditions and unreliable rainfall, require many dairy farmers to use hay and silage as methods of fodder conservation to supplement pasture and natural sources of stock feed<sup>15</sup>.

### 5.2 Conclusions of the microbiological risk assessment

Based on the available information, the risk assessment (**Attachment 2**) concluded that the presence of *B. cereus* in low numbers in infant formula is unlikely to cause harm.

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9 ICMSF (2002) *Microorganisms in Food 7. Microbiological Testing in Food Safety Management*. Blackwell Scientific, London.

10 Waes, G. (1987) Boterzuurbacteriën in melk en in kuilvoer. *Landbouwtijdschrift* 40:925-932.

te Giffle, M.C., R.R. Beumer, R. Slaghuis, and F.M. Rombouts (1995) Occurrence and characterization of (psychrotrophic) *Bacillus cereus* on farms in the Netherlands. *Neth. Milk Dairy J.* 49:125-

11 Doyle, M., Beuchat, L., and Montville, T. (Eds) (1997) *Fundamentals in Food Microbiology* (ASM Press Washington D.C).

12 Crielly, E.M., Logan, N.A., and Anderton, A. (1994) Studies on the *Bacillus* flora of milk and milk products. *Journal Applied Bacteriology.* 77:256-263

13 Christiansson, A., Bertilsson, J., Svensson, B. (1999) *Bacillus cereus* spores in raw milk: Factors affecting the contamination of milk during the grazing period. *Journal of Dairy Science.* 82:305-314

14 Sutherland, A.D. and R. Murdoch (1994) Seasonal occurrence of psychrotrophic *Bacillus* species in raw milk, and studies on the interactions with mesophilic *Bacillus* sp. *International Journal of Food Microbiology.* 21:279-292

<sup>15</sup> Australian Bureau of Statistics (2003) Year Book Australia 2003 Agriculture Selected other crops, <http://www.abs.gov.au?Ausstats/abs@.nsf/Lookup?DA76CB47B01891B8CA256CAE>

However, if reconstituted infant formula containing low levels of *B. cereus* is subject to temperature/ time abuse, there is a potential for unsafe levels to be reached. The level of *B. cereus* that may result in illness is in the range of  $10^5$  to  $10^8$  total cells/spores.

Although there is very little epidemiological evidence linking *B. cereus* to illness in infants, diarrhoea is a significant cause of ill health and death among infants and children in developed countries. It is acknowledged that both food-borne pathogens and viruses may cause diarrhoea in infants. However, as diarrhoea is usually only a mild illness, people do not always seek medical attention, and therefore the condition is often unreported. In addition if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* infection does not require notification to health authorities. Therefore it is likely that some infants become ill from *B. cereus* when the bacteria is present in sufficient numbers.

Infant formula is designed as a human milk substitute for infants and as such may represent the sole source of nutrition in some infants i.e. the frequency of consumption is very high, especially for infants under the age of 6 months. Although *B. cereus* intoxication generally only results in mild illness, infant formula is a food class considered high risk due to the susceptibility of infants to enteric bacterial pathogens, their severe response to toxins, and increased mortality.

Evidence is available that some consumers prepare infant formula incorrectly with warm tap water, leave bottles at room temperature for more than 2 hours, and store prepared warm formula in insulated carriers when going out<sup>16</sup>.

The risk assessment modelled the growth of *B. cereus* under various preparation and storage scenarios and concluded:

- Formula stored at 4°C for 24 hours after reconstitution with cooled boiled water (25°C), boiled water (80°C) or warm tap water (37°C) does not appreciably increase the level of *B. cereus* beyond that initially present in the formula. This was also the case for formula prepared with cooled boiled water and stored at 10°C for 24 hours.
- However, formula prepared from powder with initial levels of 100 cfu/g using recently boiled water or warm tap water and stored at 10°C for 24 hours may approach the infectious dose of  $10^5$  (worst case scenario) if an infant consumed 150 ml formula in one feed. Formula prepared with initial levels of 1000 cfu/g under these conditions exceeds the infectious dose (worst case scenario).
- Infant formula prepared with cooled boiled water with initial levels of 100 cfu/g in the powder may reach the infectious dose when stored at room temperature after 6 hours, and after 4 hours when initial levels are 1000 cfu/g.
- The practice of preparing infant formula, with initial levels of 100 cfu/g, with warm tap water or boiling water is potentially unsafe as the formula may reach an infectious dose when stored at 10°C for 24 hours or when stored at room temperature for greater than 4 hours.

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16 Lilurne A, Oates, R., Thomposn, S., Tong, L (1988) Infant feeding in Sydney: a survey of mothers who bottle feed. Aust Paediatr J. 24:49-54

Beck-Fein, S, Faici, C.D. (1999) Infant formula preparation, handling, and related practices in the United States. JADA 9(10):1234-40

- The practice of storing warm formula in insulated carriers is also potentially unsafe. 150 mL of warm reconstituted formula with initial levels of 100 cfu/g in the powder stored in an insulated carrier may reach an infectious dose after 2 hours.

### **5.3 Education and guidelines for the preparation and storage of infant formula for consumers**

Infant formula powder is normally stored at ambient temperatures. Manufacturers generally instruct consumers to make up infant formula with cooled boiled water and advise that it should not be held for longer than 24 hours under refrigeration. However some manufacturers are now advising consumers to use warm water (previously boiled) to reconstitute formula and to use the reconstituted formula immediately.

The National Health & Medical Research Council (NHMRC) *Dietary Guidelines for Children and Adolescents in Australia*, include Infant Feeding Guidelines that recommend infant formula be prepared daily and stored in the refrigeration for a maximum of 24 hours.

The New Zealand Ministry of Health's *Food and Nutrition Guidelines for Health Infants and Toddlers (Aged 0 to 2 years)* also recommend infant formula be prepared daily and stored in the refrigeration for a maximum of 24 hours.

Consumers are instructed by their health care professional on correct procedures to prepare and store infant formula, based on the NHMRC and equivalent New Zealand guidelines. NHMRC guidelines also clearly state, 'partially used bottles of infant formula should be discarded after an hour' and 'the safest way to transport formula is to take cooled boiled water and powdered formula in separate containers and mix them when needed. If it is necessary to transport prepared formula, it must be icy cold when leaving home and be carried in an insulated pack to keep it cold. It can be given to the infant cold if there is no way of warming it'. The New Zealand guidelines clearly state that formula should never be carried warm in a thermos flask.

Manufacturers also prepare an assortment of literature for consumers on preparation of infant formula, which generally although not always, reflects the information contained in the Australian NHMRC and New Zealand guidelines.

### **5.4 Consumer preparation and storage of infant formula**

A US survey on the feeding practices of infants found that 33% of mothers mixed formula with warm tap water and 15% of mothers left bottles at room temperature for more than 2 hours. There was also some evidence that holding prepared formula at room temperature was related to diarrhoea in older infants<sup>17</sup>.

An infant feeding survey conducted in Sydney revealed that 14% (38/274) of mothers used boiling water to reconstitute the powder instead of using cooled boiled water while one mother used cold water straight from the tap<sup>18</sup>. The same survey also found that most mothers (94%) reconstituted more than one bottle at a time, keeping the remaining bottles in the refrigerator until required.

<sup>17</sup> Beck-Fein, S, Faici, C.D. (1999) Infant formula preparation, handling, and related practices in the United States. *JADA* 9(10):1234-40

<sup>18</sup> Lilurne A, Oates, R., Thomposn, S., Tong, L (1988) Infant feeding in Sydney: a survey of mothers who bottle feed. *Aust Paediatr J.* 24:49-54

A significant number of mothers (142/275) reported that they sometimes carried a spare bottle in an insulated carrier, and two-thirds of these mothers pre-warmed the bottle before placing it in the insulated carrier.

Current information indicates that some of these feeding practices still occur (Maori Reference Group, personal communication).

## **5.5 Measurement of *B. cereus* - Enumeration methods**

### *5.4.1 Australian Standards Method of Analysis*

In accordance with the Code, the prescribed method for analysis of *B. cereus* in infant formula is AS 1766.2.6 (published in 1991).

The method prescribes two approaches to the analysis of *B. cereus*. One is a surface spread approach where the polymyxin pyruvate egg yolk mannitol bromthymol blue agar (PEMBA medium) is used. The other is a most probably number (MPN) approach where tryptone soy polymyxin broth is used. In both approaches, confirmation of *B. cereus* is made with spore staining.

The Code permits the use of alternative and equivalent methods for the analysis of *B. cereus* in infant formula.

### *5.5.2 USA Method of Analysis*

The US FDA officially recognises the methods of analysis in the Bacteriological Analytical Manual (BAM) for pathogens in infant formula. For *B. cereus*, it refers to Chapter 14 of the BAM.

The method recommends the approach of Spread Plating on Mannitol-egg yolk-polymyxin (MYP) medium for *B. cereus* present at more than 10 cells per gram of a food sample. The method recommends the use of a MPN technique employing Trypticase soy-polymyxin broth for less than 10 (*B. cereus*) per gram of a food sample.

Confirmation of *B. cereus* involves testing the isolates for fermentative activity on glucose; nitrate reduction; production of acetymethyl-carbinol; ability to decompose tyrosine; ability of growth in the presence of lysozyme; and then mobility test, Rhizoid growth; haemolytic activity and production of protein toxin crystals. The FDA recommends that only if the APC (aerobic plate count) exceeds 100 per gram, that an analysis is performed to ensure *B. cereus* in infant formula does not exceed 100 CFU/g or 1000 MPN/g.

In addition, the FDA permits the use of alternative and equivalent methods for the analysis of *B. cereus* in infant formula.

### *5.5.3 Canadian Method of Analysis*

The official Canadian method for the 'Isolation and Enumeration of *B. cereus* in Foods' is MFLP-42, published in April 2003. The method prescribes the use PEMBA medium for spread plating to isolate *B. cereus*.

Confirmation of the isolates is carried out by testing cell mobility, Rhizoid growth, haemolytic activity, (absence of) protein toxin crystals and sporulation.

The method requires 10 replicates per dilution of a solid food sample and prescribes appropriate approaches to deal with the outcome of colony counts per PEMBA plate (20 CFU per plate;  $\geq 20$  but less than 200 CFU per plate; and some with  $<20$ , some with  $\geq 20$  per plate).

Other than the use of PEMBA medium, the specification of 10 replicates per one solid food sample, and the recommended approaches in dealing with the outcome of colony counts per PEMBA plate, the Canadian method is similar to the spread plating approach of FDA method.

#### 5.5.4 *Issues arising regarding method of analysis for B. cereus*

Application A454 suggests that the enumeration limit for *B. cereus* is 10 per gram (+/- 6 per gram) and that it is unwise to set regulatory limits close to the detection limit because of the disputes likely to arise over the testing uncertainties. The method referred to is a spread plate method that involves plating 1 ml of a 1 in 10 dilution over 3 plates. This is not the Standards Australia reference method prescribed in subclause 4(1) of Standard 1.6.1. Prescribed methods of analysis are included in the Code for the sampling plans given so that disputes won't arise over the analytical results because of variations in the testing method used.

Australian Standard 1766.2.6: Examination for specific organisms – *B. cereus*, is the reference method prescribed in the Code. This method sets out two quantitative methods, a surface spread method and a most probable number (MPN) method. The surface spread method is a more precise method but the limit of detection using this method is 100 cfu per gram (0.1 ml spread plate of a 1 in 10 dilution). This method therefore cannot be used for analysis where the microbiological limit is less than 100, as is currently the case for infant formula ('m' = 10 cfu per gram).

The MPN method is more sensitive and can be used to determine low levels of *B. cereus* ( $<10$ ), but is less precise. The MPN procedure provides an estimate of the count present and confidence limits are used to indicate the precision of the MPN estimates. For a 3 tube MPN estimate of 110 cfu per gram (the value closest to 100 provided in the Standards Australia MPN tables) the 95% confidence limits give a lower limit of 15 and an upper limit of 480. This means that while the estimate is 110 cfu per gram, the true number of organisms lies between the lower and upper limits 95% of the time.

The limits of the reference analytical methods specified need to be considered in setting a microbiological limit. Microbiological limits of 100 cfu/g can reliably be measured by the Australian Standard 1766.2.6 surface spread method. The Code also allows for equivalent methods to be used (subclause 4(2) of Standard 1.6.1).

## 6. Regulatory Options

### 6.1 Regulatory options proposed at draft assessment

Four regulatory options were proposed at the Draft Assessment:

1. Status quo - maintain the current standard so that the limit for *B. cereus* in infant formula remains as:

$n = 5, c = 2, m = 10 \text{ cfu per gram}, M = 100 \text{ cfu per gram}$

2. Amend Standard 1.6.1 to the levels in the Transitional Standard 1.1A.1 so that the *B. cereus* limit for infant formula is:

$n = 5, c = 1, m = 100 \text{ cfu per gram}, M = 1000 \text{ cfu per gram}$

3. Amend Standard 1.6.1 to adopt the sampling plan proposed by the applicant so that the *B. cereus* limit for infant formula is:

$n = 5, c = 3, m = 50 \text{ cfu per gram}, M = 100 \text{ cfu per gram}$

4. Amend Standard 1.6.1 with an alternative sampling plan that is consistent with the application so that the *B. cereus* limit for infant formula is:

$n = 5, c = 0, m = 100 \text{ cfu per gram}$

Based on the findings of the risk assessment the preferred regulatory option at draft assessment was option 4.

Following consideration of comments received in written submissions after the release of the Draft Assessment report, Option 4 is still the preferred regulatory option.

