**Supporting document 1**

# Scientific evidence informing the proposed microbiological criteria for infant formula – Proposal P1039

Microbiological Criteria for Infant Formula

# Executive summary

As part of FSANZ's review of Standard 1.6.1 in the *Australia New Zealand Food Standards Code* (Code), Proposal P1039 – Microbiological Criteria for Infant Formula has been prepared to align food safety criteria with those in international standards (Codex Alimentarius [Codex]). This document summarises the risk assessment work undertaken to inform the Codex risk management approach; in particular, the information supporting establishment of microbiological criteria.

In 2008, the Codex Committee on Food Hygiene (CCFH) revised the *Code of hygienic practice for powdered infant formulae for infants and young children (CAC/RCP 66 - 2008)* in response to the emergence of *Cronobacter* species (referred to as *Enterobacter sakazakii* prior to 2008) as an important pathogen for infants fed with powdered infant formula (PIF). The revised code introduced a set of microbiological criteria for *Cronobacter* spp. in PIF, and reconfirmed the application of a set of microbiological criteria for *Salmonella* spp. in both PIF and follow-up formula (FUF).

Codex based these criteria on scientific advice and a risk assessment model undertaken by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) through a series of joint expert meetings. The expert consultations concluded that intrinsic contamination of powdered infant formula with *E. sakazakii* (*Cronobacter* spp.) and *Salmonella* spp. had been a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequelae and death. Although the rate of incidence was low, neonates and immunocompromised infants were at the greatest risk of *Cronobacter* infection.

The FAO/WHO expert consultations identified the organisms of concern in infant formula and the relevant control measures throughout the food chain to reduce the risks for infants associated with consumption of infant formula. Guidance on how a microbiological criterion could be used to reduce relative risk was also considered in the expert consultations. This was achieved by providing examples of how effectively different sampling plans are able to reject lots through detecting elevated levels of contamination and the corresponding predicted reduction in relative risk.

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# 1 Purpose

FSANZ is undertaking a review of microbiological limits contained in Standard 1.6.1 of the *Australia New Zealand Food Standards Code* (Code). Proposal P1039 has been prepared to include food safety microbiological criteria for powdered infant formula products as part of the Code to align with international standards as outlined by the Codex Alimentarius (Codex). The proposed microbiological criteria have been developed by Codex in the *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children* (CAC/RCP 66 - 2008) (CoHP)*.*

This document provides an overview of the risk assessment work undertaken to inform the Codex risk management approach, with particular emphasis on information supporting the establishment of microbiological criteria. Relevant findings from other scientific evidence published after this work, have also been included where relevant.

# 2 Background

Responding to requests from the Codex Committee on Food Hygiene (CCFH), joint expert meetings of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations were convened to consider the risk of *Cronobacter* spp. and *Salmonella* in powdered infant formula (PIF). Reports of these expert meetings are published as:

- *Enterobacter sakazakii* and microorganisms in powdered infant formula, Meeting Report, 2004 (FAO/WHO 2004)

- *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report, 2006 (FAO/WHO, 2006)

- *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula: Meeting Report, 2008 (FAO/WHO, 2008).

The meetings in 2004 and 2006 considered scientific advice relevant to powdered infant formula and developed a quantitative risk assessment model. The consultation in 2008 focused on scientific advice relevant to *Cronobacter* spp. in powdered follow-up formula (FUF).

Findings from the consultations in 2004 and 2006 identified the organisms of concern in infant formula and the relevant control measures at various steps in the food chain to reduce the risks for infants and young children associated with the consumption of infant formula. This included consideration of labelling and preparation risk reduction strategies, and the establishment of microbiological food safety criteria for *Cronobacter* spp*.* and *Salmonella* spp.

