New Zealand Food Safety
Haumaru Kai Aotearoa

Microbiological safety of reconstituted infant formula:

Effect of water quality and storage temperature on risk

New Zealand Food Safety Technical Paper No: 2021/06

Prepared for MPI
by Dr Tanya Soboleva, Food Science & Risk Assessment Directorate

ISBN No: 978-1-99-100312-6 (online)
ISSN No: 2624-022X (online)

26 February 2021

New Zealand Government

Ministry for Primary Industries
Manatū Ahu Matua

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Requests for further copies should be directed to:

Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at http://www.mpi.govt.nz/news-and-resources/publications/

Contents

Page

1 Executive Summary

Reconstituted infant formula is not a sterile product. The manufacture of powdered infant formula (PIF) does not include a processing step that eliminates all microbiological hazards that may be present in the raw materials. However, good manufacturing practices and control measures during preparation, storage and handling of the formula by parents and caregivers minimise potential risks to infants associated with consumption of infant formula.

This paper concentrates on two aspects of safe preparation and storage of the formula, namely (a) use of pre-boiled water for reconstitution of the PIF and (b) time of storage of reconstituted PIF under refrigeration.

Current New Zealand recommendations are to boil municipal supply water for reconstituting PIF up until the infant is three months old. This contrasts to the Australia New Zealand Food Standards Code (FSC) and World Health Organization (WHO) recommendations to use pre-boiled water for reconstituting all PIF

The FSC currently legally requires that packages of PIF be labelled to advise using water that has previously been boiled. Research on parent and caregiver habits has identified a high level of reliance on label use direction in preparing PIF. Consequently, aligning the general New Zealand advice with that mandated to be on PIF labelling would ensure a single source of truth for parents and caregivers on use of pre-boiled water.

Normally microbiological contaminants are not present in controlled water supplies, so the advice to boil town supply water for infants above 3 months is largely precautionary.
Standardizing the New Zealand requirements with those of the FSC and WHO to recommend use of pre-boiled water for reconstitution of PIF to a later age will, however, add another level of protection against potential contamination in reconstituting PIF, and ensure consistent advice on preparation.

Current advice for reconstituted formula is to store in the refrigerator for up to 4 hours.
By comparing the relative risk of formula refrigerated for 4 and 24 hours there is no increase in risk for product reconstituted as recommended and then stored refrigerated for 24 hours. The general recommended temperature setting for refrigerators is 2- 4ºC. Consequently, it is appropriate to recommend 24 hours refrigerated storage at temperatures at or below 4ºC, this would also ensure a level of protection from temperature fluctuations of ±4ºC that could occur within older refrigerators.

2 Introduction

The manufacture of powdered infant formula (PIF) does not include a processing step that specifically eliminates all microbiological hazards that could be present in the raw materials, e.g. spore-forming organisms in milk such as *Bacillus* spp or *Clostridium* spp., survive pasteurization, or may infrequently cross-contaminate from the processing environment, e.g. *Cronobacter* spp.

Post-processing minimisation of microbiological risks to infants associated with consumption of infant formula, therefore, requires control measures during preparation, storage and handling of the formula by parents and caregivers.

The Australia New Zealand Food Standards Code (FSC) Standard 2.9.1 – Infant Formula Products contains provisions for infant formula that 'provide a group of risk management measures relevant to the manufacture, storage and use of infant formula to help ensure the microbiological safety of the product'[1]. Similarly, measures are described by the World Health Organization (WHO)[2], New Zealand Ministry for Primary Industries (MPI) along with KidsHealth NZ and HealthED[3], and New Zealand Ministry of Health (MoH) along with HealthEd[4].

Labelling of PIF packaging is an important source, if not the primary source, of information for parents and caregivers on the correct handling of infant formula to ensure their baby's safety. The FSC specifies labelling requirements for directions for the safe preparation, use and storage of infant formula which inform parents and caregivers on how to prepare and store infant formula to ensure they keep their babies safe.

While most of the measures described in the FSC and in WHO and New Zealand guidelines are similar, some differences exist (Table 1).

Table 1 Measures for preparation, storage and handling of infant formula that differ among international and New Zealand standards and guidelines.