## **7. Impact Analysis**

### **7.1 Affected Parties**

The affected parties are:

1. Governments in Australia and New Zealand
2. Consumers in Australia and New Zealand
3. Manufacturers, producers and importers of Infant Formula

### **7.2 Impact Analysis**

#### *7.2.1 Impact of Option 1*

This option would maintain the status quo until 20 June 2004, when the existing Transitional Standard will be rescinded. This option maintains the status quo for consumers and government but will affect industry.

##### 7.2.1.1 Consumers

Risk management measures in this option minimise the extent of *B. cereus* contamination in infant formula, therefore providing a basic level of protection for consumers. However industry has advised it is unable to fully comply with the standard, therefore there is a potential risk to infants if product unable to comply with the standard reached the retail market.

### 7.2.1.2 Industry

Industry has claimed that it is unable to consistently comply with the Standard. This will lead to a number of batches of formula being lost each year, which is costly to industry.

According to the applicant, Option 1 may cause the cessation of manufacture of some or all of the formula produced within New Zealand for the Australia and New Zealand market. At total of about 3300 – 4600 tonnes of formula is manufactured for this market per annum, being 60 – 80% of the market.

In addition Option 1 poses a threat to the export trade in nutritional products, as importing countries frequently require a statement by a competent authority that the products being exported are suitable for sale in the country of origin. The total export trade in these products is approximately 40,000 – 45,000 tonnes per annum.

### 7.2.1.3 Government

Future costs to government will be those of continuing enforcement.

## *7.2.2 Impact of Option 2*

### 7.2.2.1 Consumers

Risk management measures in this option provide an inadequate level of protection for consumers. Modelling in the risk assessment has identified levels of 1000 cfu/g stored at 10°C for 24 hours may pose a risk to infants. Although there is very little epidemiological evidence linking *B. cereus* to illness to infants (with the exception of one outbreak linked to time/temperature abuse in Chile<sup>19</sup>), diarrhoea is a significant cause of ill health and death among infants and children in developed countries. As diarrhoea is usually only a mild illness, people do not always seek medical attention, and therefore the condition is often unreported. In addition if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* does not require notification to health authorities. Therefore it is likely that some infants become ill from *B. cereus* when the bacteria is present in sufficient numbers to provide an infectious dose. Option 2 would therefore provide a net cost to consumers including costs to infants and their carers, including time away from work to look after a sick infant.

### 7.2.2.2 Industry

Industry will benefit from Option 2 in that it will consistently be able to comply with the Standard thereby reducing the number and cost to industry of batches of discarded formula.

### 7.2.2.3 Government

Future costs to Government will be those of continuing enforcement.

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19 Cohen, J., Marambio, E., Lynch, B., and Moreno, A.M (1984) Infección por *Bacillus cereus* en recién nacidos. Revista Chilena de Pediatría. 55(1):20-25

### 7.2.3 *Impact of Option 3*

Option 3 would achieve the objective of this Application.

#### 7.2.3.1 Consumers

Risk management measures in this option minimise the extent of *B. cereus* contamination in infant formula, therefore providing a comprehensive level of public health and safety protection for consumers.

#### 7.2.3.2 Industry

Information supplied by the applicant indicates that industry will consistently be able to comply with the Standard thereby reducing the number of and cost to industry of batches of discarded formula. Therefore industry will benefit from this option.

#### 7.2.3.3 Government

Future costs to Government will be those of continuing enforcement.

### 7.2.4 *Impact of Option 4*

Option 4 would achieve the objective of this Application.

#### 7.2.4.1 Consumers

Risk management measures in this option minimise the extent of *B. cereus* contamination in infant formula, therefore providing a comprehensive level of public health and safety protection for consumers.

#### 7.2.4.2 Industry

Information supplied by the applicant indicates that industry will consistently be able to comply with the Standard thereby reducing the number of and cost to industry of batches of discarded formula. Therefore industry will benefit from this option. The simpler sampling plan proposed in Option 4 will provide a lower compliance cost to industry.

#### 7.2.4.3 Government

Future costs to Government will be those of continuing enforcement.

## **8. Consultation**

Two rounds of public consultation were conducted in accordance with the process for amending the Code prescribed in the FSANZ Act, one at the release of the Initial Assessment Report (March 2003), and one at the release of the Draft Assessment Report (October 2003).

## **8.1 Public Consultation at Initial Assessment**

A total of nine submissions were received from Fonterra, Nutricia, Nestlé, The Australian Food and Grocery Council (AFGC), The Food Technology Association of Victoria (FTA), The Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), the Infant Formula Manufacturers Association of Australia (IFMAA), Heinz, and Wyeth (Attachment 3).

Seven submissions supported the regulatory option proposed at Initial Assessment to amend Standard 1.6.1 with the Transitional Standard 1.1A.1. One submission supported the applicants proposed standard and one submission deferred comment until the Draft Assessment report was available. There was no support for the retention of the existing standard in 1.6.1. (Option 1 in the Draft Assessment Report).

The submissions received focused on the justification for adopting a new standard and included: consideration of international standards; lack of epidemiological evidence of illness caused by *B. cereus* in infant formula; consideration of a preparation safety factor; limitations of current testing methods; and that current limits are not attainable on a regular basis.

Further consultation by teleconference was held with the applicant and industry submitters on 6 and 14 August 2003, to discuss the outcomes of the microbiological risk assessment and possible regulatory options. An issue that arose from these discussions was that concern was expressed that the NHMRC guidelines do not necessarily reflect manufacturer's current recommendations on infant formula preparation and storage practices. The applicant also confirmed that the preferred option was consistent with their application and that the proposed levels are achievable.

The modelling undertaken as part of the risk assessment has identified that some consumer practices in the preparation and storage of infant formula may be unsafe. However it is acknowledged that consumers are educated on safe handling practices by health professionals and guidance is provided at the national level in Australia and New Zealand by the NHMRC and New Zealand Ministry of Health respectively.

## **8.2 Public Consultation at Draft Assessment**

A total of six submissions were received from: the New Zealand Food Safety Authority (NZFSA), FTA, AFGC, Heinz, Nestlé and Queensland Public Health Services.

All of the submissions received at Draft Assessment, supported a change to amend the limit for *B. cereus* in infant formula in Standard 1.6.1. The FTA and Queensland Public Health Services supported the preferred option presented at draft assessment (Option 4 for a regulatory limit of 100 cfu/g). NZFSA supported the Applicant's proposal (Option 3), but stated that Option 4 would be acceptable. Heinz supported Option 2, but also stated that Option 4 would be acceptable and the AFGC and Nestlé supported Option 2 (Retention of the Transitional Standard 1.1A.1).

One submitter raised the following issues:

- Predictive modelling is weak due to the number of assumptions.

Typically predictive modelling is based on various assumptions and on the use of surrogate data where there is an absence of definitive data on a species or commodity. The predictive modelling used in the risk assessment was based on the best available scientific data. In the absence of further data, the assumption that the growth of *B. cereus* would be similar in reconstituted infant formula to that in whole milk is considered valid. Milk is considered to be a good culture medium for *B. cereus* and it is considered that reconstituted infant formula would provide similar conditions for the growth of *B. cereus*. The additional nutrients provided by infant formula additives are not considered likely to stimulate faster or slower growth/generation times.

- No recorded market failure in the past 20 years in Australia and worldwide. Statements relating to diarrhoeal illness and infant formula are misleading in the risk assessment.

Although there is reportedly no market failure in the past 20 years, and there is very little epidemiological evidence linking *B. cereus* to illness in infants, diarrhoea is a significant cause of ill health and death among infants and children in developed countries. It is acknowledged that both food-borne pathogens and viruses may cause diarrhoea in infants. However, as diarrhoea is usually only a mild illness and people do not always seek medical attention, the condition will often be unreported. In addition, if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* does not require notification to health authorities. Therefore it is likely that some infants may become ill from *B. cereus* when the bacteria is present in sufficient numbers to provide an infectious dose.

- Given government and health carer advice, and the detailed labelling statements as required by Standard 2.9.1, the data on time/temperature abuse by carers should not be given too great an emphasis in consideration of this issue as it was generated in 1988.

Although the evidence provided in the risk assessment on consumer feeding practices is from 1988, current information indicates that some of these feeding practices still occur.

- Transitional Standard 1.1A.1 limits would not be inconsistent with international requirements.

The Transitional Standard is more lenient than the proposed Option (Option 4). Option 4 is consistent with international regulations, and provides greater public health and safety for infants. The USA FDA has recently proposed a regulation for *B. cereus* in Infant Formula to not more than 100 *B. cereus* per gram. Within the European Union, the Netherlands and Portugal have set a regulatory limit of 100 cfu/g and Hungary and Poland have an agreed limit of 100 cfu/g. Iran and Denmark have a regulatory limit for *B. cereus* to be absent in 0.01g (approximately equivalent to less than 100/g).

### **8.3 World Trade Organization (WTO)**

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

The proposed amendments to the Code relating to the regulation of *B. cereus* in infant formula were considered minor in nature as they are consistent with other international regulation. Consequently they were unlikely to have a significant effect on international trade. Therefore the proposed changes to the existing standard were not notified to the WTO.

## 9. Conclusion and Recommendation

The issues raised in the section of the Regulatory Problem have been addressed through the risk assessment, analysis of the regulatory options, and stakeholder consultations in this Application.

The existing Standard 1.6.1 (Option 1) is too restrictive and is not technically feasible during certain manufacturing periods due to fluctuations in the level of *B. cereus* in raw milk from pasture and silage fed animals. The risk assessment concurred with this finding.

The Transitional Standard 1.1A.1 (Option 2) is more lenient than both the existing and proposed option (Option 4). Option 2 allows one sample ( $c=1$ ) to exceed 100 cfu/g but not to exceed 1000 cfu/g. However levels greater than 100 cfu/g as permitted in Option 2 may not provide adequate public health and safety for infants based on the risk assessment. Levels of 1000 cfu/g may pose a risk to infants when infant formula is stored for 24 hours under poorly regulated refrigeration temperatures of 10°C or more. In addition, a more conservative limit has been proposed due to:

- infant formula being the sole source of nutrition for infants;
- infants having increased susceptibility to illness compared to the general population; and
- *B. cereus* is capable of rapid growth under conditions of time and temperature abuse.

Option 4 does not allow any sample to exceed 100 cfu/g.

Option 3 provides a similar level of public health and safety as Option 4. The proposed 'm' value for Option 3 is the level that is acceptable and attainable by industry using good manufacturing practices. However the sampling plan proposed is unnecessarily complex and inconsistent with other international standards for *B. cereus* in infant formula.

The proposed option (Option 4) differs from that proposed by the applicant (Option 3) in that it is more lenient. However this option is consistent with the section 10 objectives of the FSANZ Act. Option 4 specifies one limit of acceptance ('m') of 100 cfu/g. The risk assessment concluded that levels of *B. cereus* at 100 cfu/g in the infant formula powder do not pose a risk to infant health when the formula is prepared and stored according to manufacturers instructions and Australian NHMRC and New Zealand Ministry of Health guidelines. A microbiological limit of 100 cfu/g can reliably be measured by Australian Standard 1766.2.6 surface spread method and therefore this option can be effectively implemented by industry.

Option 4 is the recommended option for the following reasons:

- The proposed amendment will protect the health of infants from potential illness resulting from the consumption of infant formula contaminated with *B. cereus*. The proposed amendment to the Code is therefore consistent with the section 10 objectives of the FSANZ Act, in particular, the protection of public health and safety.

- The current microbiological Standard 1.6.1 (n=5, c=2, m=10, M=100) is considered too restrictive and cannot be complied with consistently by industry under good manufacturing and hygienic practice.

The risk assessment modelled the growth of *B. cereus* under various preparation and storage scenarios and concluded the following:

- Powdered infant formula containing up to 100 cfu/g of *B. cereus* and reconstituted using the following practices, would not expose infants to an infectious dose of *B. cereus*:
  - Formula reconstituted with cooled boiled water (25°C) and stored for 24 hours at 4°C.
  - Formula reconstituted with cooled boiled water and stored for 24 hours at 10°C.
- Formula reconstituted from powder with levels of 1000 cfu/g and then stored at 10°C for 24 hours may pose a risk to infants.
- The following practices are considered unsafe as they were shown to result in rapid growth of *B. cereus* to levels that result in illness, in reconstituted formula during storage:
  - Storing formula reconstituted with warm tap water (37°C) or boiling water (80°C).
  - Storing formula at room temperature.
  - Storing warm formula in insulated carriers.

## 10. Implementation and review

Industry is currently able to comply with either Standard 1.6.1 or the Transitional Standard 1.1A.1. The Transitional Standard will remain in effect until 20 June 2004. Therefore, industry will still be able to manufacture to both of these until 20 June 2004 and the general 12 month stock and trade provisions under subclause (2) of Standard 1.1.1 will apply once this Transitional Standard ceases to have effect.