# 3 Organisms of concern

The initial 2004 FAO/WHO expert meeting considered the microorganisms or microbial toxins of concern with PIF and the strength of the evidence of a causal association between their presence in PIF and illness in infants. The meeting reviewed available scientific information on microorganisms, health consequences from consumption of infant formula, the production, distribution and preparation systems involved for powdered infant formula and considered approaches which could be used to evaluate and reduce the risk associated with PIF.

Microorganisms of concern were grouped into three categories according to the strength of evidence for causal association with illness (Table 1).

*Enterobacter sakazakii (Cronobacter* spp.) and *Salmonella* spp. were listed as Category A microorganisms – that is having clear evidence of causality to illness.

**Table 1: Microorganisms and their strength of evidence in causing illness in infants fed powdered infant formula (excerpt from FAO/WHO, 2004)**

| Category | Microorganism |
|---|---|
| **Category A**: Microorganisms with a clear evidence of causality with illness | *Enterobacter sakazakii (Cronobacter* spp.) *Salmonella* spp. |
| **Category B**: Microorganisms for which causality with illness is plausible but not yet demonstrated | *Citrobacter koseri, C. freundii* *Enterobacter agglomerans, E. cloacae* *Hafnia alvei* *Klebsiella pneumoniae, K. oxytoca* |
| **Category C**: Microorganisms for which causality with illness is less plausible or not yet demonstrated | *Bacillus cereus* *Clostridium difficile, C. perfringens, C. botulinum* *Listeria monocytogenes* *Staphylococcus aureus* |

The expert meeting concluded that intrinsic contamination of powdered infant formula with *E. sakazakii* (later reclassified as *Cronobacter* spp.) and *Salmonella* spp. had been a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequelae and death. No link had been established between illness and other microorganisms in powdered infant formula, although such a link was considered plausible for a number of other *Enterobacteriaceae*.

## 3.1 Hazard Identification

### 3.1.1 Cronobacter *spp.*

*Enterobacter sakazakii* was reclassified as a new genus, *Cronobacter,* in 2007 (Iversen et al., 2007). The subject of many revisions in recent years, the genus currently comprises 10 species including *C. sakazakii, C. malonaticus, C. universalis, C. turicensis, C. muytjensii, C. dublinensis, C. condiment, C. helveticus, C. pulveris* and *C. zurichensis.* As a result of the reclassifications and increased understanding of the taxonomy of the *Cronobacter* spp., it is unclear which specific *Cronobacter* spp. are referred to in many of the pre-2007 publications (Holý and Forsythe, 2014).

*Cronobacter* spp. are oxidase-negative, facultative anaerobic, Gram-negative, motile, non-spore-forming peritrichous (have flagella covering the surface of the cell) rods belonging to the Enterobacteriaceae family. *Cronobacter* are ubiquitous and have been isolated from a wide range of foods, domestic and hospital environments and from animal and human sources.

Growth of *Cronobacter* can occur at temperatures between 5–47$^\circ$C and at pH as low as 3 (Abdesselam and Pagotto, 2014; Holý and Forsythe, 2014). The optimum temperature for growth is approximately 37–39$^\circ$C (Holý and Forsythe, 2014), with an average generation time of around 40 min at 23$^\circ$C (Nazarowec-White and Farber, 1997). With a D-value of 0.7 seconds at 70$^\circ$C, *Cronobacter* are quickly inactivated at temperatures above 70$^\circ$C (FAO/WHO, 2004).

*Cronobacter* are resistant to desiccation, osmotic stress and acids; some strains also produce a heat stable enterotoxin that survives pasteurisation (Kalyantanda et al., 2015). They are tolerant to dry stress and have been shown to survive for two years in PIF and then grow rapidly on reconstitution (Caubilla-Barron & Forsythe, 2007). One of the species, *C. sakazakii*, is able to metabolise sialic acid, a nutrient added to PIF that facilitates the brain development of infants (Kalyantanda et al., 2015; Holý and Forsythe, 2014). Cells of *Cronobacter* are able to adhere to hydrophobic surfaces such as silicone, latex, polycarbonate, stainless steel, glass and polyvinyl chloride, and form biofilms in enteral feeding tubes (Kalyantanda et al., 2015; Holý and Forsythe, 2014; Healy et al., 2010).

