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Microbiological safety of powdered infant formula: Effect of storage temperature on risk

Safety & food technology Consultation paper

Proposal P1028—Review of the regulation of infant formula products

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Executive summary

The *Australia New Zealand Food Standards Code* (the Code) contains requirements for the manufacture, storage and use of infant formula to help ensure the microbiological safety of the product. The focus of those provisions is to ensure that the information and instructions on the label for the preparation and use of infant formula were clear and easy to follow for the general community.

In the 2016 consultation paper for this Proposal, FSANZ reviewed microbiological risk management strategies for the preparation, use and storage of powdered infant formula (PIF), with a particular focus on storage time of prepared formula, and the temperature of water used for reconstitution of powdered infant formula.

One submission to the consultation paper raised the concern that the preparation and conditions proposed by FSANZ were not sufficient to ensure the safety of powdered infant formula. It was considered that a shorter storage time than FSANZ had proposed was required to restrict pathogen growth in formula following reconstitution.

As in previous work, FSANZ has used the risk assessment model developed by the Food and Agriculture Organization/World Health Organization (FAO/WHO) to estimate the relative risk that the main microbiological hazard identified—*Cronobacter* spp. (formerly known as *Enterobacter sakazakii*)—poses to infants from intrinsically contaminated PIF.

To address the concern raised, a wider range of preparation and storage times were analysed against a baseline scenario of reconstitution of PIF with water at 37°C, followed by immediate consumption. The temperature of water used to reconstitute formula was varied between 10–50°C, and the duration of cooling and storage at 6°C was varied between 0-24 hours.

The modelling indicates that the temperature of the water used for reconstitution of PIF has a greater influence on risk than the time reconstituted formula spends under refrigerated storage. As the water temperature for PIF reconstitution is increased above 40°C, the relative

risk of illness increased between 5–15 fold compared to the baseline, due to *Cronobacter* spp. being able to grow while the reconstituted formula cooled to refrigeration temperature (6°C). No difference in relative risk was observed for PIF stored under refrigeration for 24 hours compared to 4 hours when it was reconstituted with water at 20°C or lower.

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1 Introduction

Provisions in the *Australia New Zealand Food Standards Code* (the Code) provide risk management measures relevant to the manufacture, storage and use of powdered infant formula (PIF) to help ensure the microbiological safety of the product.

The risk management approach when developing the requirements for preparation and use of infant formula focused on ensuring the information and instructions on the label were clear and easy to follow for the general community. The intent was to ensure that the essential risk reduction elements were clear: e.g. use of potable water that has previously been boiled; and advice on refrigeration of reconstituted formula for up to 24 hours and on discarding unused/leftover formula.

Risk reduction strategies aimed at reducing microbiological hazards related to the preparation, use and storage of powdered infant formula (PIF) were discussed in the FSANZ consultation paper to Proposal P1028 (refer to Attachment A2.1—*Microbiological safety of powdered infant formula*—of Supporting Document 2—*Safety and Technology*[1]). Two main issues were explored: storage time of prepared formula, and the temperature of water used for reconstitution of powdered infant formula.

In its submission to the consultation paper, the New Zealand Ministry for Primary Industries (NZMPI) raised the concern that, from a risk perspective, a shorter storage time than FSANZ had proposed was required to restrict pathogen growth in formula following reconstitution.

1.1 Scope and hazard identification

This paper further considers the effects of refrigerated storage time on the relative risk of prepared infant formula. The risk is considered by reference to the potential for growth of *Cronobacter* spp. (formerly known as *Enterobacter sakazakii*) and subsequent infection of infants.

Cronobacter spp. was identified as the hazard of greatest concern by the initial FAO/WHO expert meeting on the safety of infant formula (FAO/WHO, 2004) on the basis of its high prevalence in infant formula and demonstrated links to infection and illness in infants, including severe disease leading to serious developmental sequelae and death. The risk to infants is increased by the ability of *Cronobacter* spp. to resist desiccation, osmotic stress and acids; its ability to survive for at least two years in PIF and then grow rapidly upon reconstitution; and its capacity to adhere to hydrophobic surfaces—including silicone, latex, polycarbonate, stainless steel, glass and polyvinyl chloride—and to form biofilms in enteral feeding tubes.