Measure	Food Standards Code Standard 2.9.1	WHO	New Zealand
Boiling of preparation water	19(3)(c): The label of infant formula products must include directions that instruct that potable, previously boiled water should be used.	Boil some safe water. Pour the correct amount of boiled water (no cooler than 70ºC) into a cleaned and sterilized feeding bottle, and add powder.	Until the baby is 3 months old, boil water and cool to room temperature. Water straight from the tap can be used for infants >3 months old and who live in a city or town. Always boil and cool bore and tank water for use in infant formula until the child is >18 months old.
Storage of prepared formula	19(3)(b): If a bottle of made up formula is to be stored prior to use, it must be refrigerated and used within 24 hours.	Prepared formula should be refrigerated at no higher than 5ºC, used within 24 hours, and discarded if not.	Prepared formula can be stored at room temperature for no more than 2 hours, or refrigerated at <4ºC for no more than 4 hours.

[1] https://www.legislation.gov.au/Details/F2017C00332
[2] http://www.who.int/foodsafety/publications/micro/PIF_Bottle_en.pdf
[3] http://www.mpi.govt.nz/food-safety/food-safety-for-consumers/food-safety-for-babies/
[4] https://www.health.govt.nz/your-health/pregnancy-and-kids/first-year/helpful-advice-during-first-year/formula-feeding

Two questions therefore, arise:

1. Should the New Zealand advice to boil town supply water used for reconstituting PIF change to harmonise with that of the FSC and WHO?

2. Is it safe to store prepared infant formula in a fridge for up to 24 hours, and if so at what temperature?

Notwithstanding, the considerations of this report, both MPI and the MoH support the view that mothers should, when possible, exclusively breast feed babies until they are ready for and need extra foods. It is recognized that each baby differs, but transition to solid foods generally occurs around six months. This timeframe is similar for babies that cannot be breastfed and require infant formula. Activity, physiological and developmental patterns also change when infants reach six months of age.

The formulation of products for infants differs depending on the age of the baby/infant. Infant Formula is generally recommended up to the age of six months. After 6 months of age the definitions change to Follow-Up Formula (6-12 months) and Growing-Up Formula (12-36 months). Consideration is given in this paper only on applying changes to the advice for infant formula (0-6 months).

3 Reconstitution of Powdered Infant Formula

The current New Zealand recommendation is that non-town supply bore and tank water be boiled before use for reconstituting PIF for infants up to the age of 18 months. For reconstituting PIF from the town supply the advice is to boil the water up until the infant is three months of age, unless there are medical reasons to continue, such as the infant being immunocompromised.
In contrast, the FSC and WHO guidelines state that boiled water should be used for the preparation of infant formula, without describing any exemptions. The Codex Standard for infant formula and formulas for special medical purposes intended for infants, recommends products in powder form should be reconstituted with water that is safe or has been rendered safe, by previous boiling.

There is therefore a potential that New Zealand infants of 3-6 months of age are less protected from the risk of exposure to pathogens through infant formula than those following the FSC standard, Codex standard or WHO guideline.

3.1 LEVEL OF PROTECTION FOR INFANTS 3-6 MONTHS

To assess the risk for infants 3-6 months of age it is important to consider whether they require a similar level of protection to be in place as for infant 0-3 months of age.

An infant's intestinal immune functions are underdeveloped at birth, with consequently a greater susceptibility of developing a foodborne illness. Maturation of the immune system during the first six months of life is critical (Milani *et al*, 2016; Nguyen *et al*, 2016). While the scientific literature shows that consumption of breast milk aids in the maturation of the infant's intestinal defences, it is uncertain whether infants fed formula have the same or a lesser level of protection against intestinal infections.

The gut microbiota play an important role in activating the developing immune system and inducing protective immune responses against *E. coli* and *Salmonella* (Zeng *et al*, 2016). The MoH *Report on Maternity* between 2006 and 2015[5] reported a significant increase in the proportion of elective Caesarean sections (CS) and a significant decrease in vaginal births. It is well established that the CS mode of delivery derails the normal development of the gut microbiome at infancy (e.g. A.L. Kozyrskyi et al, 2017). The influence of the delivery mode persists for months after birth (Penders et al, 2006) and could be a reason for the increased susceptibility to pathogens observed and present in 64-82% of CS-delivered infants (Watson et al, 2006). There is no scientific evidence that at 3 months of age the gut microbiota is sufficiently developed and immune responses are stronger than in infants < 3 months of age.