*Cronobacter* are regarded as opportunistic pathogens with most clinical cases associated with *C. sakazakii* (particularly the clonal complex of *C. sakazakii*-ST4) and *C. malonaticus*, and to a less extent *C. universalis* and *C. turicensis* (Holý and Forsythe, 2014). Although rare, *Cronobacter* infection represents a serious health risk in highly vulnerable neonates[1], infants and the elderly (Holý and Forsythe, 2014). In neonates, death has been reported to occur in 40%–80% of cases (Kent et al, 2015). The microorganism has been implicated in neonatal infections leading to meningitis, necrotising enterocolitis and septicaemia (Kalyantanda et al., 2015; Holý and Forsythe, 2014). On the other hand, for the majority of *Cronobacter* infections in the adult population, the symptoms are less severe and the source of infection unknown (Holý and Forsythe, 2014).

The WHO/FAO proposed an infectious dose for *Cronobacter* of 10,000 colony forming units (cfu) in a single feeding for infants fed PIF (WHO/FAO 2008). Others consider *Cronobacter* infection to have a much lower infective dose and a short incubation period (Jason, 2012).

A number of virulence traits have been identified or speculated for *Cronobacter* pathogenesis. Some strains can invade intestinal cells (via attachment and invasion mechanisms), replicate in macrophages and cross the blood-brain barrier. The type VI secretion system (T6SS[2]) may be involved in adherence, cytotoxicity, host-cell invasion, growth inside macrophages and survival within the host, while a number of other strains encode for haemolysins which may also play a role in crossing the blood-brain barrier. *Cronobacter* tend to be sensitive to most antibiotics although resistance to ampicillin and most first and second generation cephalosporins has been noted in some species (Kent et al., 2015; Holý and Forsythe, 2014).

### 3.1.2   *Salmonella* spp.

*Salmonella* spp. are facultative anaerobic Gram-negative, non-spore forming rod-shaped bacteria. They are found in the intestinal tract of warm and cold-blooded vertebrates and in the surrounding environment (FSANZ 2013).

Growth of *Salmonella* can occur at temperatures between 5.2–46.2°C, pH of 3.8–9.5 and with a minimum water activity of 0.93 when other conditions are near optimum. *Salmonella* can survive for months or even years in low moisture foods and are able to survive frozen storage at -20°C. *Salmonella* are sensitive to normal cooking conditions, however, foods that are high in fat and low in moisture may have a protective effect against heat inactivation (FSANZ 2013; Li et al. 2013).

*Salmonella* are a serious hazard as they cause incapacitating but not usually life threatening illness of moderate duration, and sequelae are rare (ICMSF 2002). People of all ages are susceptible to salmonellosis.

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[1]  A neonate is an infant in the first 28 days after birth.

However, the elderly, infants and immunocompromised individuals are at a greater risk of infection and generally have more severe symptoms (FSANZ 2013).

The predominant symptoms of salmonellosis are gastrointestinal in nature and include abdominal cramps, nausea, diarrhoea, mild fever, vomiting, dehydration, headache and/or prostration. The onset of illness is typically 24–48 hours after infection (range of 8–72 hours) and symptoms usually last for 2–7 days. Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. The fatality rate for salmonellosis is generally less than 1% (FDA 2012; FSANZ 2013).

The particular food matrix and the strain of *Salmonella* influence the level at which *Salmonella can cause* illness. It has been reported that as low as one to 100 cells can cause illness. However in other cases, significantly more cells were required to cause illness (ICMSF 1996; Teunis et al., 2010; FDA 2012). There are no dose-response data or model for infants, and the available information indicates that illness in infants can result from very low doses of *Salmonella* cells (FAO/WHO 2006).