Cronobacter spp. can grow at temperatures in the range 5–47°C and at pH levels as low as 3. Optimum temperature for growth is approximately 37–39°C, and it is rapidly inactivated at temperatures above 70°C (FAO/WHO, 2004).

[1] https://www.foodstandards.gov.au/code/proposals/Documents/P1028-Consult-SD2-SafetyTechnology.pdf

2 Background

2.1 Overview of assessments

2.1.1 Australia

The characteristics of *Cronobacter* spp. and the risk assessment model developed by the Food and Agriculture Organisation/World Health Organisation (FAO/WHO) to estimate the relative risk that *Cronobacter* poses to infants from intrinsically contaminated PIF are described in Proposal P1039—Microbiological Review of Infant Formula—in supporting document 1 (SD1)—*Scientific evidence informing the proposed microbiological criteria for infant formula*[2].

To inform work for Proposal P1028, FSANZ used the FAO/WHO risk assessment model[3] to investigate the impact of different preparation and storage conditions on the relative risk of *Cronobacter* spp. infection in infants. Three different storage time scenarios (2, 4 and 24 hours) were modelled using three different reconstitution temperatures (10, 30 and 45°C)[4]. Prepared formula left unrefrigerated (2 hours at ambient temperatures) was also modelled for the different reconstitution temperatures. The baseline for comparison was a scenario of reconstitution at 45°C with no storage time (i.e. immediately consumed). For all refrigeration scenarios modelled, there was minimal difference in the relative risk reduction from the baseline scenario.

2.1.2 New Zealand

In 2015, the NZMPI published a report (Campbell & Soboleva, 2015) reviewing the United Kingdom Food Safety Authority's (UKFSA) use of the FAO/WHO risk assessment model for *Cronobacter spp.* (Advisory Committee on Microbiological Safety of Food, 2013) and providing additional information relative to the risk of *Cronobacter* spp. in infant formula.

Key findings include:

- From a risk-based perspective, the most relevant scenario assessed for growth of *Cronobacter spp.* was prolonged storage of bottles of PIF without refrigeration, after reconstitution with water at ambient temperature;
- Use of 70°C water for reconstituting PIF can introduce concerns including risk of scalding, quality and nutritional problems;
- Boiling and then cooling water for 30 mins results in a range of temperatures, depending on the volume of water boiled, which influences the lethality delivered to bacteria; and
- Refrigeration temperatures averaged 5.2°C in New Zealand; although a significant proportion (34%) had a mean temperature >6°C.

The report makes a number of recommendations:

- Sources of water that can be used for reconstituting PIF:
    - Drinking water sourced from the cold tap, sterilised by boiling
    - Boiled water kept in a sterilised air tight container at room temperature for up to 24 hours
- Water temperature at point of mixing with PIF:

[2] https://www.foodstandards.gov.au/code/proposals/Documents/P1039%20Micro%20criteria%20for%20infant%20formula%20SD1%20Scientific%20evidence.pdf
[3] http://tools.fstools.org/esakmodel/ESAKRAModelWizard.aspx
[4] The temperature of boiled water used to reconstitute the powdered infant formula

    - Previously boiled water, cooled to room temperature
- Maximum duration of keeping reconstituted formula at room temperature
    - PIF feed should be fed to an infant immediately after preparation
    - PIF should be kept for no more than two hours at room temperature
- Maximum duration of storage of reconstituted formula under refrigeration
    - No more than four hours, in body of refrigerator (i.e. not in door shelves)
    - Only amount required for one feed should be re-warmed
- When travelling
    - Take cooled boiled water in a sterile bottle and add measured PIF at time of feed
    - An alternative option is a sterile liquid infant formula (ready to drink feeds)

2.1.3 United Kingdom

In 2010, the UKFSA investigated the attitudes and behaviours of UK consumers and caregivers in the preparation, handling and storage of PIF inside and outside the home (Redmond and Griffiths, 2013).

