

The bacterial contamination of powdered infant formula:

What are the risks and do we need to review current instructions on safe preparation?

August 2020



The bacterial contamination of powdered infant formula: What are the risks and do we need to review current instructions for safe preparation? August 2020

Dr Helen Crawley, Susan Westland and Dr Vicky Sibson

Disclaimer:

This report is provided for information only. Individual advice on feeding an infant should always be sought from a clinician or other health professional.



First Steps Nutrition Trust
Studio 3.04
The Food Exchange
New Covent Garden Market
London SW8 5EL

Contents

	Page
Terminology	3
Summary	4
Recommendations	5
1. Introduction	6
2. Bacterial contamination of powdered infant formula	10
2.1 <i>Cronobacter</i> spp.	11
2.2 <i>Salmonella</i> spp.	14
2.3 Contamination of powdered infant formula	15
3. Risks to infant health from contaminated powdered infant formula	17
3.1 Infections caused by <i>Cronobacter</i> spp. contamination	17
3.2 Documented cases of <i>Cronobacter</i> spp. infection linked to contaminated powdered infant formula	18
3.3 Infections caused by <i>Salmonella</i> spp. contamination	22
3.4 Documented cases of <i>Salmonella</i> spp. infection linked to contaminated powdered infant formula	23
3.5 Product recalls due to contamination or suspected contamination of powdered infant formula	25
4. Why do instructions for making up powdered infant formula vary?	26
4.1 The addition of probiotics to powdered infant formula for term infants	26
4.2 The addition of probiotics to powdered infant formula for preterm infants	27
4.3 Potential risks associated with the addition of probiotics to powdered infant formula	28
4.4 Can the acidification of powdered infant formula reduce the risk of bacterial contamination?	29
5. Current UK advice on reducing the risk of infection due to contamination of powdered infant formula	30
5.1 Current practices in making up powdered infant formula	34
5.2 Does the current advice ensure that the water is above 70°C when powdered infant formula is reconstituted?	36
5.3 Using other methods for reconstituting powdered infant formula	40
6. Conclusion	40
7. References	41
Appendix 1: Reported bacterial contamination of powdered infant milk samples	51
Appendix 2: Examples of infant milk recalls due to risk of bacterial contamination	53

Terminology

There are a number of names and terms used for milks marketed to infants and young children. Whilst they are all covered by the term 'breastmilk substitutes', the terms 'artificial milks' or 'formula milks' are commonly used. The products which are considered in this report are milks sold in powdered form, manufactured and marketed for use by infants and young children as a breastmilk substitute after preparation with water.

We are using the term **powdered infant formula (PIF)** throughout this report as an umbrella term to include powdered infant formula, follow-on formula and infant milks marketed as foods for special medical purposes.

Summary

Breastfeeding protects infants from infection and a mother who breastfeeds does not introduce additional bacterial hazards through the use of powdered infant milks (PIF) or through the contamination of bottles and teats. Infants are particularly susceptible to infection via foodborne pathogens because they have immature immune systems and permeable gastrointestinal tracts.

Current manufacturing processes are unable to produce sterile PIF and the pathogens of greatest concern in PIF are *Cronobacter* spp. and *Salmonella* spp. The risk to infants of serious illness or death from the bacterial contamination of PIF is well known. Although the number of infants who may become ill, or die, from infections caused by the consumption of contaminated formula is difficult to quantify, the true number is likely to be underrepresented in the literature. There is particular risk to premature infants related to serious infections such as necrotising enterocolitis (NEC) and sepsis.

Global guidance on how to minimise this risk has been established by WHO, which includes a recommendation to use fresh water which is boiled and then cooled to no less than 70°C when the PIF is added. Whilst we believe that some of the current guidance related to waiting time once the kettle has boiled needs re-assessment, the WHO guidance that the water used to make up PIF should be at a temperature of 70°C or more remains essential.

In the UK instructions on infant formula and follow-on formula product labels, and in national guidance has maintained instructions for preparation of PIF with water at 70°C or more. PIF marketed as foods for special medical purposes have variable preparation instructions and where these products are sold over the counter no risk assessment can be made. New methods of reconstitution using preparation machines and hot taps have not been independently evaluated and may require revised instructions.

In many countries, however, the importance of reconstitution of PIF at a temperature that can kill any bacteria present in the powder is not required in on-pack instructions to families on safe preparation, or in national guidance. Recent cases of illness caused by *Salmonella* infection from PIF in Europe demonstrate that this should be reconsidered. The UK's exit from the EU may put our current, safe making-up guidance at risk, and a proliferation of PIF on the global market containing added heat labile ingredients like probiotics may further threaten this.

Recommendations

1. A clear statement that powdered infant milks are not sterile should be mandatory on all powdered infant milk products marketed in the UK.
2. Guidance to use water at a temperature of 70°C degrees or more when preparing powdered infant formula should be a requirement on product labels for all infant formula and follow-on formula marketed in the UK. Packaging should include safe preparation instructions in words and pictures.
3. Any change made to current regulations on the composition, labelling and marketing of infant formula and follow-on formula should include clear statements on the need for safe preparation information, specifying that water should be at 70°C or more when the powder is added.
4. Infant milks marketed as foods for special medical purposes should only be used under medical supervision where a risk assessment can be undertaken if making up instructions differ from standard safe guidance.
5. The safety of current reconstitution advice for powdered infant formula should be reviewed with attention paid to assessing the time advised for the water to be left in a kettle after boiling (or the safety of use of other reconstitution methods) to ensure that water at all volumes is clearly at 70°C or more when the powder is added.
6. Government advice on the safe preparation of powdered infant milks should include advice for all potential preparation methods, including the use of automated preparation machines, hot taps and baby kettles.
7. Specific Government advice should be prepared to explain safe preparation of powdered infant milks for premature, low birthweight and vulnerable infants, particularly when these are being used as an enteral feed.
8. The Food Standards Agency should, as a matter of urgency, commission an independent review to consider whether the inclusion of probiotics in infant milks has any risks or benefits to health, including assessment of risk associated with potential bacterial contamination of powdered infant milks if products are reconstituted with water at less than 70°C.
9. Manufacturers of powdered infant milks marketed in the UK should be required to provide data on the bacterial load of their products on demand, including details of methods used to identify bacterial strains. Additional independent analysis should also be undertaken annually by the Food Standards Agency to compare with data reported by manufacturers.
10. The notification/reporting of *Cronobacter* and *Salmonella* infections in infants related to bacterial contamination of infant milks should be mandatory, with clear guidance for GPs and other primary care staff on how to report this.

1.0 Introduction

Breastfeeding is known to protect the health of infants and mothers and the evidence for the importance of human milk in preventing illness and infection in vulnerable low-birthweight infants is unequivocal. Breastfeeding protects the infant from a spectrum of adverse health outcomes, including infectious diseases, and the potential for undernutrition caused by contaminated water or overdilution of breastmilk substitutes (Grummer-Strawn & Rollins, 2015).

The protective effect of a human milk diet is due to its ability to compensate for the immaturity of infant gastrointestinal and immune systems in a number of ways: lowering gastric pH, enhancing intestinal motility, decreasing epithelial permeability and altering the composition of bacterial flora in favour of commensal bacteria, thereby reducing the opportunity for colonisation by pathogenic bacteria (Maffei and Schanler, 2017). Breastmilk is also a source of commensal bacteria such as bifidobacteria (Soto et al., 2014; Harmsen et al, 2000).

Necrotising enterocolitis (NEC), in which the tissues of the intestine become inflamed and start to die, is thought to affect 5-10% of very low birthweight infants, with mortality rates ranging from 15-30% (Samuels et al, 2017). A review of human milk feeding in premature infants reported that an exclusive human milk diet provides protection against NEC and that risk is significantly decreased if more than 50% of feeds are human milk (Cacho et al, 2017). There is an association between NEC and bacterial infections which is not fully understood.

When, for whatever reason, infants do not receive human milk (from their mother or a donor) breastmilk substitutes are available. Although globally around 80% of mothers initiate breastfeeding, recent data suggests that by 12 months of age around 80% of infants in most resource rich countries including the UK, the USA and many Western European countries, have been given milks other than human milk (Victora et al, 2016). In the most recent available national information for the UK, 31% of parents introduced infant formula on the first day of their baby's life, by 6 weeks of age 73% of infants had been given infant formula and by 9 months 95% of infants had had some infant formula (McAndrew et al, 2012). More recent data collected in Scotland in 2017 reported that 75% of infants had received some infant formula by 8-12 weeks of age (Scottish Government, 2018).

As well as losing the protective effects associated with human milk feeding, unsafe use, and potential contamination of breastmilk substitutes are additional risk factors for illness and infection. Manufacturers have reported that using current manufacturing processes, the industry cannot produce powdered infant formula (PIF) free of bacterial contamination (CDC, 2015). The importance of using both sterile water, and water at a sufficient temperature to kill any bacteria present, with immediate consumption of the milk (when cooled to an appropriate temperature) have long been shown to offer the best protection for infants.