## 3.2    Epidemiology

Cases of *Cronobacter* and *Salmonella* infections in infants fed PIF are likely to be under-reported due to multiple factors including misdiagnosis and difficulties in attributing a specific source of infection (Kent et al., 2015; Holý and Forsythe, 2014). The following information describes reported outbreaks and cases of *Cronobacter* and *Salmonella* infection associated with PIF that have been published in the scientific literature.

### 3.2.1    *Cronobacter* spp.

Outbreaks of *Cronobacter* infections in (mostly young) infants associated with consumption of PIF have been reported in a number of countries (Table 2). Jason (2012) reviewed available worldwide data between 1958 and 2010 for invasive paediatric *Cronobacter* infections in infants without underlying disorders. At the onset of the symptoms, 99% of infected infants were less than 2 months of age, with 83% less than 1 month of age.  Low birth weight infants accounted for 68% of all cases. Infections occurred most often in hospitals (69%) and the home (31%). These results are consistent with data from the United States indicating an incidence of *Cronobacter* infection of 1 per 100,000 infants, increasing to 9.4 per 100,000 in infants with a very low birth weight (i.e. <1.5 kg) (FAO/WHO, 2006).

*Cronobacter* infection is not notifiable in Australia and therefore data is limited[3]. Infection caused by *C. sakazakii* became a notifiable disease in New Zealand in 2005 (NZFSA, 2009). In New Zealand in 2004, a premature baby in Waikato Hospital died of meningitis caused by *C. sakazakii* infection, although authorities were unable to definitively link the case to contaminated PIF (NZFSA, 2009). In 1991, there was an unconfirmed report in New Zealand of *Cronobacter* infection of twins, with one twin recovering and the other later suffering brain damage and spastic quadriplegia (NZFSA, 2009).

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[3] The Australian national notifiable diseases surveillance system, http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-distype.htm accessed 13 August 2015.

**Table 2: Foodborne outbreaks caused by Cronobacter spp. where PIF was implicated**

| Year | No. cases (fatalities) | Aetiology | Isolated from PIF | Country | Reference |
|---|---|---|---|---|---|
| 1998 | 12 (2) | *E. sakazakii* | Yes | Belgium | Van Acker et al., 2001 |
| 2007 | 1 | *E. sakazakii* | NA | Canada | Pagotto and Farber, 2009 |
| 1990-1992 | 3 | *E. sakazakii* | NA | Canada | Pagotto and Farber, 2009 |
| 2004 | 9 (2) | *E. sakazakii* | Yes | France | Coignard et al., 2004 FAO/WHO (2008) |
| 1994 | 13 (3) | *E. sakazakii* | Yes | France | Caubilla-Barron et al., 2007 |
| 1986-1987 | 3 (1) | *E. sakazakii* | Yes | Iceland | Biering et al., 1989 |
| 2007 | 2 (1) | *E. sakazakii* | No | India | Ray et al., 2007 |
| 1999-2000 | 2 | *E. sakazakii* | No | Israel | Block et al., 2002 |
| 2004 | 5 (1) | *E. sakazakii* | NA | New Zealand | NZFSA, 2009 FAO/WHO (2006) |
| 1991 | 2 | *not specified* | NA | New Zealand | NZFSA, 2009 |
| 1977-1983 | 8 (6) | *E. sakazakii* | No | The Netherlands | Muytjens et al., 1983 |
| 2008 | 2 (1) | *Cronobacter* spp. | Yes | US | CDC 2009 |
| 2001 | 11 (1) | *E. sakazakii* | Yes | US | Himelright et al., 2002 |
| 1990 | 1 | *E. sakazakii* | No | US | Noriega et al., 1990 |
| 1988 | 4 | *E. sakazakii* | Yes | US | Simmons et al., 1989 |
| 1979 | 1 | *E. sakazakii* | No | US | Monroe and Tift, 1979 |
| **Total** | **79 (18)** | | | | |

### 3.2.2 *Salmonella* spp.

Outbreaks of salmonellosis in infants fed with reconstituted PIF have been reported in a number of countries (Table 3). Investigations on an outbreak of *S.* Bredeney in 1997 in Australia, found contamination in unopened PIF cans and PIF manufacturing plants in Victoria (Forsyth et al., 2003).