## ATTACHMENTS

1. Draft variation or standard to the *Australia New Zealand Food Standards Code*
2. Microbiological Risk Assessment Report
3. Summary of issues raised in public submissions after Initial Assessment
4. Summary of issues raised in public submissions after Draft Assessment

## ATTACHMENT 1

### Draft Variations to the *Australia New Zealand Food Standards Code*

To commence: on gazettal

[1] *Standard 1.6.1 of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *omitting from the Schedule, under Powdered infant formula products, the entries for Bacillus cereus/g in Columns 3, 4, 5 and 6, substituting -*

|   |   |     |
|---|---|-----|
| 5 | 0 | 100 |
|---|---|-----|

[1.2] *omitting from the Schedule, under Powdered infant formula products with added lactic acid producing cultures, the entries for Bacillus cereus/g in Columns 3, 4, 5 and 6, substituting -*

|   |   |     |
|---|---|-----|
| 5 | 0 | 100 |
|---|---|-----|

### Microbiological Risk Assessment Report

#### *Bacillus cereus* in Infant formula

##### EXECUTIVE SUMMARY

This risk assessment characterises the level of risk to infants in Australia and New Zealand resulting from the consumption of infant formula contaminated with *Bacillus cereus* (*B. cereus*).

The key findings of the risk assessment are:

- Low numbers of *B. cereus* are present in infant formula due to contamination of raw milk from the environment.
- Illness resulting from contamination of food with *B. cereus* is generally mild. *B. cereus* can produce diarrhoea and vomiting following consumption of food containing  $10^5$  viable cells or spores or greater. However, the infectious dose of *B. cereus* for infants may be lower because their immune systems are not fully developed and infants are more susceptible to bacterial infections than healthy adults and older children.
- Although there is very little epidemiological evidence linking *B. cereus* to illness to infants, diarrhoea is a significant cause of ill health and death among infants and children in developed countries. As diarrhoea is usually only a mild illness, people do not always seek medical attention, and therefore the condition is often unreported. In addition if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* does not require notification to health authorities. Therefore it is likely that some infants become ill from *B. cereus* when the bacteria is present in sufficient numbers to provide an infectious dose.
- Infant formula is designed as a human milk substitute for infants and as such may represent the sole source of nutrition for some infants, i.e. the frequency of consumption is very high, especially for infants under the age of 6 months.
- Surveys have shown that some consumers prepare infant formula incorrectly with warm tap water, leave the bottle at room temperature for more than 2 hours, and store prepared warm formula in insulated carriers when going out.

The risk assessment modelled the growth of *B. cereus* under various preparation and storage scenarios and concluded that:

- Powdered infant formula containing up to 100 cfu/g of *B. cereus* and reconstituted using the following practices, would not expose infants to an infectious dose of *B. cereus*:
  - Formula reconstituted with cooled boiled water (25°C) and stored for 24 hours at 4°C.

- Formula reconstituted with cooled boiled water and stored for 24 hours at 10°C.
- Formula reconstituted from powder with levels of 1000 cfu/g and then stored at 10°C for 24 hours may pose a risk to infants.
- The following practices are considered unsafe as they were shown to result in rapid growth of *B. cereus* to levels that result in illness, in reconstituted formula during storage:
  - Storing formula reconstituted with warm tap water (37°C) or boiling water (80°C).
  - Storing formula at room temperature.
  - Storing warm formula in insulated carriers.

## INTRODUCTION

This risk assessment is to identify and characterise the level of risk to infants in Australia and New Zealand resulting from the consumption of infant formula contaminated with *B. cereus*.

*B. cereus* is found in a wide variety of foods at low levels. It is responsible for two relatively mild types of food-borne illness – one an emetic illness and the other characterised by diarrhoea. Vegetative cells of *B. cereus* are heat sensitive, but its spores are very heat resistant, hence illness usually occurs in contaminated food that has been held too long at unsatisfactory temperatures.

There are four generic components to the risk assessment process:

1. Hazard identification: the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.
2. Hazard characterisation: the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of a microbiological risk assessment, the concerns relate to microorganisms and/or their toxins.
3. Exposure assessment: the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.
4. Risk characterisation: the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment.

For this risk assessment, hazard identification describes *B. cereus* and examines its association with food poisoning. The hazard characterisation estimates the relationship between the infectious dose and the likelihood of infants developing illness. The exposure assessment estimates the likely level of *B. cereus* resulting from exposure through infant formula and examines the effect of processing conditions and consumer conditions of storage and handling after reconstitution. The risk characterisation estimates qualitatively the overall risk of developing illness by infants from consumption of infant formula contaminated with *B. cereus*.

A microbiological risk assessment can be qualitative, quantitative or a combination of both (semi-quantitative). A qualitative risk assessment is used when insufficient information is available to estimate the risk numerically; a quantitative risk assessment provides numerical expressions of risk and an indication of the attendant uncertainties. This microbiological risk assessment is qualitative, with some quantitative elements, drawing on local (Australian and New Zealand) data, where available and based on the currently available evidence.

## 1. Hazard Identification

### 1.1 *Bacillus cereus*

The genus *Bacillus* encompasses a great diversity of species and strains. *B. cereus* is a Gram-positive, facultatively aerobic spore-former whose cells are large rods and are motile by means of peritrichous flagella. *B. cereus* is widely distributed in nature, being readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments.

### 1.2 Growth characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics. Psychrotrophic strains are able to grow at 4 – 5°C but not at 30 – 35°C, whereas mesophilic strains grow between 15°C and 50 or 55°C. The optimum growth temperature ranges from 30 – 40°C. The minimum pH for growth is 5.0, maximum 8.8 and optimum 6.0-7.0 (ICMSF, 1996). The minimum range for water activity *B. cereus* survives and grows under is 0.93 (ICMSF, 1996). The maximum salt concentration that is tolerable by *B. cereus* is 7% at pH 6-7 and 30°-35°C (AIFST, 2003).

Growth is best in the presence of oxygen but growth can occur anaerobically. Toxin production is lower under anaerobic conditions (ESR, 2001).

The vegetative cells are not very resistant to environmental stress such as heat, chemicals, preservatives and radiation. However *B. cereus* spores are more resistant due to their metabolic dormancy and tough physical nature (AIFST, 2003). Spores are more resistant to dry heat than moist heat. Spores can survive for long periods in dried foods.

Heat resistances for *B. cereus* have been reported at  $D_{85^{\circ}\text{C}} = 33.8\text{-}106$  min in phosphate buffer (ICMSF, 1996). At 95°C, D-values ranged from 1.5-36.2 min in distilled water and 1.8-19.1 min in milk (ICMSF, 1996). While there is considerable strain variability, spores can be resistant to temperatures 15 to 20 times greater than the temperature required to destroy the most sensitive strains of other mesophilic spore-forming bacteria (ICMSF, 1996).

Preservatives such as 0.26 % sorbic acid at pH 5.5, and 0.39% potassium sorbate at pH 6.6 can inhibit growth. Nisin is inhibitory to *B. cereus*. Other antimicrobials which have an effect on *B. cereus* include benzoate, ethylenediaminetetraacetic acid (EDTA) and polyphosphates (AIFST, 2003).

Spores are more resistant to radiation than vegetative cells (Farkas, 1994).

*B. cereus* produces one emetic toxin and four different enterotoxins. The emetic toxin preformed in foods has been elucidated and the protein responsible for the clinical symptoms (vomiting) is cereulide (Hui *et al.*, 2001). The emetic toxin is produced by cells growing in the food. The emetic toxin is resistant to heat, pH and proteolysis but is not antigenic. The toxin is a ring form peptide of 1.2 kDa and is postulated to be an enzymatically synthesised peptide (Granum and Lund, 1997). Emetic toxins are extremely resistant to heat and can survive 90 minutes at 126°C (ESR, 2001). Diarrhoeal toxins are inactivated at 56°C in 5 minutes.

The four enterotoxins have been identified and characterised; two three-component enterotoxins (haemolysin BL and non-haemolytic), enterotoxin T and a cytotoxin. These enterotoxins are heat sensitive and are inactivated at 56°C for 5 min, (but not 45°C for 30 min). It is unstable at pH values outside the range 4 to 11 and sensitive to proteolytic enzymes (AIFST, 2003). Toxin activity is reduced after 1 to 2 days at 32°C, one week at 4°C and several weeks at -20°C (Andersson, 1995).

In summary, emetic toxins are more likely to survive the gut environment and cause illness, whereas enterotoxins are formed in the small intestine after consumption of a large number of cells which then results in illness.

### **1.3 Pathology of illness**

There are two types of *B. cereus*-mediated intoxication. The two forms of illness are caused by significantly different toxins; the diarrhoeal toxin (enterotoxin) and the emetic toxin. Neither form of illness is threatening to normal healthy patients and very few fatal cases have been reported (AIFST, 2003).

The diarrhoeal type of food poisoning is caused by complex enterotoxins, produced during vegetative growth of *B. cereus* in the small intestine. Incubation is usually 10-13 hours post ingestion although incubation periods from 8 –16 hours have been reported. Gastroenteritis is usually mild with abdominal cramps, profuse watery diarrhoea, rectal spasms and moderate nausea, usually without vomiting and recovery typically occurs within 24 hours.

The second type of infection, caused by the emetic toxin, results in illness in a shorter period of time following ingestion of the toxin-contaminated food. Acute nausea and vomiting occurs 1-5 hours post ingestion and again is not serious with recovery within 12 - 24 hours. Diarrhoeal symptoms are not normally associated with the emetic cause illness.

The characteristics of the two types of illness caused by *B. cereus* are summarised in Table 1.

In a small number of illnesses both types of symptoms (diarrhoeal and vomiting) have been recorded, and this is probably due to the production of both types of toxin.

Humans may vary in their susceptibility to *B. cereus* illness. Since most strains of *B. cereus* have the potential to produce toxins, the severity of illness is dependent on the quantity of toxins produced (Notermans and Batt, 1998).

*B. cereus* has also been associated with non-food-borne non-gastrointestinal infections such as ocular and wound infections; bacteraemia and septicaemia; central nervous system infections; respiratory tract infections; and endocarditis. Individuals who are immunocompromised either by illness or medication are more susceptible to illness induced by this organism (Hui *et al.*, 2001). However, the scope of this risk assessment is the examination of illness arising from *B. cereus* contaminated food.

**Table 1:** Characteristics of the two types of illness caused by *B. cereus* (Granum and Lund, 1997)

|   | <b>Diarrhoeal syndrome</b>   | <b>Emetic syndrome</b>  |
|---|--|---|
| <b>Infective dose</b>                   | 10 <sup>5</sup> -10 <sup>7</sup> (total)                                 | 10 <sup>5</sup> -10 <sup>8</sup> (cells/g)  |
| <b>Toxin produced</b>                   | In the small intestine of the host                                       | Preformed in foods  |
| <b>Type of toxin</b>                    | Protein  | Cyclic peptide  |
| <b>Incubation period</b>                | 8-16 h (occasionally >24 h)  | 0.5-5 h   |
| <b>Duration of illness</b>              | 12-24 h (occasionally several days)                                      | 6-24 h  |
| <b>Symptoms</b>                         | Abdominal pain, watery diarrhoea and occasionally nausea                 | Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin production?) |
| <b>Foods most frequently implicated</b> | Meat products, soups, vegetables, puddings/sauces and milk/milk products | Fried and cooked rice, pasta, pastry and noodles  |

#### 1.4 Mode of transmission

The enterotoxin (diarrhoeal syndrome) of *B. cereus* poisonings is caused by the ingestion of a large number of cells and the subsequent production of the toxin in the small intestine.

The emetic syndrome of *B. cereus* food poisoning occurs after the ingestion of food in which the organism has grown and formed its toxin(s). Most documented reports of *B. cereus* intoxication from this toxin have involved a cereal, or cereal- or spice-containing, product as the food vehicle (ICMSF, 1996).

#### 1.5 Incidence of illness

*B. cereus* food poisoning is not a reportable illness in any country, and therefore only limited incidence data is available (Granum and Lund, 1997). However, it is estimated that France, Germany and the USA report less than 0.1 cases per 10,000,000 population per annum whereas Finland, Scotland, England/Wales, Hungary and Cuba all report more than 4.0 cases per 10,000,000 per annum (AIFST, 2003).

Within Australia, during the years 1977-1984, *B. cereus* was associated with 39% of food-borne illness incidents investigated in New South Wales, and this was mostly associated with fried rice (AIFST, 2003).

However, diarrhoea remains one of the most important causes of ill-health and death among infants and children. In developed countries the incidence is much lower, but still significant (Australian Bureau of Statistics, 2002). As diarrhoea is usually only a mild illness, people do not always seek medical attention, and the condition is often unreported.

Outbreaks of emetic-type illness have resulted from consumption of rice products or starchy foods (such as potato or pasta) that have been cooled slowly and stored incorrectly. Fried or cooked rice has been implicated in approximately 95% of cases with emetic symptoms and only a small proportion of cases have been attributed to the consumption of other foods such as crumpets, vanilla slices, cream and pasta (Kramer & Gilbert 1989; Lee, 1988). Outbreaks of diarrhoeal-type illness result from eating foods in which *B. cereus* organisms have grown to large numbers and toxin is subsequently formed in the small intestine.

A wide range of foods have been associated with the diarrhoeal syndrome including meat-based dishes, soups, vegetables, puddings and sauces (Kramer & Gilbert 1989).

Powdered milk used in the preparation of vanilla slices, a milk-gelatine dessert and macaroni cheese was indicated as the source of the *B. cereus* contamination contributing to outbreaks involving these foods (Holmes, 1981; Pinegar & Buxton, 1977; Anon, 1977).

A food-borne outbreak involving 35 neonates was linked to *B. cereus* in powdered milk in Chile (Cohen *et al.*, 1984). Levels of *B. cereus* detected in the powder ranged between 50-200 spores/g, however analysis of preparation methods revealed a certain degree of time and temperature abuse. No further cases were detected following changes to preparation systems of infant formula.