In order to reduce the risk associated with contaminated infant foods, including PIF, in 1979 the Codex Alimentarius Commission (CAC) provided a "*Recommended International Code of Hygienic Practice for Foods for Infants and Children*" (CAC, 1979) which was intended as a

useful checklist of requirements for national food control or enforcement authorities. It contained:

" the minimum hygienic requirements for the handling (including production, preparation, processing, packaging, storage, transport, distribution and sale) of such food to ensure a safe sound and wholesome product." (CAC, 1979).

The efficacy of this code of practice to manage contamination of PIF was called into question at the beginning of this century after outbreaks of *Cronobacter* spp. infections in neonatal intensive care units (NICU) in Western Europe and the United States were found to have been caused by PIF contaminated with *Cronobacter* spp. at levels below the limits advised by CAC 1979, as described above (Strydom et al, 2012).

An increased awareness of the risk posed by these organisms, together with recognition that existing specifications did not offer a sufficient level of protection, prompted a decision from Codex Alimentarius to revise the 1979 recommendations. Scientific advice from the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) in a series of expert reports from meetings convened in 2004, 2006 and 2008 (FAO/WHO 2004, FAO/WHO, 2006, FAO/WHO 2008) informed the updated Codex *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children* (CAC/RCP 66, 2008) which provides guidance on the hygienic manufacture of PIF and on the hygienic preparation, handling and use of reconstituted formula products.

A review of published case reports of illnesses in infants due to microorganisms associated with PIF consumption (either microbiologically or epidemiologically) was used to assess risks from contaminated PIF. The microorganisms or microbial toxins of concern were categorised according to the strength of the evidence of a causal association. Under this system, the genus *Cronobacter* spp. and *Salmonella enterica* were identified as category "A" pathogens meaning that there was clear evidence of a causal association between their presence in PIF and illness in infants.

A number of other Enterobacteriaceae were classified as category "B" because they were well-established causes of illness in infants and were found in PIF, but contaminated PIF had not been convincingly shown to be the cause of infection in infants at that time. Category "C" organisms were those that were known to cause illness in infants but which had not been identified in PIF, or, having been identified in PIF, had not at the time been implicated as causing such illness in infants (FAO/WHO, 2004).

The classification of microorganisms was updated at the second FAO/WHO meeting in 2006, to include additional pathogens identified from reviews of further cases reported since the meeting in 2004. Table 1. shows the categories assigned to different microorganisms and microbial toxins found in PIF.

Table 1. FAO/WHO 2006 categorisation of microbiological hazards associated with PIF based on the strength of evidence of causality between their presence in PIF and illness in infants

Category	Organism
A - clear evidence of causality	* <i>Cronobacter</i> spp.; <i>Salmonella</i> spp.
B - causality plausible but not yet demonstrated	<i>Pantoea agglomerans</i> and <i>Escherichia vulneris</i> (both formally known as <i>Enterobacter agglomerans</i>), <i>Hafnia alvei</i> , <i>Klebsiella pneumoniae</i> , <i>Citrobacter koseri</i> , <i>Citrobacter freundii</i> , <i>Klebsiella oxytoca</i> , <i>Enterobacter cloacae</i> , <i>Escherichia coli</i> , <i>Serratia</i> spp. and <i>Acinetobacter</i> spp.
C - causality less plausible or not yet demonstrated	<i>Bacillus cereus</i> , <i>Clostridium difficile</i> , <i>Clostridium perfringens</i> , <i>Clostridium botulinum</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> and coagulase-negative staphylococci

* formerly known as *Enterobacter sakazakii*

The second meeting in 2006 reiterated the risks associated with the multiplication of organisms in reconstituted PIF. Using the updated risk classification, a quantitative risk assessment model was developed and used to identify risk management interventions for *Cronobacter* spp. and *Salmonella* spp in PIF. Their advice included a recommendation to develop guidelines for the safe preparation, handling and storage of PIF in order to minimise the risk to infants. The guidelines subsequently developed by WHO in 2007 are based on the quantitative microbiological risk assessment of *Cronobacter* spp. in PIF. While no risk assessment was carried out for *Salmonella*, the expert group reported that the basic risk control principles for *Cronobacter* spp. would also hold true for *Salmonella* spp. The main recommendations from the guidelines, which are still in place, are that to reduce microbial risks:

- Powdered infant formula should be reconstituted with water at temperatures > 70°C
- Feeds should be used within 2 hours of preparation.

Further detail on the safe preparation, storage and handling of powdered infant formula can be found on page 31.

The third risk assessment meeting held by FAO/WHO in 2008 addressed the issue of bacterial pathogens in powdered follow-on formula. While available evidence was reviewed and consideration given to the establishment of specific microbiological criteria for *Cronobacter* spp. for this formula, no explicit recommendations were made. The meeting sought to highlight the currently available data on pathogens in follow-on formula and how it contributes to our knowledge base and facilitates risk management decisions (FAO/WHO, 2008).

Despite the fact that the key recommendation from all international bodies to reduce risk to infants of bacterial infection is that PIF should be reconstituted with water at no less than 70°C, there has been considerable resistance to this recommendation among the infant formula industry and some segments of the medical community (Hormann, 2010).

The resistance stems from a number of beliefs:

- That this temperature might destroy some nutrients in the milk (for example, thiamin, folate, vitamin C)
- That it can increase the risk of scalding the caregiver or the infant
- That higher temperatures may cause some powder formulations to 'clump' and not pass easily through the bottle teat.

The addition of probiotics to some PIF has also impacted on the dialogue as live microorganisms added are destroyed at temperatures of 70°C (more information on probiotics can be found on page 26).

A recent paper has argued that the risk of burns from the use of hot water is much greater than any risk posed by bacterial contamination of PIF (Wilkinson et al, 2019). The authors argue that sleep deprived parents, often with other children to care for, are unable to make up infant formula following the current instructions and that scald burns are common and often fatal in young children. The authors quote data for total injury from burns in the US but do not provide any evidence that this is related to infant formula preparation or feeding.

We would argue that the risks of bacterial contamination are not insignificant and the suggestion that it is too difficult for parents and carers to make up milk safely is simply not true since the current advice is to pour the water into the bottle when it has cooled to no less than 70°C and not to handle boiling water in a bottle. In addition, the only nutrient likely to be significantly affected by the water temperature is vitamin C, and the content of this vitamin is unlikely to be reduced below recommended levels during the reconstitution process (WHO, 2007).

We also note recent work suggesting that current guidance to leave 1 litre of water that has boiled in a kettle for no more than 30 minutes and is at a temperature above 70°C when added to PIF may not ensure inactivation of any pathogens present if the water is at 70°C when added (Losio et al, 2018). This is because water cools very quickly when poured into a bottle and this is particularly true for smaller volumes.

What is the purpose of this report?

This report focuses on the risks from using PIF rather than wider aspects of safety around the sterilisation of bottles and teats. It considers the known risks associated with bacterial contamination of PIF, highlights new data suggesting that global guidance may need to be revisited, and summarises current guidance on the safe preparation of PIF.

2.0 Bacterial contamination of powdered infant formula

The majority of breastmilk substitutes used to feed infants are purchased in a powder format which is then reconstituted with water before use. Powdered infant formula (PIF) is subjected to heat treatment during processing but the final packaged product is not sterile. PIF is usually marketed in tins with a foil cap and a plastic lid or in boxes with a plastic liner. Most tins or packets of PIF are about 800g-900g meaning that once opened they will be open for a period of days and potentially weeks. Families that mixed feed their infant (i.e. breastfeed and bottle feed) may keep open containers of PIF for considerably longer.

PIF can also become contaminated during the reconstitution and handling stages, in domestic as well as hospital environments. Pathogens may come into contact with PIF from contaminated water or as a result of contact with contaminated surfaces including hands, utensils, brushes, feeding tubes and bottles and teats or as a result of inappropriate storage conditions of opened containers of PIF.

Whilst awareness of the need to use sterilised equipment when PIF are reconstituted is good, and containers of PIF provide instructions in both words and pictures to support this, many people may be unaware that the PIF itself inside a sealed tin is not sterile and may have been contaminated with harmful microorganisms during processing. Contamination can occur at any of the processing stages of production and the most likely contaminant pathogens are those from the *Cronobacter* or *Salmonella* spp.

There are three different manufacturing processes for PIFs: dry-mixing, wet-mixing/spray drying or a combination of the two. Each has different risks and benefits with respect to the potential for product contamination by harmful bacteria.

Dry-mixing

In the dry-mixing process, the ingredients are received from suppliers in a dehydrated powdered form and are mixed together to achieve a uniform blend of the ingredients necessary for a complete infant formula product. As dry blending does not involve the use of water, it reduces the chance that harmful bacteria will become established in the plant environment in sufficient numbers to cause product contamination. However, the microbiological quality of a dry-blended product is largely determined by the microbiological quality of its constituent dry ingredients. In a dry blending process 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 by even low numbers of harmful bacteria, these bacteria are likely to be present in the finished product.

Wet mixing / spray drying

In the wet-mixing / spray-drying process, ingredients are blended with water in large batches. The wet product is then homogenised, pumped to a heat exchanger for pasteurisation, and then spray-dried to produce a dry, powdered product. This process has the advantage of ensuring a uniform distribution of nutrients throughout the batch, but some nutrients are destroyed. As the pasteurisation step destroys harmful bacteria that may be present in the ingredients, this process is much less dependent on the microbiological quality of ingredients. However, it does

require that the processing equipment be regularly wet-cleaned, which provides the moisture needed by bacteria to grow and become established in the plant environment. If not controlled, these bacteria can be a source of product contamination.