**Table 3: Foodborne outbreak incidents caused by Salmonella spp. where infant formula was implicated**

| Year | No. cases | *Salmonella* spp. | Isolated from PIF | Country | Reference |
|---|---|---|---|---|---|
| 1977 | ≥24 | *S.* Bredeney | Yes | Australia | Forsyth et al., 2003 |
| 1985 | 48 | *S.* Ealing | Yes | United Kingdom | Cahill et al., 2008 |
| 1993 | >3 | S. Tennessee | Yes | United States & Canada | Cahill et al., 2008 |
| 1996 | >48 | *S.* Virchow | Yes | Spain | Cahill et al., 2008 |
| 1996-1997 | 17 | *S.* Anatum | No | France & United Kingdom | Cahill et al., 2008 |
| 2000 | 30 | *S.* London | Yes | Korea | Park et al., 2004 |
| 2004-2005 | 141 | *S.* Agona | Yes | France | Cahill et al., 2008 |
| 2008 | 6 | *S.* Give | No | France | Jourdan et al., 2008 |
| 2008 | 31 | *S.* Kedougou | No | Spain | Rodríguez-Urrego et al., 2008 |
| **Total** | **>348** | | | | |

No deaths have been reported associated with reported outbreaks of *Salmonella* in PIF. This indicates that infections in infants caused by *Salmonella* have less severe consequences than those associated with *Cronobacter* spp.

## 3.3 Prevalence in PIF

### 3.3.1 *Cronobacter* spp.

There is limited published information on the extent and frequency of *Cronobacter* testing of PIFs produced in Australia and New Zealand.

However based on a small number of retail surveys, and international data, the prevalence of this organism is expected to be very low.

**Table 4:    Prevalence of Cronobacter spp. in PIF**

| Year | Organism | No. samples tested | No. of positive samples (prevalence) | Country | Reference |
|------|----------|-------------------|--------------------------------------|---------|-----------|
| 1996 | *E. sakazakii* | 120 | 8 (6.7%) | Canada | Nazarowec-White & Farber, 1997 |
| 2002 | *E. sakazakii* | 22 | 5 (22.7%) | US | Jason, 2012 |
| 2003 | *E. sakazakii* | 82 | 2 (2.4%) | UK | Iversen and Forsythe, 2004 |
| 2005# | *E. sakazakii* | 74 | 10 (13.5%) | Indonesia | Estuningsih et al., 2006 |
| 2005# | *E. sakazakii* | 8 | 2 (25%) | Jordan | Shaker et al., 2007 |
| 2006 | *E. sakazakii* | 186 | 1 (0.5%) | Brazil | Palcich et al., 2009 |
| 2006 | *E. sakazakii* | 719 | 0 | Ireland | FSAI, 2007 |
| 2007 | *E. sakazakii* | 141 | 20 (14.2%) | The Netherlands and 35 countries | Muytjens et al., 1998 |
| 2008# | *Cronobacter* spp. | 155 | 22 (14.2%) | Brazil, Korea, Indonesia, Jordan, Malaysia, Portugal, UK | Chap et al., 2009 |
| 2010 | *C. sakazakii* | 91 | 0 | Australia | NSWFA, 2011 |

# Assumed year of the survey was conducted.

While the natural reservoir of *Cronobacter* in reconstituted PIF has not been confirmed, they have been found in a wide range of foods, household environments, livestock establishments, food factories and PIF production facilities (Kent et al., 2015; Healy et al., 2010).