A *B. cereus* outbreak involving Rottweiler puppies in Germany was linked to contaminated milk powder used in formula to feed puppies. This resulted in a number of the puppies being ill with vomiting and diarrhoea and the death of 5 animals. The *B. cereus* level found in the milk powder was  $2.7 - 3.5 \times 10^4$  cfu/g in the milk powder (Gareis & Walz, 1994).

## **1.6 Occurrence in foods**

Foods are commonly contaminated with *B. cereus* owing to the organisms widespread distribution in the environment and therefore raw foods of plant origin are a major source of *B. cereus*. Cereal products are a source, but numbers are rarely high (AIFST, 2003). Rice is a well recognised source with most samples containing low levels of the organism (AIFST, 2003). Spices are also frequently contaminated with *B. cereus* (AIFST, 2003).

A survey by Nygren (1962) of the incidence of *B. cereus* in food materials revealed that 52% of 1546 food ingredients, 44% of 1911 cream and dessert dishes and 52% of 431 meat and vegetable products were contaminated, illustrating its widespread distribution. A study of milk and dairy products showed contamination rates of 9-48% and UHT-treated milk was contaminated in approximately 50% of samples (ICMSF, 1996).

The available data indicates that under normal circumstances, *B. cereus* is found in food at concentrations  $<10^3$ /g and mostly  $<10^2$ /g (ICMFS, 1996).

The presence of *B. cereus* in processed foods results from contamination of raw materials and the subsequent spore resistance to heat treatment processes during manufacture.

## 2. Hazard Characterisation

### 2.1 Virulence and infectivity of *B. cereus*

The pathogenic mechanism for the emetic toxin has been elucidated. The emetic toxin is a dodecadepsinpeptide, named cereulide, which causes vacuole formation in Hep-2 cells and emesis.

The pathogenic mechanism for the diarrhoeal form of illness has not been clearly elucidated although it is known that at least four different enterotoxins are involved (AIFST, 2003).

One of these enterotoxins, Haemolysin BL (HBL), consists of three protein components, and causes the destruction of red blood cells. HBL consists three protein components: L<sub>2</sub>, L<sub>1</sub>, and B. The second enterotoxin, non-haemolytic enterotoxin (NHE), consists of 3 protein moieties: B, L<sub>1</sub> and L<sub>2</sub>, and all components are needed for maximum cytotoxicity. Both HBL and NHE have been responsible for outbreaks of diarrhoeal food poisoning. The third enterotoxin, Enterotoxin T, consists of a single protein that is cytotoxic positive in the mouse ileal loop assay and possesses vascular permeability activity but does not appear to be involved in food poisoning (Hui *et al.*, 2001). The role of enterotoxin T is unclear (AIFST, 2003).

Lund *et al.*, (2000) recently identified the fourth enterotoxin which is single cytotoxin protein (CytK). CytK is necrotic and haemolytic. This toxin was implicated in a severe food poisoning outbreak in France resulting in three deaths (Lund *et al.*, 2000).

Since diarrhoeal enterotoxins are unstable and are inactivated by low pH and digestive enzymes, these toxins should be destroyed during passage through the stomach and not likely to cause illness (Notermans and Batt, 1998; Granum and Lund, 1997).

Other potential virulence factors associated with diarrhoeal illness that have been identified include sphingomyelinase, phosphatidylinositol- and phosphatidylcholine-specific phospholipases and haemolysins I and II (AIFST, 2003).

The involvement of the intestinal receptor site(s) of the tripartite enterotoxins in diarrhoeal symptoms has not been fully elucidated. It has been postulated that the enterotoxin disrupts the membrane of epithelial cells (Notermans and Batt, 1998). The mechanisms for cereulide synthesis is also unclear but, data suggest the peptide is enzymatically produced (Hui *et al.*, 2001).

### 2.2 Infectious dose

Kramer and Gilbert (1989) have summarised a large number of outbreaks caused by *B. cereus*. The concentration of *B. cereus* in foods implicated in diarrhoeal illness ranged from  $1.2 \times 10^3 - 10^8$  org/g.

It has also been reported that 10% of outbreaks have been associated with food containing less than  $10^5$  cfu/g (Kramer and Gilbert, 1989).

A study indicated that concentrations of *B. cereus* of  $10^3$  to  $10^5$ /g can result in illness in infants or aged and infirm individuals, although these were rare (Becker *et al.*, 1994).

Granum and Lund (1997) reported that concentrations ranging from 200 to  $10^9$  /g (or / ml) *B. cereus* have been reported in food implicated in food poisoning, giving total infective doses ranging from about  $5 \times 10^4$  to  $10^{11}$ . Partly due to the large differences in the amount of enterotoxin produced by different strains, the total infective dose seems to vary between about  $10^5$  and  $10^8$  viable cells or spores. Thus, Granum and Lund (1997) suggest an average serving of food containing more than  $10^3$  *B. cereus*/g cannot be considered completely safe for consumption.

Rowan *et al.*, (1997b) and Notermans and Batt (1998) also suggest that the infectious dose for *B. cereus* may vary from about  $1 \times 10^5$  to  $1 \times 10^8$  viable cells or spores. Notermans and Batt (1998) also suggest food servings containing greater than  $1 \times 10^4$  *B. cereus*/g may not be safe for consumption.

From the available data we conclude that the minimum total infectious dose is  $10^5$  viable cells or spores.

### **2.3 Immune status**

All people are believed to be susceptible to *B. cereus* food poisoning. *B. cereus* has the potential to cause mild food-poisoning which does not, as a rule last more than 12-24 hours. However some individuals, especially young children are particularly susceptible and may be more severely affected (ICMSF, 1986). Infants therefore may be susceptible to illness from a lower infectious dose but there is no available data to support this.

More severe symptoms have been associated with young athletes (< 19 years old) and the elderly (>60 years). Young athletes had more severe symptoms compared to coaches and officials who were not affected in an outbreak of *B. cereus* in Norway. In addition the time to onset for some patients was greater than 24 hours and duration of the illness was 2 to several days (Doyle *et al.*, 1997).

### **2.4 Food matrix**

The impact of the food matrix on the heat resistance of spores has been investigated. *B. cereus* spores are moderately heat resistant, however resistance is increased in high-fat and oily foods (e.g. for soybean oil, the  $D_{121}^{\circ C} = 30$  min) and foods generally with lower water activity (AIFST, 2003).

### 3 Exposure Assessment

#### 3.1 Sources and routes of contamination of infant formula with *B. cereus*

##### 3.1.1 Farm environment

*B. cereus* organisms from the farm environment can easily find their way into raw milk. Spores of *B. cereus* contaminate milk via faecal contamination of the udder. High numbers of Bacillus spores in raw milk have been associated with high spore numbers in fresh cow faeces (Waes 1987; te Giffel *et al.*, 1995). *B. cereus* is also spread from soil and grass to the udders of cows and into raw milk (Doyle *et al.*, 1997).

Another source of contamination is poorly made or stored silage. Silage is the term used for forage with a high moisture content preserved by fermentation and is used in seasons when fresh forage is unavailable. Silage is preserved through rapid pH reduction and maintenance of anaerobic conditions. Proliferation of Bacillus spp. (*B. cereus*, *B. lentus*, *B. firmus*, *B. sphaericus*, *B. licheniformis*, and *B. polymyxa*) usually occurs during the later stages of aerobic spoilage of silage. A study carried out by Crielly *et al.*, (1994) reported *B. cereus* levels up to  $10^7$  cfu/g in or on bedding and up to  $10^5$  cfu/g in silage and concluded that *B. cereus* contamination of cattle feed was a seasonal occurrence. Sutherland and Murdoch (1994) also suggest contamination of cows udders was due to summer pasture and soiled winter bedding in the UK.

Christiansson *et al.*, (1999) however found that the most important source of *B. cereus* contamination in raw milk was carriage of soil via dirty teats. The levels of *B. cereus* in different feeds (silage, hay and concentrates), cow dung and milking equipment was found to be very low compared to the levels found in soil. The spore content of the soil varied from <50 to 380,000/g.

Previous studies reported hay, dust, soil, feed, faeces, and milking equipment to be important sources of contamination in raw milk (Christiansson *et al.*, 1999)

Christiansson *et al.*, (1999) reported that the contribution of *B. cereus* from the exterior of the udder decreased following cleaning and disinfecting the animal surface before milking.

##### 3.1.2 Raw Milk

Raw milk is frequently contaminated with Bacillus spores. Milk is often contaminated at the farm from the udder, milking equipment or the environment and also at the dairy factory. However, as discussed above, sanitation of teats prior to milking is successful in reducing *B. cereus* in raw milk (AIFST, 2003).

Although *B. cereus* spores in raw milk from soil, dung, straw, hay and other fodder are usually fast germinating and spores from milking equipment are mainly slow germinating, milking equipment plays an important role in the contamination of milk with *B. cereus* (Becker *et al.*, 1994). The combination of improperly cleaned equipment and high temperatures provides ideal conditions for contamination of raw milk with *B. cereus* and subsequent growth to potentially high levels.

A study of *B. cereus* levels in 144 raw milk samples from individual cows showed levels of <10 spores/L up to 880 spores/L (mean of 41 spores/L) (Christiansson *et al.*, 1999). Further studies on raw milk samples showed levels of less than 100 cfu/mL in 2 samples out of 298 milk samples (Odumeru *et al.*, 1997).

A survey of 48 milk samples from Victorian manufacturers showed that 9/24 samples of raw milk, 6/12 samples of pasteurised milk and 4/10 samples of milk powder were positive for *B. cereus*. The levels of *B. cereus* contamination in raw milk, pasteurised milk, and milk powder were <10 cfu/ml, <10 to 28 cfu/ml and 30 to 960 cfu/ml respectively (Rangasamy *et al.*, 1993).

The levels of *B. cereus* in raw milk are seasonally dependent with highest levels occurring in spring and summer.

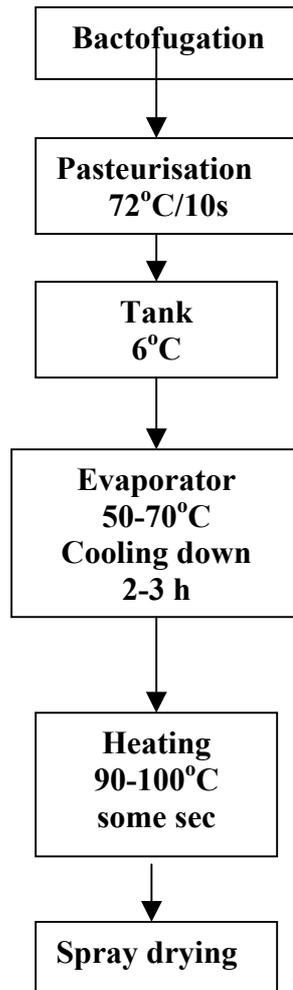
### 3.1.3 Manufacturing process for Infant Formula

There are two general types of processes for manufacturing infant formula, a dry blending process and a wet mixing-spray drying process.

In the dry blending process, the ingredients are received from suppliers in a dehydrated powdered form and are mixed together to achieve a uniform blend of the macro and micronutrients necessary for a complete infant formula product. This process does not involve the use of water in the manufacturing process, and therefore the processing line can be kept dry for long periods of time. In a dry environment, *B. cereus* are denied the water needed to support growth, reducing the possibility of *B. cereus* becoming established in the plant environment in sufficient numbers to cause further product contamination. However, the microbiological quality of a dry-blended product is largely determined by the microbiological quality of the constituent dry ingredients as there is no heat treatment to destroy bacteria in the final product. Thus, if one or more ingredients in a dry-blended product are contaminated with low numbers of *B. cereus* these bacteria are likely to be present in the finished product.

The wet-mixing-spray drying process involves blending of ingredients, homogenisation, pasteurisation and spray drying to produce a powdered product (Figure 1).

The ingredients are blended with water in large batches and then pumped into a heat exchanger for pasteurisation. The severity of the pasteurisation process varies among manufacturers, but is always sufficient to destroy the vegetative cells of harmful bacteria. Liquid is then concentrated by passing through an evaporator or pumped directly to the spray dryer. In some cases, the concentrated liquid may be cooled and stored in a large tank until needed. Prior to spray drying, the product is pre-heated and passed through a high-pressure pump to the spray dryer nozzles. After spray drying the product may be agglomerated to increase the particle size and improve its solubility. The finished powder is passed through a sifter, and then transferred to bags, totes or silos for storage. In some cases, the powder may be transferred directly to the powder packaging line. At the packaging line, the powder is transferred to a filler hopper that feeds powder into the can filling line. Filled cans are flushed with inert gas, seamed, labelled, coded and packed into cartons.



**Fig. 1 Example of the spray drying process (Becker *et al.*, 1994)**

The following steps are used routinely in the wet-mixing process, however the raw materials in the dry-blending process also undergo these steps to varying degrees.

#### 3.1.4 Initial heat treatment

The initial heat treatment step applied in the production of infant formula is very important for the activation and germination of *B. cereus* spores. Whereas raw milk does not support germination of spores, a high temperature short time treatment (HTST) is conducive for spore germination. Germination of spores is robust and frequencies of up to 100% have been reported (Notermans & Batt, 1998). The killing rate of *B. cereus* by the initial heat treatment depends on the temperature applied and heating milk for 10-20 seconds at 125°C is considered necessary to completely inactivate *B. cereus* spores (Stahouders *et al.*, 1982). If spores persist, they will be free from competition from vegetative cells of other organisms which are inactivated by pasteurisation (Doyle *et al.*, 1997).