Chapter 7 of the report describes time-temperature data from 'in-use' reconstituted PIF feeds prepared in advance of feeding, inside and outside the home. Data loggers (on bottles, on benchtops and in refrigerators) and questionnaires were used to obtain and record information. The growth of *Cronobacter* spp. based on these 'in-use' practices was then modelled using the FAO/WHO risk assessment model for *Cronobacter* and reported in Chapter 8 of the document.

A number of scenarios were modelled, including: three different scenarios for preparation in day nurseries; three scenarios for preparation in parents' homes; and the UK standard 'best practice' preparation recommended by the FSA[5] (refer to section 8.3.2 Predictions, Chapter 8 in Redmond and Griffiths, 2013).

The baseline scenario was chosen to be reconstitution of PIF with lukewarm water (37^{o}C), followed by immediate consumption.

Day Nurseries
Nursery A: prepared all formula on-site (70^{o}C, 60^{o}C, 50^{o}C) followed by 5hrs refrigerated storage prior to warming and feeding.
Nursery B: used pre-prepared formula supplied by the parent—either prepared the night before or that morning, followed by refrigerated storage on receipt.

Parents
Parent 1: prepared with ambient temperature water and stored on the bench for 11 hrs
Parent 2 and 3 (various times/temperatures): prepared with boiled water, left to cool on the bench, then refrigerated prior to warming and feeding.

Results
Scenarios where the temperature of the water used for reconstitution was greater than 70^{o}C produced a relative risk reduction the same as the 'best practice' scenario (>1 x 10^{5}). All scenarios with reconstitution at 40^{o}C and 60^{o}C led to a relative risk reduction that was in the same order of magnitude as the baseline scenario (0.34-1.55). Storage in the nurseries' refrigerator did not have a large effect on the relative risk compared to fresh preparation of each bottle with lukewarm water followed by immediate consumption.

[5] 'Best practise' scenario consists of reconstitution of the PIF at 70^{o}C on the bench at 20^{o}C, rapid cooling to 37^{o}C in 15 minutes followed by immediate consumption.

The scenario with the highest risk was the 'Parent 1' scenario, where PIF had been prepared with 25°C water then stored at ambient temperature on the bench for 11 hours (relative risk reduction of 0.00819, which is 122 fold higher than baseline).

3 Assessment

3.1 Reconstitution temperature

A key finding of the FAO/WHO expert meetings on infant formula held in 2004 and 2006, was that reconstituting PIF with mixing water at a temperature >70˚C, would provide a high level of protection (due to rapid inactivation of the organism at this temperature). It was also recognised that the time period from reconstitution of the PIF to completion of feeding may allow growth of any *Cronobacter* present to levels that can cause illness.

Section IX of the *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children* (CAC/RCP66-2008; Codex, 2008) notes that the use of good hygienic practices during reconstitution, handling and feeding (including storage) is important in minimising the risk of foodborne illness. A range of hygienic measures can achieve significant risk reduction. The code of practice notes that various risk reduction strategies are provided in the WHO PIF guidelines—for example, where there is a high degree of confidence in the microbiological quality of the infant formula and adherence with good hygienic practices in the preparation, handling and use of the formula, then alternative risk reduction strategies are available which do not involve the use of water at 70°C for reconstitution.

There are a number of concerns regarding use of water at 70°C or above, including risk of scalds, destruction of nutrients, and quality considerations (see section 3.2 in Attachment A2.1 to P1028). The current recommendation in the Code is to use cooled, previously boiled water to prepare infant formula.