An Australian survey found *Cronobacter* widely dispersed in the milk powder production environment with 32% of 298 environmental samples from five processing plants, positive for *Cronobacter*. The study suggested the wide distribution of *Cronobacter* within the processing environment may have been assisted by the movement of air, milk powder and personnel (Craven et al., 2010). A study conducted in Germany found approximately 94% of samples positive for *Cronobacter* in a PIF processing environment were attributed to powder collected from throughout the processing environment (Reich et al., 2010).

According to Holý and Forsythe (2014), general prevalence of *Cronobacter* in PIF varies between 2–14%, with reported contamination levels generally less than 1 cell per 100 g. Contamination levels exceeding 1 cell per 1 g of PIF have so far not been reported. In a review of nine surveys published between 1997 and 2009, the reported prevalence of *Cronobacter* in PIF ranged from zero in an Irish survey, to 25% in a Jordan survey (Table 4). No *C. sakazakii* was found in 91 PIFs and three ready-made formulas surveyed by the New South Wales Food Authority in 2010 (NSWFA, 2011).

### 3.3.2    *Salmonella* spp.

Limited information exists on the prevalence of *Salmonella* in PIF. No *Salmonella* was detected in 20 samples of PIF that were tested in a South Australian survey conducted in 2010 (Thompson, 2010). Similarly, *Salmonella* was not found in a survey of 91 samples of PIF and 3 ready-made infant formulas conducted in New South Wales in 2009/2010 (NSWFA, 2011).

Surveys conducted in The Netherlands (n=141 from PIF collected from 35 countries) (Muyjens et al., 1988), the United Kingdom (n=82) (Iversen & Forsythe, 2004), Indonesia (n=74) (Estuningsih et al., 2006) and Ireland (n=719) (FSAI, 2007), all found no *Salmonella*. These survey outcomes are consistent with the observation by FAO/WHO (2006) and Kent et al. (2015), that *Salmonella* in PIF is rarely detected. If present it generally occurs in very low numbers.

# 4 International risk assessments

## 4.1 Key risk assessment findings

The FAO/WHO expert meetings held in 2004 and 2006 found that:

- *Enterobacter sakazakii (Cronobacter* spp*.)* and *Salmonella* were classified as category "A" organisms (clear evidence of causality to illness)
- all infants (<12 months of age) are at particular risk for *Cronobacter* infections
- amongst infants, neonates and infants less than 2 months of age, are at the greatest risk of *Cronobacter* infection
- immunocompromised infants are more susceptible to *Cronobacter* infection
- reconstituting PIF with mixing water at a temperature of >70°C, would provide a high level of protection (due to rapid inactivation of the organism at this temperature).

In addition, the 2006 FAO/WHO expert meeting reviewed a quantitative risk assessment model developed to estimate the relative risk that *Cronobacter* posed to infants from intrinsically contaminated PIF. The meeting found that the model adequately predicted:

- use of 70°C mixing water to reconstitute PIF reduces the risk of *Cronobacter* infection significantly
- reconstitution of PIF with 40-50°C water; holding the bottle containing the reconstituted PIF at room temperature; and long feeding periods, are considered conditions that would lead to an increased relative risk of *Cronobacter* infections in infants fed PIF.

### 4.1.1 *Enterobacter sakazakii (Cronobacter* spp.) in powdered follow-up formula

The 2008 expert meeting considered the value of establishing a microbiological criterion for *Cronobacter* in FUF. While the scientific evidence identified all infants (<12 months) as the population at risk, neonates (<28 days), immunocompromised and infants less than 2 months were the group at greatest risk. As such, Codex did not propose criteria for *Cronobacter* in follow-up formula.

### 4.1.2 Sampling plan

The 2006 expert meeting also provided guidance on how a microbiological criterion could be used to reduce relative risk, by providing examples of how effectively different sampling plans are able to reject lots with elevated levels of contamination and the corresponding predicted reduction in relative risk.