Combined process

In the combined process, some of the constituents of the PIF are wet processed and then dried and other ingredients are added in a dry form after the heat treatment. The microbiological quality of these ingredients is critical, since the product may not receive further heating sufficient to destroy harmful bacteria.

PIF is packed in containers, flushed with inert gas, sealed with an airtight cap, cooled and labelled. Samples from each batch of formula undergo analysis for uniformity, nutritional content and microbiological safety, but this data is not publicly available. Using current mix technology, it is not possible to produce commercially sterile powders or to completely eliminate the potential of contamination (FAO/WHO, 2004).

Since the mid-20th century there have been numerous well documented cases of invasive bacterial infections in infants, in which contaminated PIF has been implicated both epidemiologically (i.e. correlation between contaminated PIF use and infection of children) and microbiologically (i.e. evidence of bacterial contamination in PIF and in the infected children) as the source and/or vehicle of infection. *Cronobacter* spp. and *Salmonella* spp. have been the most frequently identified illness-causing pathogens in PIF.

2.1 *Cronobacter* spp.

Cronobacter are gram-negative, rod shaped, non-spore forming, flagellated and therefore motile bacteria and are members of the *Enterobacteriaceae* family (Kalyantanda et al, 2015).

Before 2008, the genus *Cronobacter* spp. was referred to singularly as *Enterobacter sakazakii* until it was noted that there was significant variability among the various *E. sakazakii* microorganisms. This finding led to their reclassification to the *Cronobacter* genus (Kalyantanda et al, 2015). Farmer III (2015) suggests that there are 10 recognised species of the genus *Cronobacter* that appear in the literature, these are:

Cronobacter sakazakii

Cronobacter malonaticus

Cronobacter turicensis

Cronobacter mytjensii

Cronobacter dublinensis (sub species *C. dublinensis dublinensis*, *C. dublinensis lactaridj*, *C. dublinensis lausannensis*)

Cronobacter condimenti

Cronobacter universalis

Cronobacter helveticus

Cronobacter pulveris

Cronobacter zurichensis

In this report we use the term *Cronobacter spp.* when discussing studies that have previously used the name *E.sakazakii*.

Cronobacter spp. have been associated with life-threatening conditions in neonates, particularly in preterm and/or low birthweight infants and are considered pathogenic. *C. sakazakii* is one of several species of *Cronobacter* that can invade human intestinal cells, replicate in white blood cells called macrophages and invade the blood-brain barrier (Kucerova et al, 2011). It is therefore *Cronobacter sakazakii* that is of greatest concern in respect of infant and neonatal infections.

Cronobacter spp. has developed mechanisms that aid its survival in its natural habitat of plant based materials. These same mechanisms also aid its survival through some of the PIF production processes and also support its growth in the reconstituted product, its persistence in the feeding environment and contribute to its virulence in those who consume it.

Sources of *Cronobacter spp.*

Whilst there is now a much greater understanding of the taxonomy and biochemical characteristics of *Cronobacter spp.*, the primary reservoir and mode of transmission of the species is not yet fully understood. They have been described as ubiquitous (El-Sharoud et al, 2009; Kandhai, et al, 2010) and have been isolated from a wide range of different specimens including the human and animal gut, human skin and faeces, plant and animal based foods, hospitals, clinical samples, food production lines, water waste, soil and domestic environments (Norberg et al, 2012, Beuchat et al, 2009). The most probable natural habitat for *Cronobacter spp.* is plants and plant based materials (Walsh et al, 2011; Osaili and Forsythe, 2009, Schmid et al, 2009). As they do not occur naturally in animals and humans, the principle sources of food contamination are most likely to be soil, water and vegetables and rodents and flies may serve as a secondary route of contamination (Iversen and Forsythe, 2003).

Thermotolerance

The range of temperatures over which *Cronobacter spp.* will grow is 6-47°C with an optimum temperature around 39°C (Iversen & Forsythe, 2003). While *Cronobacter spp.* are unlikely to survive the pasteurisation process (Edelson-Mammel and Buchanan, 2004, Breeuwer et al, 2003), they have been shown to survive industrial drying processes (Arku et al, 2008) and to be present in previously unopened cans of PIF (Himelright et al, 2002; van Acker et al, 2001).

Acid tolerance

The ability of a pathogen to survive in foods depends in part on its ability to survive acid or alkaline conditions. Acid resistance studies indicate that *Cronobacter spp.* can survive at 36°C at a pH as low as 3.5 for more than 5 hours. Dancer et al, reported that some species can grow at a pH of 3.9 in laboratory medium (Dancer et al, 2009). However below pH 3, their survival was found to be transitory with substantial diversity in acid resistance existing among different strains (Edelson-Mammel et al, 2006).

Cronobacter species have been reported to survive in cantaloupe melon (pH 6.8), watermelon (pH 5.0), and tomato, (pH 4.4) but not in apple juice (pH 3.9) or strawberry juice (pH 3.6) when stored at 25°C (Kim and Beuchat, 2005). In another study, the growth and survival of *Cronobacter sakazakii* in an infant rice cereal after reconstitution with various liquids (milk, water, apple juice, and infant formula) was investigated. Reconstitution with apple juice prevented the growth of *Cronobacter* even when the cereal was left at room temperature, possibly because of the low pH of the apple juice. However, when the cereal was reconstituted with milk, water, or infant formula, growth was observed at 12 to 30°C and was directly proportional to temperature (Richards et al, 2005).

Desiccation resistance

Cronobacter spp. have been isolated from a range of dried foods including herbs and spices, rice cereals and PIF indicating that they can survive for a period of time in dry environments (Muytjens et al., 1988; Gurtler and Beuchat, 2007). More specifically, desiccated *Cronobacter* cells in stored PIF have been shown to survive for periods of up to 2 years, with encapsulated strains able to survive for up to 2 ½ years (Caubilla-Barron and Forsythe, 2007). The long survival times achieved by *Cronobacter* spp. suggest that it may persist during the entire shelf life of some products.

Dormant *Cronobacter* spp. cells that have survived long periods of time desiccated can grow rapidly (Osaili and Forsythe, 2009). The generation time (doubling time) of *Cronobacter* spp. in reconstituted PIF at room temperature (21°C), has been reported as 40-94 minutes (Kucerova et al, 2011) and 37-44 minutes at 22°C (FSAI, 2011).

Formation of Biofilms

Cronobacter spp. have been shown to be capable of adhering to a wide variety of surfaces including glass, silicon, latex, polyvinyl chloride (PVC), polycarbonate and to a lesser extent to stainless steel. Attachment occurs more readily on hydrophobic surfaces (Lehner et al, 2006, Iversen and Forsythe, 2003). This characteristic aids survival on feeding bottles, tubes and equipment where it can form biofilms - multicellular communities held together by a self-produced extracellular matrix

Biofilm formation has been shown to be influenced by the growth medium; PIF is particularly suitable (Oh et al, 2007). Furthermore, PIF has been shown to have a protective effect on *Cronobacter* spp. by increasing its resistance to cleaning agents and disinfectants (Hurrell et al, 2009). Disinfectants used in hospitals, day-care centres, and food service kitchens have been shown to be ineffective in eliminating *Cronobacter* spp. cells dried onto a stainless steel surface (Kim et al. 2007).

The formation of biofilms increases the risk of infections on enteral feeding tubes which may contaminate subsequent feeds (Hurrell et al, 2009). As the biofilm ages clumps of cells may be shed and these may survive passage through the neonate's stomach due to encapsulation offering protection from the acidity (Kim et al, 2006). Due to neonates' low immune status and lack of competing intestinal bacterial flora these organisms could result in infections (Townsend and Forsythe, 2008). The preparation of feeds that will be used for nasogastric or PEG

(percutaneous endoscopic gastrostomy) feeding an infant or young child therefore needs particular scrutiny.

Utilisation of Sialic Acid

Some formula manufacturers add sialic acid to their products but *C. sakazakii* is unique within the *Cronobacter* genus in that it can utilise sialic acid from breastmilk, infant formula, milk oligosaccharides, mucins lining the intestinal wall and even brain gangliosides, for growth (Joseph et al. 2013). This property enables *C. sakazakii* to remain viable in PIF that are both contaminated with the microorganism and fortified with sialic acid (Kalyantanda et al, 2015). In the UK, while there are currently no PIF that contain added sialic acid, it may be intrinsically present in other milk based ingredients.

Virulence

Studies in *Cronobacter sakazakii* have demonstrated that virulence is strain specific, which may explain why the same species can be pathogenic or non-pathogenic. The same microbe might also be harmless in a full-term infant but pathogenic in a pre-term infant (Grishin et al, 2013).

2.2 Salmonella spp.

Salmonella are gram-negative, rod shaped, non-spore forming, predominantly motile and flagellated bacteria and are members of the *Enterobacteriaceae* family (Fàbrega and Vila, 2013).