*B. cereus* apparently can also develop and produce spores on the surfaces of the equipment of dried milk producing plants resulting in cross-contamination of product (Becker *et al.*, 1994).

### 3.1.5 *Bactofugation*

Bactofugation is used by some producers and removes bacteria, especially spores, from milk by high speed centrifugation. This process can remove up to 95% of the spores. After a HTST treatment at 72°C for 10s, the milk is stored at 6°C and then passed to the evaporator.

### 3.1.6 *Evaporation*

Evaporation is the removal of water using a heat exchange process. During evaporation the temperature increases up to 50-70°C, and, in the case of a discontinuously operating evaporator, the time required to cool the concentrate can be 2-3 h. Normally, this period of time is shorter, but in some cases (if there are any problems with the spray tower) the time can be longer and growth of *B. cereus* cannot be excluded.

### 3.1.7 *Spray drying process for milk and infant formula*

Following evaporation, the concentrate passes to the dryer. Before drying it may be heated again at 90-100°C for several seconds followed by a few minutes at 40-45°C then concentrate is fed to an atomiser. The inlet air temperatures of spray towers are normally between 150°C and 220°C (Becker *et al.*, 1994). Due to the cooking of the rapidly evaporating water, the inner temperature of the sprayed particles only reach 40-50°C (Kessler, 1988) or up to 70°C (Eschamann, 1970).

The spray drying process requires processing equipment to be regularly wet cleaned, therefore providing a moist environment for bacterial growth in the plant environment. If not controlled, these bacteria can be a source of product contamination. *B. cereus* spores are able to adhere to several types of surfaces and hence it is difficult to remove them from equipment during cleaning. These spores also possess appendages and/or pili that are, at least in part, involved with adhesion (Doyle *et al.*, 1997).

After spray drying the product may be agglomerated to increase the particle size and improve its solubility. The product is then re-dried to 5% moisture or less. Accidental microbiological contamination can occur during the agglomeration process.

### 3.1.8 *Cooling, packaging*

Subsequent processing steps such as cooling, intermediate storage, mixing and packaging may also influence the microbiological quality by recontamination from the production line or the environment. During storage of infant formula, surviving organisms slowly die, but spore-formers, being the most resistant, retain viability for long periods of time (ICMSF, 1998).

### 3.2.9 *Overall impact of processing*

Heat treatments applied during processing inactivate vegetative cells of *B. cereus*. However, spores can survive these heat treatments and heat treatment in itself will cause spore germination. The process does provide opportunity for contamination, especially if *B. cereus* colonises on processing equipment. The final product, has low water activity and will not support the growth of *B. cereus*.

## 3.2 Prevalence of *B. cereus* in Infant Formula

### 3.2.1 Prevalence of *B. cereus* in Infant formula – powder

Table 2 shows the prevalence of *B. cereus* in milk based infant foods compiled from literature by Becker *et al.*, (1994). Levels of *B. cereus* range from 0-3000 *B. cereus*/g.

**Table 2:** Incidence of *B. cereus* in milk based infant Food (extracted from Becker *et al.*, 1994)

| Country | Type of Product               | Samples examined | Samples positive (%) | <i>B. cereus</i> /g | Authors                        |
|---------|-------------------------------|------------------|----------------------|---------------------|--------------------------------|
| Rumania | Milk products <sup>a</sup>    | ?                | (25.8)               | ?                   | Ionescu and Ionescu, 1971      |
| FRG     | Infant Food                   | 60               | 8 (13.3)             | 100-400             | Könning, 1972                  |
| Korea   | Dried milk <sup>a</sup>       | 3 Brands         | ?                    | 1.5-100             | Kwun <i>et al.</i> , 1979      |
| India   | Infant food                   | 10               | 9 (90.0)             | 200-2000            | Singh <i>et al.</i> , 1980     |
| Poland  | Infant food                   | 25               | 15 (60.0)            | 10-1000             | Stec and Burzynska, 1980       |
| USSR    | Infant food                   | ?                | ?                    | 5-650               | Kirilenko <i>et al.</i> , 1983 |
| FRG     | Infant food                   | 90               | 39 (43.3)            | <1500               | Döll, 1983                     |
| Egypt   | Infant food                   | 10               | 10 (100)             | 10-3000             | Helmy <i>et al.</i> , 1984     |
| FRG     | Infant food                   | 140              | 54 (38.6)            | 3-460               | Becker <i>et al.</i> , 1984    |
|         | Whey powder <sup>a</sup>      | 10               | 6 (60.0)             | 3-100               |                                |
|         | Skim milk powder <sup>a</sup> | 6                | 4 (66.7)             | 3-100               |                                |
|         | Lactose <sup>a</sup>          | 50               | 0                    | 0                   |                                |
| Egypt   | Infant food                   | 30               | 24(80.0)             | <500                | Moustafa <i>et al.</i> , 1984  |

<sup>a</sup>

intended for infant feeding

Studies by Becker *et al.*, (1994) examined infant formula distributed in 17 countries and found 54% of 261 samples were contaminated with *B. cereus* with levels ranging from 0.3 to 600/g. The majority of samples (44%) had counts from 0.3 to 10 *B. cereus*/g, 10% had counts higher than 10/g and four samples (1.5%) had counts >100/g. Comparison of different formula types revealed 50% of milk-based formula and follow-on formula (formula for babies over 6 months of age) were contaminated with *B. cereus*, 69% of soy-based formula were contaminated and 92% of special dietetic foods were contaminated (Table 3).

**Table 3:** Incidence of *B. cereus* in infant formula (Extracted from Becker *et al.*, 1994)

| Product                       | Positive/total samples examined | MPN/g  |         |      |
|-------------------------------|---------------------------------|--------|---------|------|
|                               |                                 | 0.3-10 | >10-100 | >100 |
| Infant formula (milk protein) | 48/92 (52%)                     | 37     | 10      | 1    |
| Infant formula (soy protein)  | 11/16 (69%)                     | 11     | -       | -    |
| Follow-on formula             | 45/86 (52%)                     | 35     | 7       | 3    |

A 1995 New Zealand infant formula survey 'Chemical and Microbiological Composition of Infant Formulae – A report for the Ministry of Health/Public Health conducted by ESR commission' surveyed 20 commercially available infant formulae products, including standard formula, soy based formula, specially modified formula, low birth weight formula and follow-on formula.

This study reported eight products with low numbers of *B. cereus* (7 had counts of 20 cfu/g and 1 had a count of 40 cfu/g. All others had counts of < or equal to 10 cfu/g.

Data supplied by Anchor Products for the period 2000- 2001, showed 82.2% of formula had levels < 10 cfu/g, 15.8% of products had levels ranging from 10 – 90 cfu/g, 0.8% of product had levels ranging from 100-140 cfu/g and 1.2% had levels ranging from 150-1000 cfu/g

### 3.2.2 *Prevalence of B. cereus in reconstituted infant formula*

A study in the United Kingdom showed 17 out of 100 samples of reconstituted infant formula contained *B. cereus* (mean  $1.3 \times 10^2$  cfu/g, maximum  $4.8 \times 10^2$  cfu/g) (Rowan *et al*, 1997a). A study of 24 pasteurised infant feed formulas tested within 1 hr of preparation recorded 2 positive samples. One of these positives had levels of *B. cereus* of  $1.4 \times 10^3$  cfu/g (Rowan *et al*, 1997b).

*B. cereus* has been detected in infant milk formulae (51%) at levels of  $<10^2$  cfu/mL after reconstitution with 56°C water (Driessen, 1993)

Crielly *et al.*(1994) found 53% of 45 milk-based powders reconstituted in special feeds positive for *B. cereus*.

Thirty-eight diarrhoeal-type *B. cereus* isolates were recovered from 100 samples of reconstituted milk-based infant formula available in many European Economic Community countries (Rowan and Anderson, 1998).

## 3.3 **Growth of *B. cereus* in Reconstituted Infant Formula**

### 3.3.1 *Generation times of B. cereus*

Some strains of *B. cereus* have generation times in milk of 31 minutes at 30°C (AIFST, 2003). At 42°C some stains have a generation time of 11 minutes (AIFST, 2003). Growth and enterotoxin production have been observed in milk at 6°C; milk stored 11-12 d and 24 d at 7 and 4°C respectively and in milk within 72 h at 10°C or 24 h at 15°C. Some strains have been shown to produce detectable toxin in aerated milk within 66.5 h at 8°C. The conclusions from these studies are that most *B. cereus* strains isolated from dairy products are able to grow and produce toxins below 10°C (ESR, 1995).

### 3.3.2 *Growth in reconstituted infant formula*

Several studies have examined the growth of *B. cereus* in infant formula.

Rowen *et al.*, (1997a) studied reconstituted infant formula powder using water at 56°C and 90°C. The formula was then cooled to room temperature either under running cold water, or immediately refrigerating for 30 minutes. All samples were then stored at various temperatures for 25 hours. This study demonstrated that the temperature of water used for formula preparation and or subsequent cooling conditions did not affect the level of *B. cereus* in the formula.

Infant formula powder with counts of 100 *B. cereus*/g reconstituted using the manufacturers instructions and incubated for 7-9 hours at 24°C resulted in reconstituted formula with levels >10<sup>5</sup> cfu/g (Becker *et al.*, 1994).

Rowan *et al.*, (1997a) found that incubation at <10°C for 24 hours had no effect on microbial numbers in reconstituted infant formula. The factors influencing the bacteriological quality of infant formula were the number of organisms initially present and the temperature and duration of incubation. It was reported that formula containing approximately 10<sup>2</sup> *B. cereus* spores per g may become unfit for consumption when subjected to storage at or above 25°C for 14 h, reaching levels of 1.3 x 10<sup>3</sup> cfu/g.

Another study by Rowen *et al.*, (1997b) looked at the bacteriological quality of hospital-prepared infant feeds. Sixty per cent of reconstituted feeds incubated at 25°C and 90% of feeds incubated at 30°C had *B. cereus* counts exceeding 1.0 x 10<sup>3</sup> cfu/g after 14 hours. Formula containing levels at 100 *B. cereus* cells/g after the initial preparation and stored at 25°C for 8 hours showed levels of 1.4 x 10<sup>3</sup> cfu/g which was considered unacceptable for consumption.

Crielly *et al.*, (1994) examined *Bacillus* populations in milk and milk products. Milk based powders reconstituted at 5°C and 15°C for 24 hour showed no further growth of *B. cereus*. However the number of *B. cereus* increased in the range 10<sup>0</sup> – 10<sup>6</sup> cfu/ml between 8 and 24 hours at 20°C. Samples incubated at 26°C also showed a similar result.

In summary, as reported in the literature, when infant formula was reconstituted with initial levels of *B. cereus* at 100 cfu/g and stored at temperatures of 24°C, levels of *B. cereus* ranged from 10<sup>3</sup> cfu/g to >10<sup>5</sup> cfu/g after 7-14 hours. There was no apparent growth of *B. cereus* when reconstituted infant formula was held at less than 10°C for 24 hours.

### 3.3.3 Toxin production in Infant Formula

The emetic and diarrhoeal toxins have been detected in reconstituted infant formula. *B. cereus* will produce emetic toxin (cereulide) in skimmed milk at 30°C and production increases under aerated conditions (Finlay *et al.*, 1999; Szabo *et al.*, 1991).

Rowen *et al.*, (1997a) examined 100 reconstituted infant formula samples and found, 17% positive for *B. cereus*. Six of these positives were found to contain the enteric toxin, whereas the diarrhoeal toxin was not detected. Although diarrhoeal enterotoxin was not detected in any feeds analysed immediately after reconstitution, diarrhoeal enterotoxin was detected in 4 infant formulas stored for 14 hours at >25°C.

Rowen *et al.*, (1998) also examined 100 reconstituted milk-based infant formulas for the presence of diarrhoeal enterotoxin produced by psychrotrophic *B. cereus*. Thirty-five diarrhoeal-type *B. cereus* isolates were recovered.

In summary both the *B. cereus* emetic and diarrhoeal enterotoxins have been detected in reconstituted infant formula.

### **3.4 Consumer preparation and storage of infant formula**

Infant formula powder is normally stored at ambient temperatures. Manufacturers instruct consumers to make up infant formula with cooled boiled water. However once reconstituted, formula may be subjected to conditions of time and temperature abuse resulting in growth of *B. cereus*.

The Australian NHMRC Dietary Guidelines for Children and Adolescents, incorporating Infant Feeding Guidelines for Health Workers, recommend infant formula be prepared daily with cooled boiled water and stored in the refrigerator for a maximum of 24 hours (NHMRC, 2003).

The New Zealand Ministry of Health 'Food and Nutrition Guidelines for Healthy Infants and Toddlers (Aged 0 to 2 years) also recommended that infant formula should be prepared daily with cooled boiled water and stored in the refrigerator for a maximum of 24 hours (Ministry of Health, 2000).

A US survey on the feeding practices of infants found 33% of mothers mixed formula with warm tap water and 15% of mothers left bottles at room temperature for more than 2 hours (Beck-Fein and Falci, 1999). There is also some evidence indicating that holding prepared formula at room temperature is related to diarrhoea in older infants (Beck-Fein and Falci, 1999).

An survey of infant feeding practices conducted in Sydney revealed that 14% (38/274) of mothers used recently boiled water to reconstitute the powder instead of cooled boiled water while one mother used cold water straight from the tap (Lilburne *et al.*, 1988). The same survey also found that most mothers (94%) reconstituted more than one bottle at a time, keeping the remaining bottles in the refrigerator until required. A significant number of mothers (142/275) reported that they sometimes carried a spare bottle in an insulated carrier, and two-thirds of these mothers pre-warmed the reconstituted formula before placing it in the insulated carrier (Lilburne *et al.*, 1988).