In the risk assessment model, calculation of rejection rates and relative risk reductions requires data for three variables: mean log concentration (MLC); between-lot standard deviation ($\sigma_b$); and within-lot standard deviation ($\sigma_w$)

Contamination levels were informed by prevalence data from studies conducted from 1998-2004 that indicated a prevalence of *Cronobacter* in PIF of 1.4% (272/29,229). Within the data, sample sizes ranged from 10 to 1332 g with a mean concentration of *Cronobacter* in the positive samples of $-3.84 \pm 0.70$ $\log_{10}$ cfu/g (equivalent to a mean of 1.44 cfu/10 kg), and a range of $-5.39$ to $-2.79$ $\log_{10}$ cfu/g.

Using the risk assessment model, a total of 162 sampling plan scenarios were performed encompassing the variable combinations of three values for MLC (-5, -4 and -3 log[cfu/g]), two $\sigma_b$ (0.5 and 0.8), three $\sigma_w$ (0.1, 0.5 and 0.8) and nine sample plans (1 and 10 g sample sizes and 3, 5, 10, 30 or 50 samples).

Key findings included:

- the higher the MLC, the greater the rejection rate
- the higher the between-lot variation ($t_b$), the greater the rejection rate
- the larger the sample size, the greater the rejection rate
- the higher the sample number, the greater the rejection rate
- changes in the within-lot variation ($\sigma_w$), does not impact on the rejection rate.

In practice, a higher rejection rate means a greater level of risk reduction for *Cronobacter* infections resulting from consumption of contaminated PIF. In order to reduce the potential for rejection of product, PIF manufacturers should focus on ensuring the MLC is low and reducing the between-lot variability.

The 2006 FAO/WHO expert meeting did not recommend any specific microbiological criteria or sampling plans.

### 4.1.3 Microbiological criteria

In 2008, the CCFH developed microbiological criteria for *Cronobacter* and *Salmonella* in PIF to accompany the *Code of hygienic practice for powdered infant formulae for infants and young children – CAC/RCP 66 - 2008* (Codex, 2008). The criteria were established based on the evidence presented by the three FAO/WHO expert meetings.

The criteria are applied to finished/packaged PIF on a lot-by-lot basis. Codex prescribed that a lot failing to meet the criteria must be prevented from release for human consumption or recalled if it had already been released. In addition, a follow-up assessment must be conducted to determine the cause of the contamination and appropriate corrective actions to prevent similar contamination occurring in the future.

The criterion for *Cronobacter* was presented in a two-class sampling plan with 30 samples of 10 g each per batch of finished PIF. The reference method of detection is ISO/TS 22964:2006 and its latest version, *Milk and milk products — Detection of* Enterobacter sakazakii. Other validated methods that provide equivalent sensitivity, reproducibility and reliability to ISO/TS 22964:2006 can be applied. *Cronobacter* should not be detected in any of the 30 samples. With this sampling plan, *Cronobacter* is detectable at a mean concentration of 1 cfu/340 g, with a standard deviation of 0.8 and probability of detection at 95%.

A criterion for *Salmonella* in both PIF and FUF was presented in a two-class sampling plan with 60 samples of 25 g each per batch of finished PIF or FUF. The reference method of detection is ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of* Salmonella *spp.* Other validated methods that provide equivalent sensitivity, reproducibility and reliability to ISO 6579 can be applied.

*Salmonella* should not be detected in any of the 60 samples. Under this sampling plan, *Salmonella* is detectable at a mean concentration of 1 cfu/526 g, with a standard deviation of 0.8 and probability of detection at 95%.

The underlying assumption for these criteria is that the history of the lot is unknown, and the criteria are being used on a lot-by-lot basis. In instances where the history of the product is known (e.g. the product is produced under a fully documented HACCP system), alternate sampling criteria involving between-lot process control testing may be feasible.

### 4.1.4    Summary

While a range of potential pathogenic microorganisms were initially considered by the FAO/WHO expert meetings (Table 1), *Cronobacter* spp. (formally *E. sakazakii*) and *Salmonella* spp. were identified as having clear evidence of a causal association with illness in infants fed PIF.