Salmonella

The genus *Salmonella* contains 2 species - *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* and its 6 sub species are the most clinically significant as they are important agents of foodborne illness. More than 2,500 serotypes have been described but, because they are rare, scientists know very little about most of them. Less than 100 serotypes account for most human infections (CDC, 2015).

Sources of Salmonella

Salmonella spp. live in the intestinal tract of humans and other warm-blooded animals, including farm animals and pets, as well as reptiles and birds. It is shed in the faeces and poor hygiene practices can result in transmission. Contamination can occur through many routes; in food production environments by cross contamination from raw foods, contaminated ingredients or infected handlers. The most usual cause of salmonellosis in humans is from consumption of contaminated foods (FSAI, 2011). Many foods have been identified as vehicles for the transmission of *Salmonella* spp. including eggs, poultry, chocolate, fruit, milk, vegetables and PIF.

Characteristics of *salmonella*

Like *Cronobacter* spp., *Salmonella* spp. has a degree of thermotolerance, influenced by factors including the pH and water availability of the substance in which it is suspended (Campbell and Soboleva, 2015). Heat resistance has also been shown to increase with increasing concentration of milk solids, therefore PIF has a protective effect on *Salmonella* spp. (FSAI, 2011).

Whilst *Salmonella* are able to resist dry stress they are less resistant to both osmotic and dry stress than *Cronobacter* spp. (Breeuwer et al, 2003). Also like *Cronobacter* spp., *Salmonella* serotypes may become encapsulated and produce biofilms on surfaces such as PVC, polyurethane and silver impregnated enteral feeding tubes (Hurrell et al, 2009) and could therefore act as a channel for transmission of bacterial cells into the stomach of the neonate, regardless of whether the current feed is contaminated prior to entering the feeding tube (Kalyantanda, 2015).

2.3 Contamination of Powered Infant Formula

The only specific food that has been epidemiologically associated with *Cronobacter* spp. infection outbreaks is PIF and it is suggested that reconstituted PIF is a common vehicle for transmitting *Salmonella* to infants (FAO/WHO, 2004). Better isolation of pathogens in PIF may be because infections in infants are often more serious, they are more likely to receive medical care and have samples taken for analysis and also because their diet is much more limited than the adult diet making it more straightforward to identify any potential food-based contaminants (Patrick et al, 2014).

It is accepted that *Cronobacter* spp. do not survive the pasteurisation process, therefore contamination during the production of PIF (intrinsic contamination) is most likely to occur after pasteurisation via the drying, filling and packing stages, or via the addition of contaminated ingredients as may occur in the 'dry mixing' and 'combination' processing methods (Mullane et al, 2006; FAO/WHO, 2004). As *Cronobacter* spp. has been isolated from human skin, faeces and environmental samples, it may also be feasible that staff at factories may act as vehicles for infection (Kent et al, 2015).

Like *Cronobacter* spp., *Salmonella* spp. also does not survive the pasteurisation process and therefore contamination is likely to occur post pasteurisation. However, unlike *Cronobacter* spp., contamination is more likely to occur from the preparer or preparation environment than from the manufacturing process.

In their 2004 report, FAO/WHO state that:

"Practical experience and data show that it is possible to control Salmonella in processing environments to an extent where it will be virtually completely absent. Under these conditions, the risk of recontamination is extremely low and it is possible to manufacture products fulfilling the most stringent microbiological requirements [] as recommended by the Codex Alimentarius".

There have been a number of surveys over the past decades which have isolated pathogens in PIF. A summary table of some of these studies can be found in Appendix 1. The proportion of positive samples in studies is highly variable, but the majority of studies isolated pathogens in some of the PIF samples tested. There is likely to have been considerable variation in detection methods used so results are not all directly comparable. It is also not known where the milks tested in different countries originated and in markets such as China there are a wide range of products for sale including those imported from large multi-national manufacturers.

As part of FAO/WHO risk assessments on the microbiological safety of PIF in 2004, the panel reviewed data provided to them by the breastmilk substitute industry on the frequency and levels of *Cronobacter spp.* Out of 30 sets of samples provided by 11 manufacturers, *Cronobacter spp.* was detected in 21 (70%) although the proportion of positive results was much higher in some samples than others, ranging from 0.2% to 33.3% of samples. The likelihood of detecting the presence of *Cronobacter* does not appear to depend on either the size of individual samples nor the number of samples taken, probably because contamination can occur at very low levels and the distribution of bacterial cells is heterogenous.

Information was also provided to the committee on contaminants found in ingredients used in breastmilk substitute manufacture. Contamination of individual ingredients was generally low, with the highest recorded rate being in starch (3%) which may be used in some specialist infant milks.

Unlike *Cronobacter sakazakii* and other *Enterobacteriaceae*, *Salmonella* has more rarely been looked for in surveys of PIF and reported incidences have been less frequent. Rates of *Salmonella* infection are highest in infants less than one year old and in the US it has been reported that incidence rates are eight times higher in the infant population than amongst adults (Kent et al, 2015). In 1985, an outbreak of *Salmonella ealing* infection in the United Kingdom was linked to one brand of infant formula, and contamination was traced back to problems in the spray drier. At least five outbreaks of contamination were reported between 1985 and 2005, and an outbreak in France in 2005 led to 141 confirmed cases of illness (Kent et al, 2015). More recently persistent *Salmonella* contamination in an infant formula manufacturing facility in Spain has been identified, however, the source of the infection was identified by tracking back from infected infants and reported formula consumption rather than from identification of contaminated PIF. Samples of PIF did not reveal contamination but the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) noted in their Rapid Outbreak Assessment document that the detection of *Salmonella* in dry products is difficult due to low and non-homogenous contamination, the sensitivity of the sampling procedures and the analytical methods used (ECDC and EFSA, 2019).

3.0 Risks to infant health from contaminated powdered infant formula

Infants are at greatest risk of infection during the first weeks of life and because of the immaturity of their immune systems, exposure to foodborne pathogens in PIF or from other sources during this time may quickly lead to invasive infections. Breastfeeding is known to be protective against infection, particularly during the neonatal period. One of the most significant risk factors for *Cronobacter sakazakii* infection in neonates is feeding PIF (Lai et al, 2001; FAO/WHO, 2004; Bowen and Braden, 2006; Jason 2012).

Published reviews of cases of invasive *Cronobacter* spp. infection in infants globally have implicated PIF in outbreaks of *Cronobacter* spp. infection. It has been reported that 92% and 90% of infected infants had received PIF (Bowen and Braden, 2006; Jason, 2012), despite intrinsic or extrinsic contamination of PIF not always being identified epidemiologically and microbiologically as the vehicle of infection. Individual case reports have also implicated PIF as the source of infection.

3.1 Infections caused by *Cronobacter* spp. contamination

Illness and infections caused by *Cronobacter sakazakii* occur across all age groups and primarily in adults where the outcomes are less severe than in infants (Patrick et al, 2014). However, existing data suggest that very young infants are at a greater risk of severe disease and death from *Cronobacter sakazakii* infection, with those <2 months of age, particularly pre-term or low-birthweight infants and those who are immunocompromised being most at risk (FAO/WHO, 2004). Invasive *Cronobacter sakazakii* infection in infants causes meningitis, bacteraemia and septicaemia and has been associated with NEC (Peter et al, 1999, Patrick et al, 2014, Farmer III, 2015, Kent et al, 2015).

The primary manifestations of *Cronobacter* infection in infants, meningitis and bacteraemia, tend to vary with age. Meningitis tends to develop in infants with a greater gestational age and birth weight and infection occurs at a younger age (<28 days) than infants with bacteraemia alone. Bacteraemia tends to develop in premature infants outside of the neonatal period with most cases occurring in infants less <2 months of age (Bowen and Braden 2006, Lai, 2001). NEC does not tend to develop until several weeks after birth and there is a propensity for the disease to develop between 28 and 31 days postpartum age (Neu, 2005). However, infants with immunocompromising conditions have developed bloodstream infections as late as 10 months of age and previously healthy infants have also developed invasive disease outside the neonatal period (FAO/WHO, 2004).

Meningitis is the most frequently reported condition in neonatal *Cronobacter* spp. infections and around 90% of those infected develop brain abscesses (Burdette and Santos, 2000). Mortality rates for infants who develop invasive infections have been suggested to be as high as 80% (Nazarowec-White and Farber, 1997), and the majority of infants that recover from the infection may still require prolonged hospitalisation for related complications including intestinal obstruction from scarring, liver failure due to the prolonged requirement for total parenteral nutrition, short bowel syndrome with intestinal failure and associated nutritional deficiencies, poor neurological development and hydrocephalus (Hunter, 2008, Bowen and Braden, 2006, Lai, 2001).