3.2 Storage time of prepared formula

Paragraph 2.9.1—19(3)(b) of the Code requires a direction instructing that if a bottle of made up formula is to be stored before use, it must be refrigerated and used within 24 hours. This differs from the New Zealand infant feeding guidance, which states that prepared formula may be stored at up to 4°C in the lower half of the fridge, at the back, but should be kept for a maximum of four hours (NZ Ministry of Health, 2008).

In this report, the FAO/WHO risk assessment model for *Cronobacter*[6] was again used to assess refrigerated storage of 'no more than 4 hours' and '24 hours' when all other inputs are the same.

3.3 Use of the FAO/WHO risk assessment model

The risk assessment model estimates the risk of illness from *Cronobacter* spp. posed to infants from intrinsically contaminated PIF, and does not consider post-manufacture contamination or recontamination from the preparation environment or other sources, or the impact thereof. The model describes the effect that each of the preparation and storage stages have upon the microbiological quality of the reconstituted PIF, in terms of

[6] http://tools.fstools.org/esakmodel/ESAKRAModelWizard.aspx

Cronobacter spp., and examines the risks of different preparation and handling scenarios in terms of the relative risk posed to infants.[7]

Experimental studies suggest that background levels of contamination of PIF with *Cronobacter* spp. are low, with reports in the literature suggesting typical contamination levels of less than 1 cfu/g (Muytjens, Roelofs-Willemse & Jasper, 1988).

Cronobacter spp. has an observed growth range between 5.5°C and (for some strains) 47°C (Nazarowec-White & Farber, 1997). Studies at room temperature (21°C), demonstrated a doubling time for *Cronobacter* spp. of 40-94 minutes, while decimal reduction times and z-values varied considerably between strains, i.e. D_{55} 2-49 minutes, z-values 2-14°C (Nazarowec-White & Farber, 1997).

These growth characteristics provide the opportunity for growth of any contaminating populations during the preparation of infant formula, resulting in higher levels of *Cronobacter* spp. at feeding.

The model assumes an initial contamination level of 1 cfu of *Cronobacter* spp. per serving (prior to storage and growth) in the dry product. This initial level of 1 cfu per serving is adjusted to take into account any growth or decline that may occur due to the conditions of preparation, holding and feeding, to give an estimate of the dose ingested. Dose depends not only on the variability of the level in dry PIF, but also on the variability in bottle preparation, storage conditions of the bottles, and duration of feeding that may allow the numbers of *Cronobacter* spp. to increase.

The probability of infection resulting from consumption of an estimated dose of C*ronobacter* spp. can be described by the exponential dose-response model (Haas, Rose & Gerba, 1999). The value of the dose-response parameter has not been established with any certainty, although values between 10^{-10} and 10^{-5} have been suggested (FAO/WHO, 2006). If both growth and inactivation of cells are neglected, the probability of infection for one infant during its neonatal period can be estimated to range from 1×10^{-5} (1 in 100,000) to 1×10^{-10} (1 in 10,000,000,000).

3.3.1 Model Inputs

The baseline scenario selected was reconstitution of PIF with water at 37°C, followed by immediate consumption. All other preparation parameters were assumed to occur quickly. Selecting these parameters as the baseline allows comparison of scenarios with increased and decreased risk. If the FAO/WHO recommended water temperature of 70°C had been selected, then all scenarios with reconstitution temperatures below this would have an increased risk.

The mean log concentration was the default value set by the model, as the focus is on preparation and handling scenarios. The cooling temperature selected was 6°C. In 2010, 25% of fridges surveyed in New Zealand (n=158) operated between 2-4°C, while 39% recorded temperatures between 4-6°C. Temperatures above 5.5°C may allow growth of *Cronobacter* spp., albeit slowly. Feeding duration used was 30 minutes. Feeding times of less than 15 minutes were reported in a New Zealand study (NZ Ministry of Health, 2008) for 75% of feeds surveyed.

The baseline scenario and inputs selected are consistent with those used in the UKFSA project (see Section 2.1.3).

[7] Refer to the FAO/WHO meeting report Enterobacter sakazakii and Salmonella in powdered infant formula (FAO/WHO, 2006) for a detailed description of the model.