All infants (<12 months of age) were identified as the population at particular risk for *Cronobacter* infections. Among this group, those at greatest risk are neonates (<28 days), particularly pre-term, low-birthweight (<2500 g), and immunocompromised infants, and those less than 2 months of age. A significant proportion of *Cronobacter* infections in this population group can lead to severe consequences including meningitis, necrotising enterocolitis, septicaemia and death.

The presence of *Cronobacter* in PIF and in the general environment, including on humans, as well as the high affinity that *Cronobacter* cells have to hydrophobic surfaces, enable two routes of exposure to be postulated. Intrinsic contamination occurs where *Cronobacter* originates from the PIF, while extrinsic contamination occurs from *Cronobacter* originating from the environment such as hospital air, infant enteral feeding tubes or bottles of infant formula (Holý and Forsythe, 2014).

The current understanding of factors involved in *Cronobacter* infection in infants includes:

- PIF is not a sterile product
- PIF, the environment, and the apparatus used in reconstituting and feeding the reconstituted PIF to infants, can be contaminated with *Cronobacter*
- PIF reconstituted with water at a temperature of less than 70$^{\circ}$C will not inactive *Cronobacter*
- the time period from reconstituting the PIF to completion of feeding may allow growth of any *Cronobacter* present in the reconstituted PIF to levels that can cause illness
- *Cronobacter* infections in neonates and infants can lead to severe consequences such as meningitis, necrotising enterocolitis and septicaemia.

PIF with a low mean log concentration of *Cronobacter* and low between-lot variations during manufacture, present a lower risk to infants and has a lower tendency for rejection when a microbiological criterion for *Cronobacter* is applied.

## 4.2    Additional published reviews

A number of more recent reviews have been published on *Cronobacter* and PIF since the work of the FAO/WHO. This has been driven partly by a rapid expansion in knowledge on *Cronobacter* and partly by increased awareness of the serious health impact of *Cronobacter* infection in young infants.

## 4.3    Review Papers

- *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen (Healy et al. 2010)
- persistence and survival of pathogens in dry foods and dry food processing environments (Beuchat et al. 2011)
- prevention of invasive *Cronobacter* infections in young infants fed powdered infant formulas (Jason 2012)
- *Cronobacter* spp. in powdered infant formula (Norberg et al. 2012)
- *Cronobacter* spp. as emerging causes of healthcare-associated infection (Holý and Forsythe, 2014)
- *Cronobacter* species contamination of powdered infant formula and the implications for neonatal health (Kalyantanda et al. 2015)
- novel approaches to improve the intrinsic microbiological safety of powdered infant milk formula (Kent et al. 2015)
- new insights into the emergent bacterial pathogen *Cronobacter* (Forsythe 2015).

Key information from these review papers includes:

- advances in taxonomy of *Cronobacter* spp.
- improved understandings on the physiology of *Cronobacter* spp.
- new insights into the virulence factors of *Cronobacter* infections
- further understanding on the epidemiology of PIF-associated *Cronobacter* infections.

# 5    Conclusion

The FAO/WHO expert consultations identified the organisms of concern in infant formula. They concluded that intrinsic contamination of powdered infant formula with *E. sakazakii* (*Cronobacter* spp.) and *Salmonella* spp. had been a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequelae and death. Although the rate of incidence is low, neonates and immunocompromised infants are at the greatest risk of *Cronobacter* infection.

Relevant control measures throughout the food chain were also identified and included consideration of labelling and preparation risk reduction strategies and the establishment food safety criteria for *Cronobacter* and *Salmonella*, although they did not recommend any specific criteria or sampling plans.

CCFH revised the *Code of hygienic practice for powdered infant formulae for infants and young children – CAC/RCP 66 - 2008* to introduce microbiological criteria for *Cronobacter* spp. in PIF, and re-confirmed the application of microbiological criteria on *Salmonella* spp. in PIF and FUF.

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