There is very limited evidence available from which to draw the conclusion that at 3 months of age the resistance of infants to infection increases sufficiently to remove the additional level of protection,

[5] http://www.health.govt.nz/system/files/documents/publications/report-on-maternity-2015.docx

suggesting in some respects that 3 months of age may not be the most appropriate age for providing changing advice on PIF preparation practices.

There is considerable uncertainty in being able to make a judgment that susceptibilities to foodborne illness will be different or comparable between infants of 0-3 months of age and those 3-6 months of age. The uncertainty in the data regarding susceptibility to infections in 3-6 month old infants suggests the approach currently recommended for infants 0-3 months of age should be precautionarily extended to cover infants 0-6 months of age.

3.2 DRINKING WATER STANDARDS IN NEW ZEALAND

It is most likely that water used for the reconstituting PIF in New Zealand homes or institutions will come from sources intended for drinking water, e.g. taps. Drinking water, irrespective of its source must be potable and comply with the *Drinking Water Standards for New Zealand 2005 (Revised 2008)* (DWSNZ)[6]. The DWSNZ functions to protect public health, giving a high priority to health risks arising from microbial contaminants because they can lead to rapid and major outbreaks of illness. As a consequence, generally town water supplies will be of low microbial risk to all the New Zealand population.

The DWSNZ controls, however, do not purport to specify a concentration of a microbial contaminant at which risk is eliminated because a degree of uncertainty over the magnitude of the risk always exists. They simply minimise the risk by minimising the probability that the potable water is contaminated. Although unlikely to be necessary in most cases there is no doubt that boiling water prior to use for reconstituting PIF adds a robust control step for pathogens.

3.3 PREPARATION INSTRUCTIONS ON PACKAGING

Parents/caregivers usually follow advice on the labels of PIF packaging. A 2008 survey of consumers' behaviour states that "all participants get information that they know is relevant and useful from the formula tin' and there is a high degree of trust from all participants in the information provided on the tin (Winstanley & Cressey, 2008). A contemporary survey of caregiver beliefs identified 92% of all respondents (n=1333) viewed it as extremely important or important to follow label preparation instructions, and only 1% of respondents noted they had never used the preparation instructions. (NZFS, 2020). Specifically, 79% of respondents reported using only boiled water every or most of the time in reconstituting PIF (NZFS, 2020).

The FSC currently requires that the label on PIF packaging includes directions that potable, previously boiled water, should be used in the preparation of infant formula. The FSC does not provide exemptions dependent on the age of the infant, water supply, or whether the infant is partially breast fed or not.

As a result, it is a legal requirement that labels on all PIF packaging available in New Zealand recommend using previously boiled water for reconstituting the PIF. All parents in an observational study in the UK boiled water before using it to reconstitute PIF (Redmond & Griffin, 2013).

Recommending a single preparation instruction for the full feeding period with PIF ensures consistency in advice with the labelling requirements, as well as limiting any risks or confusion that could be introduced as parent/ caregivers change from one set of instructions to alternative guidance.

3.4 CONCLUSION

The FSC legally requires that PIF packaging be labelled with used directions for reconstitution only with previously boiled water. Consumer research shows most parents and caregivers consider it important to follow label preparation instructions, and very few did not refer to these. Standardizing all New Zealand infant feeding guidance with those of the FSC and WHO would harmonize recommendations with labelling already on product packaging and ensure a single source of truth for parents and caregivers.

[6] https://www.health.govt.nz/system/files/documents/publications/drinking-water-standards-2008-jun14.pdf

The advice to use boiled water is currently applied to advice for infants 0-3 months of age. Further to ensuing consistent advice to parents and caregivers extending the boil water recommendation to infants 3- 6 months of age aligns with a precautionary approach to account for the uncertainty in susceptibility of these infants to foodborne illness.