In summary, studies have indicated that some consumers prepare infant formula incorrectly with warm tap water, leave bottles at room temperature for more than 2 hours and store prepared warm infant formula in insulated carriers when travelling.

### **3.5 Consumption data for Infant Formula**

As the 1995 National Nutrition Survey (NNS) did not survey children under the age of two, it is difficult to obtain data on the consumption of infant formula in Australia. However a report based on the results of the NNS on the introduction of breast milk substitutes and solid foods to Australian children between 1992 and 1995 revealed that by the age of 26 weeks, the majority of children had been given infant formula (56.9%) (Donath and Amir, 2002).

The pattern of infant feed at four weeks, 12 weeks and 24 weeks of life, using a sub-sample of children aged over six months, is shown in Table 5.

**Table 5:** Patterns of feeding in the first six months, children aged six months and over, n=2,874 (Donath and Amir, 2002)

|   | At 4 weeks | At 12 weeks | At 24 weeks |
|---|------------|-------------|-------------|
| Breast milk only  | 70.3%      | 57.8%       | 17.5%       |
| Infant formula <sup>a</sup> only                        | 25.1%      | 32.2%       | 15.8%       |
| Breast milk and infant formula <sup>a</sup>             | 2.7%       | 3.1%        | 2.2%        |
| Breast milk and solid food                              | 0.7%       | 2.7%        | 23.3%       |
| Infant formula <sup>a</sup> and solid food              | 1.0%       | 4.2%        | 36.2%       |
| Breast milk, infant formula <sup>a</sup> and solid food | 0.0%       | 0.1%        | 3.2%        |

<sup>a</sup> includes milk substitutes other than infant formula

The recommended guidelines for feeding infants less than 3 months of age are 150 mL/kg/day (NHMRC, 2003). This equates to 137 – 157 mL of formula per feed (based on an infant weight of 5.5 – 6.3 kg and 6 feeds per day) (Butt, 2002). The recommended guidelines for feeding infants between 3 and 6 months require 120 mL/kg/day (NHMRC, 2003). Based on an infant net weight of 6.3 – 8 kg (Butte, 2002), and 6 feeds a day, approximately 126 – 160 mL are required for each feed. Infants aged 6 to 12 months require between 90 – 100 mL/kg/day (NHMRC, 2003), however at this age, infants are likely to be receiving solids as well as formula. The number of feeds per day is reduced for this age group, as formula is no longer the sole source of nutrition for these infants.

### 3.6 Predicted growth of *B. cereus* in infant formula

#### 3.6.1 Storage and handling scenarios

The possible exposure of infants to *B. cereus* as a consequence of the consumption of contaminated infant formula was estimated using a number of scenarios for the preparation and storage of infant formula (Table 6). These scenarios were based on recommended NHMRC and Ministry of Health guidelines on how to prepare formula safely, and possible consumer practice.

**Table 6:** Storage scenarios considered in estimating the possible exposure as a result of the consumption of contaminated infant formula

| Scenario | Storage conditions                   | Temperature/Time  | Temperature of water used to make up formula |
|----------|--------------------------------------|---|--|
| 1        | Refrigerate<br>4°C                   | 4°C/24 hours  | Cooled boiled water                          |
|          |                                      |   | Boiled water                                 |
|          |                                      |   | Warm tap water                               |
| 2        | Refrigerate<br>10°C                  | 10°C /24 hours  | Cooled boiled water                          |
|          |                                      |   | Boiled water                                 |
|          |                                      |   | Warm tap water                               |
| 3        | Room temperature                     | 25°C/24 hours   | Cooled boiled water                          |
|          |                                      |   | Boiled water                                 |
|          |                                      |   | Warm tap water                               |
| 4        | Insulated container/room temperature | Insulated container for 4 hours followed by 25°C/20 hours | Warm tap water                               |

|   |   |   |                |
|---|---|---|----------------|
| 5 | Insulated container/refrigerate<br>4°C  | Insulated container for 4<br>hours followed by<br>4°C/20 hours  | Warm tap water |
| 6 | Insulated container/refrigerate<br>10°C | Insulated container for 4<br>hours followed by<br>10°C/20 hours | Warm tap water |

### 3.6.2 Time and temperature histories of milk stored or cooled under various scenarios

A number of experiments were carried out by Food Science Australia to estimate the cooling rate of prepared formula under the various scenarios listed in Table 6.

Whole milk was heated in 250 ml baby feeding bottles to an initial temperature of 25 °C, 37 °C and 80 °C to represent formula reconstituted with cooled water, warmed water and boiling water, respectively. The milk was then cooled or stored under similar conditions to those specified in Table 5. The temperature of the milk was recorded using a copper constantan thermocouple and an electronic temperature recording device (Squirrel 1250, Grant Instruments, Cambridge). Cooling curves are given for selected scenarios in later sections of this report. For milk held in insulated containers temperatures reported were the average of duplicate samples. For scenarios 4, 5 and 6 (Table 6) temperature histories for milk cooled out of the insulated containers were estimated from the temperature histories obtained for warmed milk cooled at 25 °C, 10 °C and 4 °C.

### 3.6.3 Growth of *B. cereus* in Milk

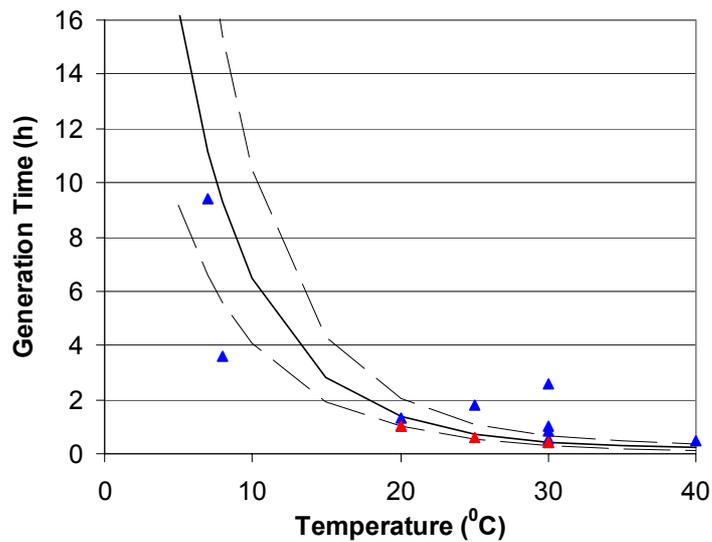
Data from the USDA Pathogen Modelling Program (PMP) was used to develop a model for estimating the growth of *B. cereus* in milk over the temperatures of interest. The parameters used for predicting the growth rate were a pH of 7 and a salt concentration of 0.9%. While these values do not necessarily reflect the conditions in reconstituted infant formula they give growth rate predictions similar to those reported in the literature. The values obtained from the PMP for the growth rate and lag period of *B. cereus* are given in Table 7. The ranges given in Table 7 represent the 95% confidence intervals for the lag and generation time as calculated in the PMP.

**Table 7:** Predicted growth rate and lag period for *B. cereus* growing over a range of temperatures.

| Temperature<br>(°C) | Lag period (h) |         |       | Generation time<br>(h/gen) |         |       |
|---------------------|----------------|---------|-------|----------------------------|---------|-------|
|                     | lower          | average | Upper | Lower                      | average | upper |
| 5                   | 232.3          | 414.7   | 740.1 | 8.8                        | 16.1    | 29.6  |
| 10                  | 46.4           | 70.2    | 106.2 | 4.2                        | 6.5     | 10    |
| 15                  | 11.4           | 16.3    | 23.2  | 2                          | 2.8     | 4.1   |
| 17.5                | 6.2            | 8.8     | 12.5  | 1.4                        | 2       | 2.8   |
| 20                  | 3.6            | 5.2     | 7.4   | 1                          | 1.4     | 2     |
| 22.5                | 2.3            | 3.3     | 4.7   | 0.7                        | 1       | 1.4   |
| 25                  | 1.6            | 2.2     | 3.2   | 0.5                        | 0.7     | 1.1   |
| 30                  | 0.9            | 1.3     | 1.9   | 0.3                        | 0.4     | 0.6   |
| 35                  | 0.8            | 1.1     | 1.5   | 0.2                        | 0.3     | 0.4   |

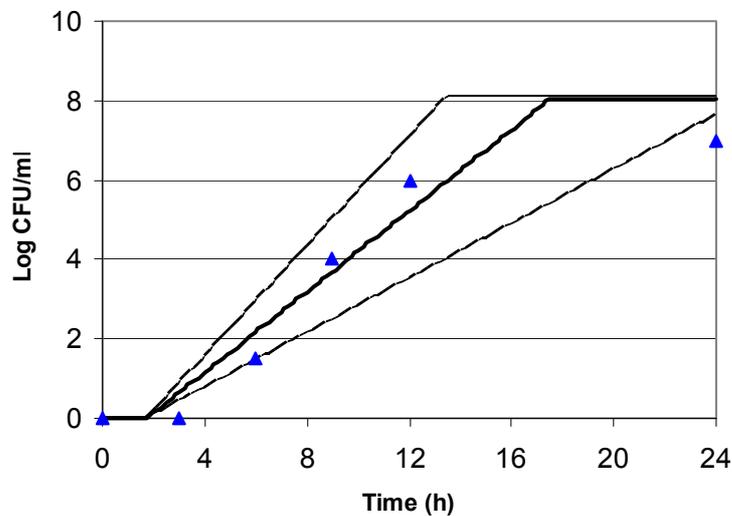
These data were  $\log_{10}$  transformed and fitted to quadratic equations. The equations were then used to predict the growth of *B. cereus* under changing temperature conditions using a spreadsheet model. To determine the validity of the predictions the model outputs were compared to published data for the growth rate of *B. cereus* (Figure 2). Predicted generation times for *B. cereus* were similar to those reported in the literature. It was assumed that the growth rates for milk would be similar to those of infant formula.

**Figure 2:** Comparison of current model with generation times from published studies. Dotted lines represent the 95% confidence interval for the generation time given in the PMP. ▲ Generation times published in ICMSF (1996) for *B. cereus* in milk. ▲ Generation times predicted using a published broth model (Chorin et al, 1997).



Becker et al. (1994) estimated the growth of *B. cereus* in naturally contaminated reconstituted infant food (assumed to be infant formula), incubated at 27 °C for 24h. There was good agreement between the model and the results obtained by Becker et al. (1994) (Figure 3).

**Figure 3:** Comparison of predicted growth of *B. cereus* at 27 °C to the published data of Becker et al. (1994) for reconstituted infant food. ▲ Average growth of *B. cereus* from Becker et al. (1994) Figure 2. Solid line is the average growth predicted using the model. Dotted lines represent best and worst case predictions.



Apart from those studies given in Figures 2 and 3, there is little published data on the generation time and lag time of *B. cereus* growing in milk. Most publications deal with the prevalence of *B. cereus* after incubation under abusive conditions i.e. >20 °C for 24h.

The PMP model is only valid over the temperature range of 5 to 42 °C. Therefore the spreadsheet model was designed to stop growth outside this range. It is recognised that some strains of *B. cereus* are able to grow at temperatures outside this range, especially at the higher end of the range (i.e. above 42 °C). However, based on the cooling curves generated in this study it appears that the time milk spends above 42 °C is short and therefore it is unlikely that restricting the range will result in a significant underestimation of the growth.

There is little published information on the lag period for *B. cereus* at various temperatures. Notermans *et al.* (1997) estimated the lag and growth rate of *B. cereus* in pasteurised milk at 6, 8, 10 and 12 °C. A large amount of data was generated during the study. Unfortunately the data were only graphed making comparisons with the current model difficult. When the predicted lag and growth were plotted against the published data (not shown) there was general agreement between the model and the data for the growth of *B. cereus* at the four temperatures included in the study. However, the predicted lag period was longer than that reported, especially at lower temperatures (i.e. 6 °C). Generally the lag period predicted using the model was longer than that reported for *B. cereus* growing in milk at various temperatures. This is only of concern at low temperatures since at higher temperatures the lag does not contribute significantly to the predicted growth (i.e. the lag period is short in comparison to the time spent at high temperatures).

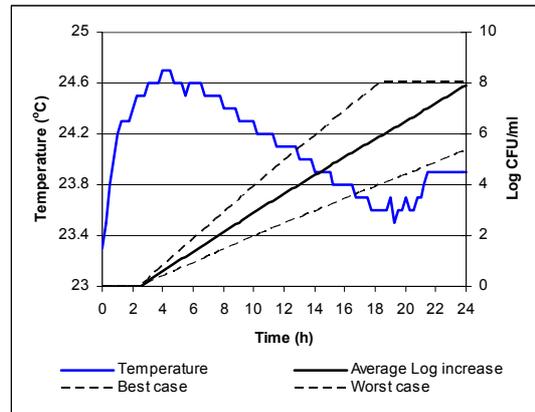
#### 3.6.4 Predicted growth of *B. cereus* under different cooling scenarios

Time and temperature data generated during the cooling trials described earlier were incorporated into a spreadsheet model developed to predict the likely increase in *B. cereus* numbers. The model was modified to restrict growth to 8-log<sub>10</sub> units. This was done for convenience and is not meant to be a true indication of the final concentration of *B. cereus* in milk.