The pathogenesis of NEC is complex and not yet well defined. It is characterised by ischaemia, bacterial colonisation of the intestinal tract and increased levels of protein in the gastrointestinal lumen; the latter often attributable to the consumption of infant formula (Iversen and Forsythe, 2003). A well supported theory is that damage to the intestine after birth results in invasion of the intestine by bacteria which in turn initiates a cascade of inflammation that leads to further destruction or perforation of the intestine and then to systemic infection (Hunter et al, 2008). *Salmonella* and *Cronobacter* spp. are amongst the pathogens most commonly associated with NEC (Hunter, 2008). A positive correlation between NEC and oral infant formula feeding has also been reported (Kosloske, 1984; Iversen and Forsythe, 2003; Van Acker et al., 2001). A prospective multicentre study of pre-term infants reported an almost 10-fold increase in the incidence of NEC in formula-fed infants compared to those who were fed breastmilk (Lucas and Cole 1990). More recently, a study of very low birthweight infants showed that implementing an exclusive human milk diet led to a significant decrease in the incidence of NEC, increased feeding tolerance, decreased time to full feeds, shorter lengths of hospital stay and resulted in considerable cost savings (Assad et al, 2016).

Incidence rates of infection from *Cronobacter* spp.

Overall, there are too few data sets available to give a reliable picture of the likely number of infections attributable to *Cronobacter* spp., or the variation in incidence between countries or regions. The main challenge is a lack of active national surveillance systems for *Cronobacter* spp. disease. *Cronobacter sakazakii* infection is notifiable in only a small number of countries. Invasive *Cronobacter* spp. disease based on clinical and laboratory data is notifiable in New Zealand and in one state in the USA (Minnesota), for infants only. The majority of countries have a foodborne disease surveillance system that should include *Cronobacter* spp. but cases are frequently reported by outbreak or through voluntary passive systems (FAO/WHO, 2004). In addition, where data is available it is not reported consistently, hindering comparison; for example, by age group, including differentiating between neonates and older infants, or including clinical data or outcomes.

Estimates of the incidence of *Cronobacter* spp. infection in infants under 12 months from several sources are shown in Table 2. There is likely to be significant under reporting of cases (Jason, 2012, FAO/WHO, 2004) and those cases that have been reported are likely to be the tip of the iceberg. Jason (2012) reviewed infections without underlying disorders and reported that 99% of infected infants were less than 2 months old, 83% less than one month old, and that low birthweight infants accounted for 68% of all cases.

The reported incidence of infection with *Cronobacter* spp. varies from 0.21–1.81 cases per 100,000 infants with younger infants and those born low birthweight at much higher risk. A figure of 1/100,000 is often cited as a potential incidence rate meaning that the risk is not insignificant in a population such as in England where there is significant formula feeding, birth rates of around 700,000 a year and a low birthweight rate of 7% (ONS, 2019).

Table 2. Estimates of incidence of *Cronobacter* spp. infection in infants <12 months old

Study group and reference	Country	What was measured?	Estimate of incidence (cases per 100,000 infants)
US Centers for Disease Control (CDC) (Patrick et al, 2014)	US	<i>Cronobacter</i> spp. from any infection site from 22 infants, between 2003-2009.	1.81
		<i>Cronobacter</i> spp from blood and cerebrospinal fluid from 6 infants, between 2003-2009.	0.49
FAO/WHO working group (FAO/WHO, 2008)	England and Wales	<i>Cronobacter</i> spp from blood and cerebrospinal fluid from 18 infants, between 1992-2007.	0.21
		<i>Cronobacter</i> spp from blood and cerebrospinal fluid from 14 infants <1m of age	1.76
US Centers for Disease Control (CDC) Foodnet Survey 2002. (https://www.cdc.gov/cronobacter/technical.html#how-common)	US	Infants aged <1 year	1.0
		Low birth weight infants (<2,500g)	8.7
		Very low birthweight (<1500g)	9.4

CDC reviewed invasive *Cronobacter* infections among infants between 1961-2018 and reported that global cases reported were significantly higher during the final quarter of the study (2004-2018) increasing from a mean of 1.2 cases a year before 2004 to 8.7 cases a year from 2004 (Stryko et al, 2020). They also reported that among US cases during this period a significantly higher proportion occurred among full-term and non-hospitalised infants

The consequences of infection can be devastating. In a statistical analysis of more than 100 cases of microbiologically confirmed cases of neonatal *Cronobacter* spp. infection, the overall mortality rate of the 67 invasive infections was 26.9% (Friedemann 2009). The lethality of *Cronobacter* spp., meningitis, bacteraemia and NEC was calculated to be 41.9%, <10% and 19.0% respectively (Friedemann, 2009).

Lai et al, 2001 also reported higher fatality rates for meningitis rather than non-meningitis infections in infants, 33% versus 45% respectively. It is interesting to note that a review of 17 cases of neonatal meningitis revealed that patients with *Cronobacter* spp. infections had more severe outcomes than those with more frequently occurring meningitis caused by other Gram-negative bacteria, including *Enterobacter cloacae* (Willis and Robinson, 1988).

3.2 Documented cases of *Cronobacter* spp. infection linked to contaminated PIF

Documented cases of *Cronobacter* spp. infection in infants and young children have been collated by different authors and groups. An overview of all cases since those first recorded in

1958, until 2008, was presented by the working group of the FAO/WHO, 2008. Around 120 documented cases of *Cronobacter* spp. infection and at least 27 deaths from all parts of the world were reported.

One of the first instances in which cases of neonatal meningitis were directly attributed to contamination of PIF was reported by Muytjens et al (1983). In environmental samples from a hospital in The Netherlands where 5 of 8 cases of neonatal meningitis were treated, *Cronobacter* spp. was isolated from prepared infant formula but not from the PIF itself. A spoon and dish brush used in the preparation of formula also tested positive for *Cronobacter* spp. These cases may therefore have been caused by a lack of hygiene and handling practices (Muytjens et al, 1983). This report suggests that PIF was the vehicle rather than the source of infection.

Several years later, *Cronobacter* spp. were isolated from unopened cans of PIF being used in a hospital in Iceland where three infants with *Cronobacter* spp. infection were being treated. The strains isolated from the PIF were indistinguishable from the strain isolated from the patients. *Cronobacter* spp. were not isolated from any environmental sources in the neonatal wards or in the milk kitchen. Although the PIF was always prepared following the manufacturer's guidelines and given to the infants within two hours of preparation, anecdotal evidence suggested that occasionally, formula bottles were left in heaters at 35 to 37°C for undisclosed periods of time, which may have allowed the pathogen to proliferate in the reconstituted PIF (Biering et al, 1989). This report suggests that PIF was both the vehicle and source of the infections.

An outbreak of *Cronobacter* spp. infections in infants in Mexico in 2010 was reevaluated (Jackson et al, 2015) and it was reported that *Cronobacter sakazakii* was recovered from both the powdered and reconstituted PIF fed to infants, and their faecal samples. All strains of *Cronobacter sakazakii* recovered from these sources showed identical biotypes, adhesion and invasiveness factors, and pulsed-field gel electrophoresis profiles. The authors identified two issues in this incident that deserved attention:

- (i) the PIF was reconstituted at around 40°C, i.e. well below the 70°C recommended by the WHO
- (ii) PIF was recognised as the primary contamination source of *Cronobacter sakazakii*

In some case reviews conducted where infants became ill or died with an infection thought to be caused by contamination of PIF it has not been possible to trace the bacteria directly to the product. In a case review following the death of an infant in Brazil from meningitis in 2017 it was known that PIF had been fed but no sample was available and the investigators concluded that '*Because contaminated PIF from opened cans has been identified as the vehicle in nearly all infant Cronobacter infections in the past decade for which a source has been found, this lack of testing is probably the most significant limitation of this investigation.*' (Chaves et al, 2018).

The role of *Cronobacter* spp. in PIF was recently highlighted among Iraqi infants with neonatal sepsis (Hassan and Naser, 2018) where the authors reported a significant association between PIF feeding and incidence of *C. Sakazakii* infection, and high mortality (75%) in *C.sakazakii*-positive children compared with those who were *C.sakazakii*-negative (21%).

A case study of contaminated powdered infant formula in Belgium (Van Acker et al, 2001)

One of the first cases to provide clear epidemiological and microbiological evidence that contaminated PIF can be the source of infection was reported by the microbiology department of a hospital in Belgium, where 12 neonates with birthweights <2000g developed NEC in June-July 1998. All had been fed PIF before becoming symptomatic and 10 of the 12 had received the same brand (Nestlé Alfaré) compared to four out of 38 infants without NEC. Six of the 12 infants with NEC had positive cultures for *Cronobacter* spp. compared to none of the 38 infants without. Furthermore, of the 14 infants who had received the implicated infant formula, six had positive cultures for *Cronobacter* spp. compared to none of the 36 who did not receive that formula.

PIF was prepared in the NICU by weighing and mixing powder using sterilised equipment. The blender head was rinsed in cooled tap water between preparations. PIF was made up with chilled, distilled mineral water and left on cooling tables before being distributed and stored in fridges.

Cronobacter spp. were isolated from several bottles of the reconstituted Alfaré in the hospital milk kitchen, whereas cultures from another brand were negative. Samples from the mineral water used to make up the formula and the water used to clean the blender head were also negative. However, it is not known whether any environmental samples were taken from work surfaces in the milk kitchen. *Cronobacter* spp. were isolated from a single, unopened batch of the implicated PIF. Molecular typing confirmed strain similarity between all PIF isolates and three patient isolates.

After the use of the contaminated PIF was stopped, no further cases of NEC were observed.