The outputs are expressed as relative risk reduction compared to the selected baseline scenario which is set at 1.0. The outputs provide a comparison across the range of water temperatures used for reconstitution of PIF and storage times and all risk reductions are relative to the baseline.

Fixed inputs (for all scenarios):

Table A1.1: *Risk assessment model inputs*

Stage	Temperature (°C)	Duration (h)
Preparation of formula	20	0.5
Holding/Cooling	6	variable
Active rewarming/cooling	37	0.25
Feeding period	25	0.5

Variable inputs:

Temperature of water used to reconstitute formula (°C): 50°C, 40°C, 37°C, 20°C, 10°C
Duration of Holding/Cooling (h): 0, 2, 4, 12, 24

Variable inputs selected for Baseline: 37°C, 0hrs

3.3.2 Results

Table A1.2: *Model outputs presented as relative risk reduction. Baseline scenario bolded*

Reconstitution temperature	Duration of Holding/Cooling	Relative Risk Reduction*
50	24	6.59×10^{-2}
50	12	9.93×10^{-2}
50	4	0.14
50	2	0.19
50	0	0.53
40	24	0.53
40	12	0.66
37	24	0.66
40	2	0.81
40	4	0.81
20	0	1
20	2	1
20	4	1
20	12	1
20	24	1
37	**0**	**1**
37	2	1
37	4	1
40	0	1
10	0	1
10	2	1
10	4	1
10	12	1
10	24	1

* Compared to the baseline: A relative risk reduction of less than 1 represents an increased relative risk

3.3.3 Discussion

The baseline scenario assumes that no-to-minimal growth occurs during preparation and feeding. Therefore, with no growth or decline, the model predicts a probability of infection for this 'best case' scenario as between 1 in 100,000 and 1 in 10,000,000,000 per infant feeding.

There was no difference in relative risk compared to the baseline for all scenarios with reconstitution water temperatures of 20°C and 10°C; formula reconstituted with 37°C water temperature and refrigerated storage for 2 and 4 hours; and formula prepared with 40°C water with no refrigerated storage.

There was a slight increase in relative risk compared to the baseline for all refrigerated storage scenarios when PIF was reconstituted with 40°C water; and for formula prepared with 37°C water and stored for 24 hours (1.2–1.9 fold increase).

The temperature of the reconstitution water appears to have more influence on the relative risk than the time in refrigerated storage. Both the 40°C for 12hr and 37°C for 24hr scenarios had the same relative risk reduction (0.81: i.e. a 1.2-fold increase in risk of illness). This would result in a shift in the probability of infection from between 1 in 100,000 and 1 in 10,000,000,000 to between 1.2 in 100,000 and 1.2 in 10,000,000,000.

As the water temperature for PIF reconstitution increased above 40°C, the relative risk increased between 5 to 15 fold (0.19 to 6.59×10^{-2}) compared to the baseline. This is due to *Cronobacter* species being able to grow while the temperature of the reconstituted formula cooled to 6°C. Once the temperature of the reconstituted formula reaches refrigeration temperature, there will be no-to-limited growth of the organism.

All bar two scenarios (50°C for 12 and 24hrs) had a relative risk within the same order of magnitude as the baseline scenario.

3.3.4 Conclusion

Based on the analysis, there is no difference in relative risk for prepared infant formula stored under refrigeration for 24 hours compared to 4 hours when reconstituted with water at a temperature of 20°C or lower.

4 Summary and Conclusion

The key risk reduction measure in the preparation, handling and storage of PIF is minimising the time the formula spends within the temperature range which permits growth of *Cronobacter* spp.

The modelling undertaken indicates that the temperature of the water used for reconstitution of PIF has a greater influence on risk than the time reconstituted formula spends under refrigerated storage. No difference in relative risk was observed for PIF stored under refrigeration for 24 hours compared to 4 hours when it was reconstituted with water at 20°C or lower.

5 References

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