4 Storage of Prepared Infant Formula

A 2015 review by Campbell & Soboleva[7] recommended that reconstituted PIF should be used immediately after preparation. However, prepared infant formula may not all be consumed in one feeding and may require storage for a short time. This storage would generally be no longer than two hours at room temperature or, if longer, under refrigeration.

The temperature and time of refrigerated storage determine the microbiological safety of the prepared infant formula. New Zealand's MPI and MoH specify storage from 2-4°C (at the back of the fridge) for no longer than 4 hours.

However, the FSC/WHO differ in their recommendations. The FSC specifies refrigeration for no longer than 24 hours, and the WHO recommends storage at <5°C for no longer than 24 hours. More broadly the Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children recommends any formula prepared for later use should be refrigerated immediately following reconstitution and used within 24 hours.

4.1 HOUSEHOLD REFRIGERATION TEMPERATURES

In 2010, the New Zealand Food Safety Authority surveyed temperatures in 158 household refrigerators across New Zealand. The temperatures were also monitored after assistance with any refrigerator adjustments and an educational campaign[8]. While only 37% of refrigerators were running at <4°C, after adjustment this improved to 50% operating at <4°C and 87% operating at <6°C. Worn seals were the most common defect in the refrigerators running just above the ideal temperature. *Cronobacter* will only grow slowly at 6ºC and is not considered a health risk in such refrigerators.

A third of the refrigerators unable to be run at temperatures <6ºC were over 20 years old. It is considered likely with the improving manufacturing quality that many of these refrigerators will now have been replaced with modern, better temperature controlled, refrigerators.

4.2 MICROBIAL GROWTH AFTER 24 HOURS STORAGE

To assess the impact of increasing a 4 hour storage time at 6ºC to 24 hours the FAO/WHO risk assessment model was used. The output from this model is then benchmarked against the risks present with a feeding immediately after preparation to inform whether different storage times increase the relative risk.

Current New Zealand guidance recommends using water that has been boiled and cooled to room temperature to prepare formula for consumption. To cover the typical range of room temperatures in New Zealand 20ºC and 30ºC were considered for reconstituting PIF. For each temperature the relative risk was compared with the baseline scenario that assumes immediate feeding after preparation. It was assumed that prepared formula was stored in individual bottles and each was kept refrigerated prior to use and all inputs other than storage time are the same. The feeding duration used was 30 minutes. [9]

[7] http://www.health.govt.nz/our-work/environmental-health/food/microbiological-safety-reconstituting-powdered-infant-formula
[8] http://www.foodsafety.govt.nz/elibrary/Refrigerator_Temperature_Survey.pdf.
[9] According to the Food Standards Agency survey feeding duration was <15 minutes for 74% of feeds with a maximum feeding time of 45 minutes (Redmond and Griffith, 2013)

The outputs of the model for storage at **6ºC** are presented below

Reconstitution temperature	Duration of holding (hours)	Relative risk reduction*	Proportional increase in risk
20ºC	**0**	**1**	
	4	1	0
	24	1	0
30ºC	**0**	**1**	
	4	1	0
	24	1	0

*Compared to the baseline, which is immediate feeding. A relative risk reduction of less than 1 represents an increased relative risk.

This means that there is no difference in relative risk for storage for 4 or 24 hours at 6ºC compared to immediate consumption of the formula reconstituted at 20ºC or 30ºC. Similarly, as demonstrated in the table below, storage at 8ºC for 4 or 24 hours will result in the level of risk for formula reconstituted at 20ºC or 30ºC, i.e.

Reconstitution temperature	Duration of holding (hours)	Relative risk reduction	Proportional increase in risk
20ºC	**0**	**1**	
	4	1	0
	24	1	0
30ºC	**0**	**1**	
	4	1	0
	24	1	0

Some parents may reconstitute PIF with preboiled water before it has fully cooled to room temperature (nominally 37ºC) for preparation of infant formula. Although this temperature is higher than the usual range of room temperatures in New Zealand, it might still be considered by a parent or caregiver as suitable for immediate feeding. The recent survey of consumer practices identified that only about half of parents and caregivers reported using cool or lukewarm water most or every time in reconstituting formula, although 86% reported checking the temperature every or most of the time before feeding (NZFS, 2020)