The authors identified that whilst the manufacturers microbiological quality control data for the contaminated batch fulfilled the requirements of the Codex Alimentarius code of practice for foods for infants and children (CAC/RCP 21-1979) at that time, it did not fulfill the more stringent requirements of Belgian law. The batch was recalled, the production facility upgraded, appropriate hygienic measures were taken and more stringent release norms for dietetic specialities (now known as Foods for Special Medical Purposes (FSMP) were applied by Nestlé.

Mechanism of infection by *Cronobacter* spp.

Cronobacter has a range of physiological attributes that enable it to survive in dry PIF and grow and multiply in reconstituted PIF. In order for it to cause systemic infections it must also possess a range of virulence factors to enable it to attach to and invade host cells and spread throughout the body. It is thought that the most likely route of infection with *Cronobacter sakazakii* in infants is through ingestion of contaminated PIF. Following ingestion, infection is likely to occur after colonisation by attachment and invasion of cells in the mucous membranes and gastric and intestinal epithelial tissues, prior to internalisation within the enterocytes or movement through

the epithelial layer into the bloodstream and across the blood brain barrier (Jaradat et al, 2014; Kent et al, 2015; Almajed and Forsythe, 2016).

Despite knowing that *Cronobacter sakazakii* has the capacity to cause systemic infection, the exact mechanism is still not fully understood. Some *Cronobacter* spp. have been shown to produce an *enterotoxin* that is heat-stable and able to survive pasteurisation and therefore remain active in PIF (Jaradat et al, 2014). However the importance of enterotoxin in the pathogenicity of *Cronobacter* spp. is not known (Jaradat et al, 2014). *Cronobacter* spp. along with other gram-negative bacteria are associated with the production of endotoxins or lipopolysaccharide (LPS). The presence of LPS in infant milk, enhances the permeability of the neonatal intestinal epithelium and consequently increases bacterial translocation from the gut and across the blood-brain barrier (Townsend et al, 2007). LPS is also heat-stable and persists during the processing of PIF, remaining viable for long periods in reconstituted PIF (Jaradat et al, 2014, Townsend et al, 2007).

Risk factors for *Cronobacter* spp. infection

Some of the factors that make neonatal infants susceptible hosts include the immaturity of their gastro-intestinal and immune systems, the lack of a mature gut microbiota and a higher than usual gastric pH. It has been suggested that some strains of *Cronobacter* spp. elicit the type 2 immune response¹, which is known to be inefficient in fighting intracellular infections. The bias of neonatal immune response towards this type of immune response may help to explain neonatal inability to eliminate the pathogen (Townsend et al, 2007). Enteral feeding, especially the administration of infant formula, can lead to microbial colonisation of the gut with both commensal species and others that are capable of causing damage. The presence of harmful microbes in the gut of susceptible neonates can cause mucosal inflammation, which results in the production of high levels of inflammatory factors including cytokines, nitric oxide, platelet activating factor and prostanooids to name a few, which further damage the epithelial barrier. Bacterial translocation across the compromised barrier exacerbates the inflammatory response, leading to more epithelial damage, more bacterial translocation, and ultimately, intestinal necrosis (Grishin et al, 2013).

Cronobacter sakazakii (and *Salmonella*) have some tolerance to acid environments and it has been reported that 72 strains of *Cronobacter* spp. were able to grow at pH 4.5 whilst some could grow at a pH as low as 3.9 (Dancer et al, 2009). The majority of preterm infants are able to maintain a gastric pH less than or equal to 4, which should provide a barrier to many strains of *Cronobacter* spp. (and *Salmonellae*) (Hyman et al, 1985). However, hyposecretion of stomach acid during the immediate neonatal period could result in a less acidic gastric environment (Euler et al, 1977), which may support growth of some strains of *Cronobacter* spp. enhancing the susceptibility of the neonate to infection. A gastric pH of 5 has been reported in neonates (Zhu et al, 2013).

3.3 Infections caused by *Salmonella* spp. contamination

¹ This immune response characterised by the production of specific immune factors (interleukin-4 (IL-4), IL-5 and IL-13) against helminths invading cutaneous or mucosal site and in allergic diseases.

There are nearly 2,000 strains of the *Salmonella* bacteria that can cause illness in humans. Symptoms include diarrhoea, fever and vomiting, and infection can cause serious illness in infants. When *Salmonella* infections become invasive, they can affect the bloodstream, bone, joint, brain and nervous system, and other internal organs. Invasive *Salmonella* infections may cause serious conditions such as bacteraemia, meningitis and osteomyelitis (CDC, 2018).

Incidence rates of infection from *Salmonella* spp.

The incidence of salmonellosis (from all sources) is more common among infants and young children but it is unclear whether the increased rates results from greater susceptibility, or whether infants are more likely than persons in other age groups to be brought for medical care, or to have stool cultures performed for symptoms of salmonellosis. In many regions of the world where *Salmonella* serotyping is not routinely performed and *Salmonella* surveillance networks are not established, identification of geographically and/or temporally diffuse outbreaks of *Salmonella* infection is difficult (Cahill et al, 2008) and, as for *Cronobacter* spp., incidence rates are likely to be under estimates.

Table 3 provides some estimates of incidence of *Salmonella* infection in infants, but these cases are not necessarily related to PIF. Whilst data on *Salmonella* spp. infections are reported regularly in the UK these do not consider rates of infection separately in infants and so more recent data to approximate incidence is not available.

Table 3. Estimates of incidence of *Salmonella* spp. infection in infants <12 months old

Study group and reference	Country	What was measured?	Estimate of incidence (cases per 100,000 infants)
US Centres for Disease Control (CDC, 2016)	US	<i>Salmonella</i> spp. Infection reported in 2015 through 10 US FoodNet sites for 5,126 infants.	129.1
Cheng et al (2013) Infant Salmonellosis in the US 1996-2008	US	<i>Salmonella</i> spp. Infections reported through the Foodborne Disease Active Surveillance Network.	177.8
Weinberger et al, 2006	Israel	<i>Salmonella</i> spp. Infection data submitted to Central Government Laboratories, during 1997–2002 for infants.	92.8
Skirrow, 1987	England	<i>Salmonella</i> spp. Infection data from 5 public health laboratories during 1983–1984 for infants	181

3.4 Documented cases of *Salmonella* infection linked to contaminated powdered infant formula

Table 4 outlines *Salmonella* spp infection in infants who were known to consume PIF. In some outbreaks it has not been able to identify the *Salmonella* bacteria in the PIF itself but there have been clear epidemiological links. It is important to consider the challenge of detecting low level *Salmonella* contamination and the limits of microbiological testing. This is important as it means that whilst constant testing of products and production facilities for bacteria remain essential, it is the safe preparation of PIF with water at a temperature which can kill any bacteria present which will protect infant health.

Table 4. Salmonellosis infections in infants known to have consumed powdered infant formula, 1985-2018.

Reference	Year	Location	<i>Salmonella</i> serotype	No. of cases	<i>Salmonella</i> isolated from PIF	PIF epidemiologically implicated
Rowe et al, 1987	1985	UK	<i>ealing</i>	48	✓	✓
CDC, 1993	1993	USA & Canada	<i>tennessee</i>	>3	✓	✓
Usera et al, 1996	1994	Spain	<i>virchow</i>	48	✓	✓
Threlfall et al, 1998	1996-1997	UK and France	<i>anatum</i>	17	x	✓
Park et al, 2004	2000	Republic of Korea	<i>london</i>	30	✓ from open package	✓
Brouard et al, 2007	2004-2005	France and export countries	<i>agona</i>	141	✓	✓
Rodriguez-Urrego et al, 2010	2008	Spain	<i>kedougou</i>	31	x	✓
Jourdan et al, 2008	2008	France	<i>give</i>	8	x	✓
National Centre for Epidemiology 2011	2010-2011	Spain	<i>poona</i>	280	NK	✓
Jourdan-da Silva et al, 2018	2017-2018	France and 83 export countries at Jan 2018	<i>agona</i>	37	x	✓
Jones et al, 2019	2019	France (Spanish formula)	<i>poona</i>	33	x	✓

Recent *Salmonella* outbreaks in Europe resulting from bacterial contamination of powdered infant formula

In France in 2017/2018 at least 37 infants became infected with *Salmonella agona* as a result of consuming contaminated PIF. This outbreak involved the recall of 12 million boxes of product across 83 countries. All cases had gastrointestinal symptoms and there were no cases of bloodstream infection or meningitis. The median age of the infants involved was four months.

The identification of eight isolates of *Salmonella agona* in infants within a period of eight days alerted the French National Research Centre to the outbreak. Prompt investigation provided strong epidemiological evidence pointing to infant milk products manufactured by the same company as the source of the outbreak. In the three days before the onset of symptoms 35 out of 36 cases had consumed infant milk products manufactured by Lactalis Nutrition Santé group in France.

A previous *Salmonella agona* outbreak affecting 141 confirmed cases occurred in France in 2005 and was associated with two different products manufactured within the same facility implicated in the more recent outbreak (Brouard et al, 2007). During the 2005 outbreak, samples of the implicated products and environmental samples from the facility yielded isolates with the same Pulsed-Field Gel Electrophoresis (PFGE²) pattern as the clinical isolates, the level of contamination was considered to be low. The production dates of the food samples which tested positive for *Salmonella* suggested a persistent environmental contamination, the source of which was not identified in the facility.