### 3.6.5 Predicted growth in milk cooled under constant temperature conditions

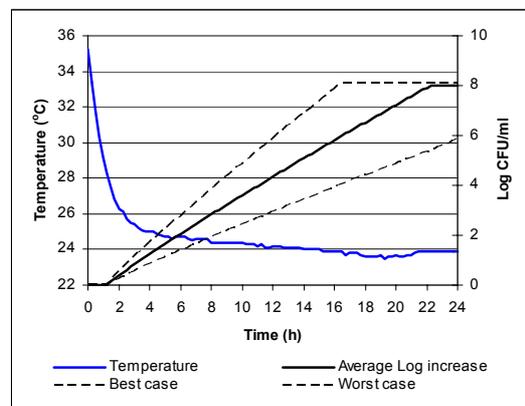
When milk reconstituted with cooled water was placed at 25 °C the model predicted between a 5 and 8-log<sub>10</sub> increase in *B. cereus* over a 24 h period (Figure 4).

**Figure 4:** Predicted growth of *B. cereus* in milk reconstituted at room temperature and held at 25 °C.



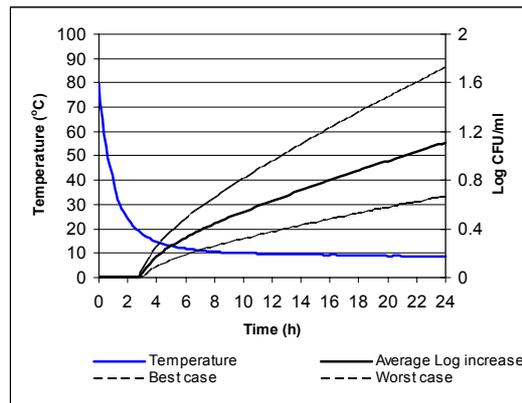
When milk warmed to 80 °C was cooled under similar conditions to those given in Figure 3 an increase of between 6 and 8-logs was predicted, a similar increase was calculated for milk warmed to 37 °C (Figure 5).

**Figure 5:** Predicted growth of *B. cereus* in milk reconstituted at 37 °C and held at 25 °C.



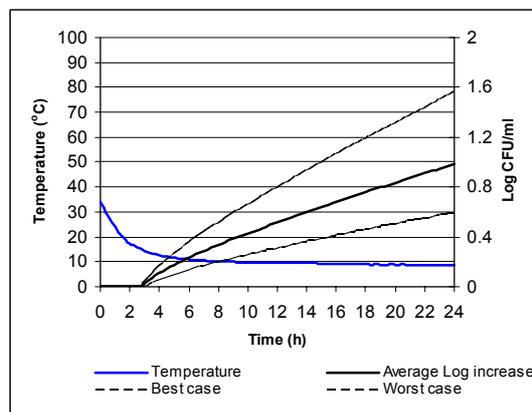
Cooling milk at 10 °C resulted in lower predicted increases in *B. cereus*. When milk reconstituted at 80 °C was cooled at 10 °C a 0.7 to 1.8-log<sub>10</sub> increase in *B. cereus* was predicted (Figure 6).

**Figure 6:** Predicted growth of *B. cereus* in milk reconstituted at 80 °C and held at 10 °C.



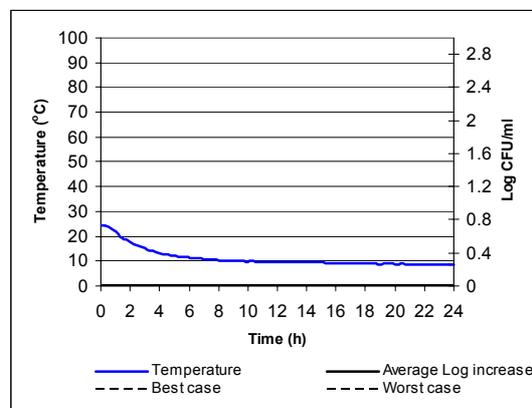
When milk was reconstituted at 37 °C and held under similar conditions a 0.5 to 1.6 log<sub>10</sub> increase was predicted (Figure 7).

**Figure 7:** Predicted growth of *B. cereus* in milk reconstituted at 37 °C and held at 10 °C.



When milk at 25 °C was held at 10 °C for 24h no growth of *B. cereus* was predicted; *B. cereus* was still in lag after the 24h period (Figure 8).

**Figure 8:** Predicted growth of *B. cereus* in milk reconstituted at 25 °C and held at 10 °C.

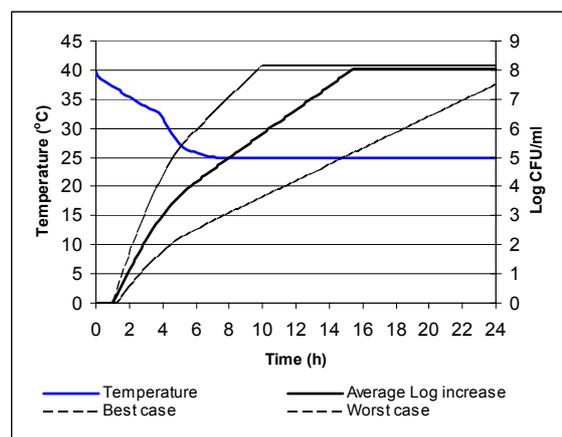


When milk was cooled at 4 °C or lower there was no predicted increase in *B. cereus* numbers under any of the conditions modelled i.e. the temperature of the milk after reconstitution did not have any effect on the growth after 24h when milk was cooled at 4 °C.

### 3.6.6 Predicted growth of *B. cereus* in milk stored in insulated containers prior to or during cooling.

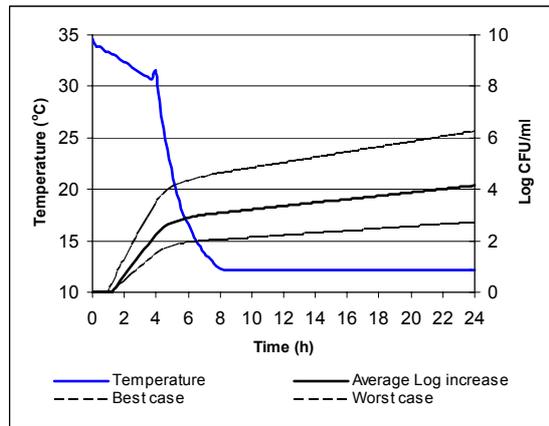
In order to model the growth of *B. cereus* in milk stored in insulated containers, milk (37 °C) in baby bottles was placed into commercially available insulated containers (capable of holding two bottles). The container was then held at room temperature for 4h before milk was removed and cooled at 25, 10 or 4 °C. The effect of leaving bottles in the container during cooling was also modelled. When milk was held in the container at room temperature for 4 h before transferring to 25 °C, a ~8- $\log_{10}$  increase in *B. cereus* numbers (Figure 9) was predicted. It is likely that the milk would have been inedible at this time due to growth of spoilage bacteria.

**Figure 9:** Predicted growth of *B. cereus* in milk reconstituted at 37 °C and held for 4h at 25 °C before being removed from the container and stored at 25 °C.



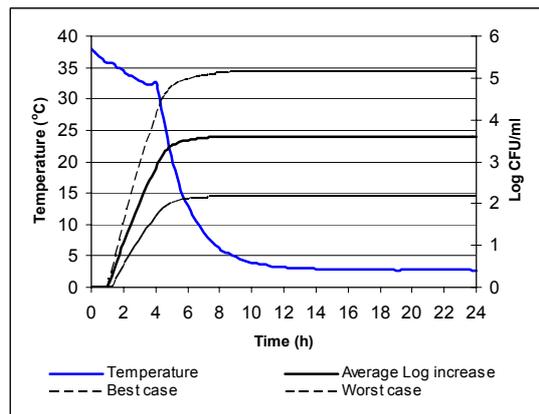
When milk was held in the insulated container for 4h before being removed and stored at ~10 °C, there was a predicted 2.7 to 6.2- $\log_{10}$  increase in *B. cereus* (Figure 10). The time spent in the insulated container prior to cooling was critical in determining the amount of growth of *B. cereus*. After 4 h storage at room temperature (25 °C) numbers of *B. cereus* were predicted to increase by between 1.5 and 3.5- $\log_{10}$  units.

**Figure 10:** Predicted growth of *B. cereus* in milk reconstituted at 37 °C and held in an insulated container for 4h at 25 °C and then removed and cooled at 10 °C for 20h.



When milk was placed at 4 °C after holding in an insulated container for 4 h there was a 2 to 5-log<sub>10</sub> predicted increase in *B. cereus* (Figure 11).

**Figure 11:** Predicted growth of *B. cereus* in milk reconstituted at 37 °C and held in an insulated container for 4h at 25 °C and then removed and cooled at 4 °C for 20h.



A summary of predicted increases calculated for different scenarios is given in Table 7.

**Table 7:** Summary of predicted increases in *B. cereus* under various preparation and storage scenarios

| Scenario | Temperature/Time  | Temperature of water used to make up formula | Predicted Log <sub>10</sub> increase |
|----------|---|--|--------------------------------------|
| 1        | 4°C/24 hours  | Cooled boiled water                          | 0                                    |
|          |   | Boiled water                                 | 0                                    |
|          |   | Warm tap water                               | 0                                    |
| 2        | 10°C /24 hours  | Cooled boiled water                          | 0                                    |
|          |   | Boiled water                                 | 0.7 – 1.7                            |
|          |   | Warm tap water                               | 0.6 – 1.6                            |
| 3        | 25°C/24 hours   | Cooled boiled water                          | 5.4 – 8.0                            |
|          |   | Boiled water                                 | 5.5 – 8.0                            |
|          |   | Warm tap water                               | 5.8 – 8.0                            |
| 4        | Insulated container for 4 hours followed by 25°C/20 hours | Warm tap water                               | 7.5 – 8.0                            |
| 5        | Insulated container for 4 hours followed by 4°C/20 hours  | Warm tap water                               | 2.2 – 5.1                            |
| 6        | Insulated container for 4 hours followed by 10°C/20 hours | Warm tap water                               | 2.7 – 6.2                            |

### 3.6.7 Estimated levels of *B. cereus* in infant formula under various scenarios

Using the predicted increases in *B. cereus* from the modelling, final levels of *B. cereus* have been estimated when initial levels of *B. cereus* of 100 cfu/g and 1000 cfu/g are present in the infant formula powder (Table 8). As infants less than 6 months of age consume on average approximately 150 mL formula/feed, the final total concentration of *B. cereus* that could be present in a bottle of reconstituted formula are also estimated in Table 8.

The estimated levels of *B. cereus* in infant formula (Table 8), modelled from the growth of *B. cereus* in milk, correlates with levels of *B. cereus* reported in the literature. The modelling showed no predicted increase in the number of *B. cereus* when infant formula is stored at 4°C for 24 hours. This compares with the work of Crilley *et al.*, (1994) who also report that no growth occurred in milk based infant formula stored at 5°C for 24 hours.

Becker *et al.*, (1994) reported that when infant formula was reconstituted with initial levels of *B. cereus* at 100 cfu/g and stored at 24°C, levels of >10<sup>5</sup> cfu/mL were reached after 7-9 hours. The estimated levels from the predictive model ranged from >10<sup>2</sup> - <10<sup>4</sup> cfu/mL after 7 hours to >10<sup>3</sup> - <10<sup>5</sup> cfu/mL after 9 hours.

**Table 8:** Summary of estimated levels in *B. cereus* using initial levels of 100 cfu/g and 1000 cfu/g in infant formula powder