Increasing the reconstitution temperature to 37ºC will have an impact on the relative risk of formula that is subsequently stored at 8ºC (this temperature allows slow growth of *Cronobacter*) for 24 hours compared with 4 hours. The reason is that any *Cronobacter* potentially present in PIF will increase in numbers during the preparation and initial cooling of the formula. This is demonstrated with the following table:

Reconstitution temperature	Duration of holding (hours)	Relative risk	Proportional increase in risk
37ºC	**0**	**1**	
	4	1	0
	24	0.7	1.4

Storage at 8ºC for 24 hours will result in a 1.4 fold risk increase, shifting the probability of infection from 1 in 100,000 to 10,000,000 to 1.4 in 100,000 to 10,000,000. The risk however remains within the same order of magnitude and the range of uncertainty in the elevation in risk would not fall outside the inherent uncertainty of the model.

4.3 CONCLUSION

Using previously boiled water cooled to room temperature for reconstitution of PIF will ensure that microorganisms potentially present in the water are eliminated and will ensure the microbiological safety of the formula for younger infants.

Based on comparison of the relative risk related to consumption of formula refrigerated for 4 and 24 hours there is no proportional increase in risk for product reconstituted at 20 or 30ºC and then stored at up to 8ºC for 24 hours. As the general recommended temperature setting for refrigerators is up to 4ºC it is appropriate to recommend 24 hours refrigerated storage at temperatures at or below 4ºC, this

also ensures a level of protection from temperature fluctuations of ±4°C that could occur within older refrigerators.

5 References

Kozyrskyi A.L., Bridgman, S.L., Tun, M.H., 2017. Impact of birth and postnatal medical interventions on infant gut microbiota. Chapter 4 In: Microbiota in health and disease: from pregnancy to childhood, Wageningen Academic Publishers, 2017

Milani, C., Turroni, F., Duranti, S., Lugli, G.A., Mancabelli, L., Ferrario, C., van Sinderen, D., Ventura, M.,2016. Genomics of the Genus Bifidobacterium Reveals Species-Specific Adaptation to the Glycan-Rich Gut Environment. Appl Environ Microbiol. 82(4):980.

NZFS (New Zealand Food Safety), 2020. Caregivers' beliefs about the risk of improper infant formula preparation and their understanding of infant formula preparation risks. New Zealand Food Safety Paper No: 2020/05. https://www.mpi.govt.nz/dmsdocument/39917-Caregivers-beliefs-about-the-risk-of-improper-infant-formula-preparation-and-their-understanding-of-infant-formula-preparation-risks-Report

Nguyen, Q.N., Himes, J.E., Martinez, D.R., Permar, S.R., 2016. The Impact of the Gut Microbiota on Humoral Immunity to Pathogens and Vaccination in Early Infancy. PLoS Pathog 12(12): e1005997.

Penders, J., Thijs, C., Vink, C., Stelma, F.F., Snijders, B., Kummeling, I., van den Brandt, P.A., Stobberingh, E.E., 2006. Factors influencing the composition of the intestinal microbiota in early infancy. Paediatrics, 118, 511-21.

Redmond, E., Griffith, C., 2013. Parent handling, preparation and storage of powdered infant formula feeds: observation and microbiological analysis. Chapter 6 In: An investigation into the attitudes and behaviours of consumers and caregivers in the preparation, handling and storage of powdered infant formula inside and outside the home. Food Standards Agency Research Project B13008: 284–321.

Watson, K., Jones, R.C., Cortes, C., Gerber, S.I., 2006. Community associated methicillin-resistant *S.aureus* infection among healthy newborns. MMWR 2006; 55: 329-32

Winstanley, A., Cressey, P., 2008. Information sources and practices – preparation of powdered infant formula in New Zealand - qualitative research. http://www.foodsafety.govt.nz/elibrary/industry/powdered-infant-formula/Information_Sources-Report_Examines.pdf

Zeng, M.Y., Cisalpino, D., Varadarajan, S., Hellman, J., Warren, H.S., Cascalho, M., Inohara, N., Núñez, G., 2016. Gut Microbiota-Induced Immunoglobulin G. Controls Systemic Infection by Symbiotic Bacteria and Pathogens. Immunity. 44(3):647-658.