In January 2018 a cluster of four isolates of *Salmonella poona* in infants in France were isolated and by March 2019 30 confirmed cases had been identified across 11 regions, and three further cases were isolated in Spain. Half of all cases were hospitalised (Jones et al, 2019). Parents and guardians all reported using a particular brand of formula (but different types: infant formula, follow-on formula and milk marketed as a food for special medical purposes) based on protein hydrolysates from rice. These formula had been exported widely and infection was traced back to a drying tower in the formula factory.

3.5 Product recalls due to contamination or suspected contamination of PIF

Globally, voluntary recalls of PIF are issued in response to contamination or suspected contamination with bacteria, particularly *Salmonella* spp. and *Cronobacter* spp. Some of those documented over the past 20 years are shown in Appendix 2. This highlights the frequency with which product recalls occur. Whilst companies act swiftly to recall products that are found to be contaminated or may be at risk of being contaminated, the need for transparency from manufacturers as well as regular testing of products by independent bodies, as well as safe instructions for preparation, is essential.

² A laboratory technique used to produce a DNA fingerprint for a bacterial isolate; i.e. a group of the same type of bacteria.

4.0 Why do instructions for making up powdered infant formula vary?

It has been suggested as a myth that manufacturer's instruction for preparing PIF are complete and definitive in telling caregivers how to avoid the risks of infection by *Cronobacter spp* and other pathogens (Farmer III, 2015). Reflecting on 40 years of work with *Cronobacter spp*. Farmer concludes that the breastmilk substitute industry has done a poor job in providing complete information and instructions to consumers and highlights that:

'...there is no universal standard or wording that commercial manufacturers of PIF are required to follow'.

The WHO guidance is clear that water at a temperature of at least 70°C is needed to kill any pathogenic bacteria in the PIF (WHO, 2007). In many countries however it has become acceptable that PIF instructions suggest that infant formula can be made up with water at room temperature. Silano et al (2016) reviewed evidence for the risk of contamination of PIF and global guidance on how infant formula should be safely prepared and concluded that there is no clear consensus on the recommendation for home preparation of PIF and several contradictory guidelines. These authors advised a precautionary approach following guidance from WHO since there is clear evidence of risk.

In some areas there is contradictory advice from health bodies – in the US the Food and Drug Administration (FDA) suggest that families should follow manufacturers instructions, where advice to make formula with water at room temperature is given. The US Center for Disease Control, however, suggests that PIF is made up with water at temperatures above 70°C (CDC, 2019). In Europe there is no clear recommendation from EFSA and the influential industry-involved European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) does not consider the use of water >70°C necessary.

In the UK, WHO guidance for PIF reconstitution has been maintained for infant formula and follow-on formula, but products marketed as foods for special medical purposes (FSMP) are exempt from this guidance. This is the case even if they are sold over the counter without guidance to families about potential risks of not following this guidance. Information on the current FSMP market and all the products that currently recommend reconstitution temperatures <70°C, with the rationale given by the manufacture (if one was given), can be found in the report *Specialised infant milks in the UK* at <https://www.firststepsnutrition.org/composition-claims-and-costs>.

4.1 The addition of probiotics to powdered infant formula for term infants

Probiotics are live micro-organisms that, when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Human breastmilk contains probiotics as well as hundreds of different types of prebiotic oligosaccharides. Cows' milk, the basis of most PIF, contains virtually none (Teitelbaum and Walker, 2002). In their desire to create infant formulas which mimic the bifidogenic activity of breastmilk some manufacturers have supplemented their products with prebiotics and/or probiotics; in combination these may also be called synbiotics. The rationale for their addition to infant formula is that they may be capable of modifying the balance of intestinal microflora in favour of commensal (beneficial) bacteria over pathogenic

bacteria, which it is suggested may offer a protective effect. The hypothesised benefits that are most frequently studied in healthy, term infants include improved stool frequency/consistency, relief of gastro-intestinal discomfort, reduced risk of common childhood infections and reduced risk of allergy.

Whilst this area of research has attracted an enormous amount of scientific and commercial interest, the studies which investigate the use of probiotics in infant formula for healthy term infants, either alone or as synbiotics (i.e. in combination with prebiotics), differ greatly with respect to the quality of the study, the probiotic strains used, dose, outcomes measured and treatment period.

In the EFSA opinion on the essential composition of infant formula and follow-on formula (EFSA, 2014) the authors reviewed a number of studies and systematic reviews on the potential benefits of probiotics in infant formula, and did not find any significant physiological or health effects among those consuming supplemented formula compared with those given unsupplemented formula. They also noted the evidence for any benefit of probiotics or synbiotics on infant health comes from single studies, as opposed to systematic reviews, and those studies have methodological limitations, They therefore conclude that there is no evidence for beneficial effect, and that these are not necessary additions to infant or follow-on formula (EFSA, 2014).

In a systematic review of randomised control trials (RCT) that compared the use of infant or follow-on formula supplemented with probiotics and/or prebiotics, the ESPGHAN Committee on Nutrition noted that, whilst there was some evidence available to suggest an association (but not a causal relationship) between the use of specific probiotics in infant formula and a reduction in the incidence of gastrointestinal infections and antibiotic use, there was too much uncertainty to draw reliable conclusions (Braegger et al, 2011). Whilst the committee found no evidence for adverse effects of probiotic use in products for infants, they did raise some specific concerns:

"First, timing, that is, the administration often begins in early infancy, sometimes at birth when the gut microbiota is not fully established, and factors that influence microbiota may permanently affect the development of the ecosystem. Second, duration, that is, the daily administration of such products is often prolonged (several weeks or months). Last but not least, delivery is in the form of a specific matrix (infant formula) that could be the only source of feeding of an infant."

There has been a large number of studies and reviews conducted on the efficacy of probiotics on infant health but whilst there is some evidence of favourable outcomes there is a consensus that variations in types of probiotics used and dosage makes it difficult for recommendations to be made. For example, a recent systematic review looking at evidence for a benefit from the addition of probiotics on infantile colic concluded that there was limited evidence of benefit in full-term breastfed infants (Karkaneh et al, 2019) and it has been concluded elsewhere that while probiotics are a multi-billion dollar industry, there is little evidence to show that supplementing healthy term infants provides any health benefits (Quin et al, 2018). In fact there is a suggestion that whilst probiotic exposure during infancy has limited effects on gut microbial composition, it is associated with increased infection later in life. These correlative findings should caution clinicians against probiotic supplementation during infancy until rigorous

controlled follow-up studies determining their safety and efficacy have occurred (Quin et al, 2018).

There are a lack of studies which examine the effect of probiotics against the clinical manifestations of *Cronobacter* spp. infection in term infants; doubtless because the neonates at greatest risk of infection are those that are born preterm or at low birthweight.

4.2 The addition of probiotics to powdered infant formula for preterm infants

Intestinal dysbiosis (or intestinal microbial imbalance) has recently been proposed as an important factor in the pathogenesis of NEC (Underwood, 2017). The bacteria that colonise the gut of preterm infants may largely be acquired from the newborn intensive care unit (NICU) environment, rather than from the mother's genital tract flora, skin, or breast milk. In addition, gastrointestinal colonisation with normal bacterial flora (eg, *Bifidobacterium* spp. and *Lactobacillus* spp.) is delayed and lacks biodiversity, whereas colonisation with potentially pathogenic bacteria is increased (eg, *Escherichia coli*, *Enterococcus* spp., and *Klebsiella pneumoniae*). Broad spectrum antibiotics may further modify the composition of the intestinal flora and predispose very preterm infants to both late-onset sepsis and NEC (Jacobs et al, 2013).

Modifying infant formula with probiotic strains of bacteria has been suggested as one way to potentially reduce the risk of developing NEC. Although their mechanisms of action are not completely understood, it is suggested that probiotics may impair the growth of pathogenic species such as *Salmonella* and *Cronobacter* spp., which are most commonly associated with NEC (Hunter et al, 2008). Some systematic reviews and meta-analyses of RCTs have suggested that oral administration of probiotics in preterm infants provides a protective effect against NEC (Deshpande et al, 2010, Alfaleh and Anabrees 2014), whilst others suggest that there is insufficient evidence to recommend the routine use of probiotics (Mihatsch et al, 2012). In addition, in some of the trials included in meta-analyses, infants in the probiotics groups had higher rates of sepsis compared to the control group (Dani et al, 2002; Lin et al, 2008). Caution should be exercised against extrapolating conclusions from heterogeneous clinical trials to specific probiotics, as clinically relevant effects on immune modulation, efficacy and safety may be strain specific (Neu 2014, Mihatsch et al, 2012).