| Scenario<br>Temperature/<br>Time   | Temperature of<br>water used to make<br>up formula | Predicted<br>Log <sub>10</sub><br>increase | Initial level<br>in powder<br>cfu/g | Estimated<br>level<br>cfu/mL*                      | Estimated level<br>total cfu/150 ml<br>bottle* |
|--|--|--|-------------------------------------|--|--|
| 1. 4°C/24 hours  | Cooled boiled water                                | 0  | 100                                 | 15   | 2250 (10 <sup>3</sup> )                        |
|  |  |  | 1000                                | 150  | 22500 (10 <sup>4</sup> )                       |
|  | Boiled water                                       | 0  | 100                                 | 15   | 2250 (10 <sup>3</sup> )                        |
|  |  |  | 1000                                | 150  | 22500 (10 <sup>4</sup> )                       |
|  | Warm tap water                                     | 0  | 100                                 | 15   | 2250 (10 <sup>3</sup> )                        |
|  |  |  | 1000                                | 150  | 22500 (10 <sup>4</sup> )                       |
| 2. 10°C /24 hours  | Cooled boiled water                                | 0  | 100                                 | 15   | 2250 (10 <sup>3</sup> )                        |
|  |  |  | 1000                                | 150  | 22500 (10 <sup>4</sup> )                       |
|  | Boiled water                                       | 0.7 – 1.7                                  | 100                                 | >10 - <10 <sup>3</sup>                             | >10 <sup>4</sup> - <10 <sup>6</sup>            |
|  |  |  | 1000                                | >10 <sup>2</sup> - <10 <sup>4</sup>                | >10 <sup>5</sup> - <10 <sup>7</sup>            |
|  | Warm tap water                                     | 0.6 – 1.6                                  | 100                                 | >10 - <10 <sup>3</sup>                             | >10 <sup>3</sup> - <10 <sup>5</sup>            |
|  |  |  | 1000                                | >10 <sup>2</sup> - <10 <sup>4</sup>                | >10 <sup>4</sup> - <10 <sup>6</sup>            |
| 3. 25°C/4 hours  | Cooled boiled water                                | 0.4 – 0.8<br>after 4 hours                 | 100                                 | >10<br>(after 4 h)                                 | >10 <sup>3</sup> - <10 <sup>5</sup>            |
|  |  |  | 1000                                | >10 <sup>2</sup><br>(after 4 h)                    | >10 <sup>4</sup> - <10 <sup>6</sup>            |
|  | Boiled water                                       | 0.5 – 1.3<br>after 4 hrs                   | 100                                 | >10 - <10 <sup>4</sup><br>(after 4 h)              | >10 <sup>3</sup> - <10 <sup>5</sup>            |
|  |  |  | 1000                                | >10 <sup>2</sup> - <10 <sup>4</sup><br>(after 4 h) | >10 <sup>4</sup> - <10 <sup>6</sup>            |
|  | Warm tap water                                     | 0.8 – 1.7<br>after 4 hours                 | 100                                 | >10 <sup>2</sup><br>(after 4 h)                    | >10 <sup>4</sup> - <10 <sup>6</sup>            |
|  |  |  | 1000                                | >10 <sup>3</sup><br>(after 4 h)                    | >10 <sup>5</sup> - <10 <sup>7</sup>            |
| 4. Insulated<br>container for 4<br>hours<br>followed by<br>25°C/20 hours | Warm tap water                                     | 0.5 - 1.7<br>after 2 hours                 | 100                                 | >10 - <10 <sup>3</sup><br>(after 2 h)              | >10 <sup>3</sup> - <10 <sup>6</sup>            |
|  |  |  | 1000                                | >10 <sup>2</sup> - <10 <sup>4</sup><br>(after 2 h) | >10 <sup>4</sup> - <10 <sup>7</sup>            |
|  |  | 1.7 – 4.3<br>after 4 hours                 | 100                                 | >10 <sup>2</sup> - <10 <sup>6</sup><br>(after 4 h) | >10 <sup>5</sup> - <10 <sup>8</sup>            |
|  |  |  | 1000                                | >10 <sup>3</sup> - <10 <sup>7</sup><br>(after 4 h) | >10 <sup>5</sup> - <10 <sup>9</sup>            |

\*using a rehydration factor of 15% in the reconstituted product

## 4 Risk characterisation

### 4.1 Uncertainty

Uncertainties in the risk assessment primarily come from the following two aspects.

#### 4.1.1 Modelling *B. cereus* growth in milk versus infant formula

The predictive model used to estimate the growth of *B. cereus* in infant formula was based on data on *B. cereus* growth in milk from the USFDA Pathogen Modelling Program (PMP). It is assumed that the growth rates of *B. cereus* in milk would be similar to those of infant formula. There is a degree of uncertainty in applying a model developed for milk to infant formula. However, there was good agreement between the model and the results obtained by Becket et al., (1994) for the growth of *B. cereus* in naturally contaminated reconstituted infant food (assumed to be infant formula) at 27°C for 24 hours.

It is assumed that milk is a good culture medium for the growth of *B. cereus* and that generally the additional nutrients provided by infant formula additives are unlikely to inhibit growth of *B. cereus*.

Data on the actual growth rates of *B. cereus* in infant formula will further validate the predictive growth estimates in the modelling.

#### 4.1.2. *The infectious dose of B. cereus in infants*

This risk assessment concluded that while the infectious dose for the general population for *B. cereus* is  $10^5$  cells/spores or greater, the infectious dose of *B. cereus* for infants may be lower. It is assumed that infants would be more susceptible because their immune systems are not fully developed and infants are more susceptible to bacterial infections compared to healthy adults and older children. However there is limited data to support infant susceptibility to *B. cereus*. The dependency of infants on formula in their first few months of life was a major consideration in determining the likelihood of exposure to *B. cereus* infection from reconstituted infant formula.

In conclusion:

- infants are generally more susceptible to bacterial infections;
- infants have poorly developed immune systems; and
- as infant formula may be the sole source of nutrition for some infants a high level of safety must be ensured.

## 4.2 Variability

The scenarios used to predict the growth for *B. cereus* in infant formula were based on the conditions recommended in the NHMRC and Ministry of Health guidelines on how to prepare infant formula safely, and also on possible consumer practice (based on survey results on consumer behaviour when preparing infant formula). The following assumptions were used to predict the growth rates for *B. cereus* in infant formula:

- The temperature of the water used to prepare infant formula was 25°C, 80°C and 37°C for cooled boiled water, recently boiled water (boiled water) and warm tap water respectively.
- The temperature of 4°C was used to model conditions reflecting a domestic refrigerator with good temperature control. The temperature of 10°C was used to model conditions reflecting a domestic refrigerator with poor temperature control.

## 4.3 Conclusions of this risk assessment

The final quality of infant formula depends on the quality of the raw ingredients, correct processing, ensuring the product is not contaminated after processing, and safe storage after reconstitution.

The presence of *B. cereus* in infant formula is associated with contamination of raw milk and the subsequent survival of the spores following heat treatment.

Heat treatments will cause spore germination, and in the absence of competing flora, *B. cereus* can grow well (Granum and Lund, 1997).

There has been one reported outbreak of food poisoning due to *B. cereus* directly attributed to infant formula and there have been three outbreaks (vanilla slices, milk-gelatine dessert and macaroni cheese) linked to dried milk powder which were thought to be contaminated with *B. cereus*.

Diarrhoea remains one of the most important causes of ill-health and death among infants and children. In developed countries the incidence is much lower, but still significant (Australian Bureau of Statistics, 2002). As diarrhoea is usually only a mild illness, people do not always seek medical attention, and the condition is often unreported. In addition if medical attention was sought it is unlikely infants would be tested for *B. cereus* as illness resulting from *B. cereus* is not required to be notified to health authorities. Therefore it is likely that some infants become ill from *B. cereus*, although there is no epidemiological evidence directly linking diarrhoeal illness to *B. cereus*.

The infectious dose of *B. cereus* is in the range of a  $10^5$  to  $10^8$  viable cells or spores. It has been therefore suggested that levels of  $10^3$  cfu/g in a 100 g serving of food may not be completely safe to consume (Granum and Lund, 1997).

Infant formula is specifically designed as a human milk substitute for infants. Infants are a particularly vulnerable group due to their underdeveloped immune systems and more importantly, the high frequency of consumption of infant formula (i.e. formula may represent their sole source of nutrition). The majority of infants consume infant formula to some degree during their first twelve months. In general reconstituted infant formula is considered to be a high risk food due to the susceptibility of infants to enteric bacterial pathogens, their severe response to toxins, and increased mortality (ICMSF, 2002).

NHMRC and Ministry of Health guidelines recommend that infant formula be reconstituted with cooled boiled water then stored in the refrigerator for a maximum of 24 hours. However, studies have indicated that some consumers prepare infant formula incorrectly with warm tap water, leave bottles at room temperature for more than 2 hours, and store prepared warm infant formula in insulated carriers when going out (Beck-Fein and Falci, 1999; Lilburne *et al.*, 1988).

Formula stored at  $4^{\circ}\text{C}$  for 24 hours after reconstitution with cooled boiled water ( $25^{\circ}\text{C}$ ), boiled water or warm tap water does not appreciably increase the level of *B. cereus* beyond that initially present in the formula. This was also the case for formula prepared with cooled boiled water and stored at  $10^{\circ}\text{C}$  for 24 hours.

Formula prepared from powder with initial levels of 100 cfu/g using recently boiled water ( $80^{\circ}\text{C}$ ) or warm tap water ( $37^{\circ}\text{C}$ ) and stored at  $10^{\circ}\text{C}$  for 24 hours may approach the infectious dose of  $10^5$  (worst case scenario) if an infant consumed 150 ml formula in one feed. Formula prepared with initial levels of 1000 cfu/g under these conditions exceeds the infectious dose (worst case scenario).

Preparing infant formula with cooled boiled water with initial levels of 100 cfu/g in the powder may reach the infectious dose when stored at room temperature after 6 hours, and after 4 hours when initial levels are 1000 cfu/g.

The practice of preparing infant formula with warm tap water or boiling water is potentially unsafe. Formula prepared under these conditions with initial levels of 100 cfu/g may reach an infectious dose when stored at 10°C for 24 hours or when stored at room temperature for greater than 4 hours.

The practice of storing formula in insulated carriers is also potentially unsafe. 150 mL of warm reconstituted formula with initial levels of 100 cfu/g in the powder stored in an insulated carrier may reach an infectious dose after 2 hours.

In summary, the risk assessment modelled the growth of *B. cereus* under various preparation and storage scenarios and concluded that:

- Powdered infant formula containing up to 100 cfu/g of *B. cereus* and reconstituted using the following practices, would not expose infants to an infectious dose of *B. cereus*:
  - Formula reconstituted with cooled boiled water (25°C) and stored for 24 hours at 4°C.
  - Formula reconstituted with cooled boiled water and stored for 24 hours at 10°C.
- Formula reconstituted from powder with levels of 1000 cfu/g and then stored at 10°C for 24 hours may pose a risk to infants.
- The following practices are considered unsafe as they were shown to result in rapid growth of *B. cereus* to levels that result in illness, in reconstituted formula during storage:
  - Storing formula reconstituted with warm tap water (37°C) or boiling water (80°C).
  - Storing formula at room temperature.
  - Storing warm formula in insulated carriers.

## Acknowledgements

FSANZ acknowledges the predictive modelling contribution from Food Science Australia.

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## Summary of Issues Raised through Public Consultation on the Initial Assessment Report

| Submitter    | Comments  |
|--------------|---|
| Fonterra     | <p>Submits Standard 1.6.1 for infant formula is:</p> <ul style="list-style-type: none"> <li>• unnecessarily severe</li> <li>• out of step internationally</li> <li>• does not take into account that infant formula recombined (13-15%) before use, thereby adding a seven-fold ‘safety factor’</li> <li>• does not take into account low risk of organism</li> <li>• falls short of Codex guidelines for micro criteria for foods</li> <li>• is not consistently attainable and there is poor repeatability of <i>B. cereus</i> tests at the ‘m=10’ level</li> </ul> <p>Recommends:</p> <ul style="list-style-type: none"> <li>• ‘two class attribute’ std where unacceptable level is set 100 (i.e. n=5, c=0, m=100) or</li> <li>• transitional std adopted (1.1A.1) n=5, c=1, m=100, M=1000</li> </ul> |
| FTA Victoria | <ul style="list-style-type: none"> <li>• Supports Option 1A – sampling plan proposed by the applicant</li> </ul>  |
| Heinz        | <ul style="list-style-type: none"> <li>• Supports Option 1B (Std 1.1A.1) – to amend Standard 1.6.1 and accept an alternative sampling plan. n=5, c=1, m=100, M=1000</li> </ul>  |
| AFGC         | <ul style="list-style-type: none"> <li>• Supports A454,</li> <li>• replace limits in Std 1.6.1 with requirements applied under old R7 Std (Std 1.1A.1)</li> <li>• which would still protect public health and safety.</li> <li>• unclear why limits were changed during review</li> </ul>   |
| IFMAA        | <ul style="list-style-type: none"> <li>• endorse AFGC submission</li> <li>• support Regulatory option 1B</li> <li>• maintenance of limit in Std 1.1A.1 (formally Std R7)</li> </ul>   |
| Nutricia     | <ul style="list-style-type: none"> <li>• Support A454, specifically AFGC submission.</li> <li>• Need for harmonisation of Aust Std with international Stds to prevent their companies being unable to import product for infants with special needs,</li> <li>• should not more restrictive than EU Stds</li> </ul>   |
| DAFF         | Deferred comment until draft assessment report is available   |
| Nestlé       | <ul style="list-style-type: none"> <li>• Supports Option 1B (Std 1.1A.1)</li> <li>• Supports AFGC submission</li> </ul>   |
| Wyeth        | <ul style="list-style-type: none"> <li>• Supports Option 1B (Std 1.1A.1)</li> </ul>   |

## Summary of Issues Raised through Public Consultation on the Draft Assessment Report

| Submitter                       | Comments   |
|---------------------------------|--|
| NZFSA                           | <ul style="list-style-type: none"> <li>• Prefers option 3 although Option 4 is acceptable</li> <li>• Option 3 allows for sample variability and uncertainty</li> <li>• Concern over some of the assumptions used in the modelling</li> <li>• Vital that manufacturers actively undertake on-going education campaigns to consumers regarding preparation of formula, especially prevention of time and temperature abuse.</li> </ul>   |
| FTA Victoria                    | Supports Option 4  |
| AFGC                            | <p>Supports Option 2 – retention of the transitional std as FSANZ has supplied insufficient evidence in support of its preferred option.</p> <ul style="list-style-type: none"> <li>• Predictive modelling has too many assumptions indicating weakness of the modelling</li> <li>• No recorded market failure in past 20 years in Australia and worldwide</li> <li>• Statements relating to diarrhoeal illness and infant formula are misleading in the Risk Assessment</li> <li>• Given the govt. and health carer advice, detailed labelling statements as required by Std 2.91 the dated data on time/temperature abuse by carers should not be given too great an emphasis in consideration of this issue</li> <li>• Transitional Standard 1.1A.1 limits would not be inconsistent with international requirements</li> </ul> |
| Heinz                           | Supports Regulatory Option 2 (Transitional Standard 1.1A.1), however would find both options 2 and 4 acceptable alternatives   |
| Nestlé                          | Supports submission made by AFGC, prefers adoption of Option 2, retention of Transitional Standard 1.1A.1 limits   |
| Public Health Services<br>- QLD | Supports Option 4  |