A systematic review by van den Akker et al (2018) looking at strain-specific outcomes in preterm infants and conducting a network meta-analysis to identify strains with potentially greater efficacy considered fifty-one randomised control trials (RCT) involving 11,231 preterm infants and found that most strains or combinations of strains were only studied in a small number of trials. Only three of the 25 studies looking at probiotic treatment combinations showed significant reduction in mortality rate, seven reduced the incidence of NEC, two reduced late-onset sepsis, and three reduced time until full enteral feeding. There was no clear overlap of probiotic strains which were effective on multiple outcomes and the authors concluded that efficacy in reducing mortality and morbidity was only found in a minority of the studied strains or combinations of probiotic strains. This may be due to an inadequate number, or size, of RCTs or could be due to a true lack of effect for certain species. As has been the case from many reviews conducted it was also concluded that further large and adequately powered RCTs using

strains with the greatest apparent efficacy are needed before treatment strategies can be suggested.

Although available studies have not reported any adverse effects, we counsel caution in the introduction of any potentially infectious agent for immunologically immature very low birth weight infants. Each probiotic strain and potential combinations need to be characterised separately; efficacy and safety should be established for each product.

4.3 Potential risks associated with the addition of probiotics to powdered infant formula

It has been suggested that there is not enough available evidence suggesting that the use of probiotics in preterm infants is safe, let alone beneficial (Agostoni et al, 2010). Incorporating probiotics or bacteriocins produced by lactic acid-producing bacteria (LAB) into infant formula as a method of directly or indirectly improving their intrinsic microbiological safety is challenging as they have been shown to be heat labile, particularly at the temperatures used during production of PIF. In order to remain effective, they need to be added after any thermal processing stages, which raises important questions about the safety of PIF. As probiotics are live bacteria which, along with pathogenic bacteria, would not survive reconstitution with water at 70°C, a risk assessment weighing up the perceived benefits of feeding an infant with a probiotic infant formula or supplementing infant formula with other sources of pharmacological probiotic preparations would be required. The availability of infant milks marketed as FSMP containing probiotics in the UK has highlighted the lack of consensus surrounding the 70°C reconstitution recommendation. Whilst there has not been an independent review of benefits associated with the addition of probiotics to infant milks in the UK, evidence review by First Steps Nutrition Trust suggests that this is weak. Their review of advertising claims related to the addition of probiotics in two infant milks marketed in the UK can be reviewed in the report *'Scientific and Factual: A further review of breastmilk substitute advertising to healthcare professionals'* available at <https://www.firststepsnutrition.org/working-within-the-who-code>.

The Norwegian Scientific Committee for Food and Environment (2014) has taken a more cautious approach and in an assessment of infant formula and follow-on formula with *Lactobacillus fermentum* they concluded that whilst the manufacturer had submitted some data regarding possible long-term adverse effects of giving a probiotic strain daily as a “monoculture” over a prolonged period of time, these data were not sufficient to draw any conclusion regarding long-term safety of the strain.

'It is supposed that the early composition of the human gastro-intestinal tract microbiota can have long-lasting functional effects. If that is the case, a daily supply of a “monoculture” of a single, specific strain such as L. fermentum CECT 5716, in large quantities over a prolonged period of time to age groups where the intestinal flora is still developing may therefore have unknown, but possible long-lasting adverse effects.'

In 2016 in reviewing a probiotic supplement, this opinion was reiterated (Norwegian Scientific Committee on Food and Environment, 2016):

'As evidence is accruing that the early microbial composition of the infant gut is important for the development of the gut flora and the immune system of the growing child, it is not possible to exclude that a daily supply of a particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract, may have long-term, albeit still unknown, adverse effects on its development.'

We would also urge caution in the use of probiotics in infant milks and believe that the known risks posed by bacterial contamination should take precedent over the use of probiotics in infant formula where benefits are debated and long term risks are not known.

4.4 Can acidification of powdered infant formula reduce the risk of bacterial contamination?

The acidification of infant formula is one innovation which is intended to tackle the issue of intrinsic bacterial contamination. Acidified infant formulas are "Infant and follow-on formulae that have been fermented with LAB during the production process, but do not contain live bacteria in the final product due to inactivation of the fermenting bacteria by heat treatment or other means". (ESPGHAN, 2007). The logic is that the beneficial LAB produce inhibitory compounds such as organic acids and bacteriocins during fermentation which have an antimicrobial effect (Awaisheh et al, 2013), so acidifying the formula prevents the growth of pathogenic bacteria.

However, and despite widespread use, there is little published data available to indicate their effectiveness. The few trials that are available examine their impact on diarrhoeal disease in infants, but none examine the clinical manifestations of *Cronobacter* spp. or *Salmonella* infection. While a small number of studies have examined the antimicrobial activity of specific strains of bacteria against such pathogens in dry and reconstituted PIF, the results are mixed. For example, when stored at ambient temperatures (~21°C), although *Lactobacillus acidophilus* was found to persist in both dry and reconstituted PIF for 14 hours, it did not have any antimicrobial activity against *Cronobacter* spp. (Al-Holy et al, 2009). Similar results were obtained for *Bifidobacterium breve* in reconstituted PIF when stored for more than two hours at more than 30°C. When held for more than two hours at 37°C, *B.breve* actually appeared to stimulate the growth of *Cronobacter* spp. (Osaili et al, 2008).

Conversely, another study investigated the antibacterial activity of *Lactobacillus acidophilus* and *Lactobacillus casei* isolated from the faeces of healthy infants, in reconstituted PIF and showed that the concentration of included live LAB increased over a six hour period and inhibited the growth of some strains of *C. sakazakii*. But while the LAB produced bacteriocins had an inhibitory effect on the *C.sakazakii* strains in the reconstituted PIF, this effect was not observed when the liquid was heated to 60°C and 80°C or treated with proteolytic enzymes, suggesting that the bacteriocins produced by LAB may be inactivated by heat (Awaisheh et al, 2013).

Zhu et al (2013) reported that under neonatal gastric acid condition of pH 5.0, formula that had been slightly acidified with different organic acids did not exert an inhibitory effect on its own, but in the presence of infant gastric acid did exert an inhibitory effect on *Cronobacter* spp. populations which was visible in the neonatal stomach (Zhu et al, 2013).

In 2007 the ESPGHAN Committee on Nutrition carried out a systematic review of the literature to assess knowledge on the effects of fermented infant formula without live bacteria. They concluded that:

"the published data on the effects of fermented infant formulae without live bacteria are limited and do not allow firm conclusions"

In 2015 a systematic review by Szajewska et al also concluded that the limited available evidence suggested that the use of fermented infant formula, compared with the use of standard infant formula did not offer clear additional benefits (Szajewska et al, 2015).

5.0 Current UK advice on reducing the risk of infection due to contamination of powdered infant formula and follow-on formula.

In the UK instructions on the safe preparation, handling, storage and use of PIF are provided by the Food Standards Agency. The Food Standards Agency (FSA) issued guidelines on the safe preparation and storage of PIF in 2013 (Food Standards Agency, 2013) and this advice continues on the NHS website in 2019. The guidelines are summarised in Table 5.

Key guidance relates to the temperature of the water required for reconstitution and the statement used is:

'Boil at least 1 litre of fresh water from the cold tap in a kettle. Leave the water to cool for no more than 30 minutes'.

It is known that bacteria multiply most rapidly at temperatures between 7°C and 65°C. Even at 5°C – the temperature recommended for domestic fridges – multiplication will continue but at a much-reduced rate. The guidelines are designed to reduce the holding time between reconstituting and using feeds in order to minimise the amount of time during which bacterial multiplication can occur, as well as including recommendations for cleaning and sterilising all feeding equipment and for making up formula. Following these guidelines can reduce the risk of infection from micro-organisms in PIF.

Table 5. Guidelines on the safe preparation and storage of PIF.

General recommendations	
Recommendation	Rationale
Make up feeds one at a time as the baby needs them.	To reduce the holding time between reconstituting and using feeds in order to minimise the amount of time during which bacterial multiplication can occur.
Sterilise all bottles and equipment to be used.	The infant's immune system is not as well developed as an adults. This recommendation minimises the risk of illness and infection.
Use water from the cold tap to make up feeds. Do not use bottled or artificially softened water.	Bottled water is not sterile and may contain too much sodium or sulphate. If you must use bottled water, check on the label that the sodium (Na) level is less than 200mg/l and the sulphate (SO or SO ⁴) level is no higher than 250mg/l.

Recommendation	Rationale
Boil at least 1 litre of fresh water from the cold tap in a kettle. Do not use previously boiled water. Leave the water to cool for no more than 30 minutes.	This step should ensure that the water used to reconstitute the feed is at a temperature above 70°C, which will kill most of the pathogenic micro-organisms that may be present in powdered formula.
Clean and disinfect all equipment and work surfaces to be used and wash your hands. Keep teat and bottle cap on the up-turned lid of the steriliser. If using a cold-water steriliser, shake off excess solution and rinse bottles in cooled boiled water from the kettle. Do not use tap water.	To avoid contamination of bottles with bacteria from tap water or unclean work surfaces.
Pour the correct amount of cooled, boiled water into bottles and double-check the volume before adding the powder. Fill the scoop loosely with milk powder according to the manufacturer's instructions. Level off the scoop using the leveller provided or the back of a clean, dry knife. Always use the scoop provided with the powder you are using. Add the powder to the water in the bottle.	Scoop sizes differ between manufacturers and between different milk powders from the same manufacturer. Too much powder may result in constipation or dehydration.
Holding the edge of the teat, put it on the bottle and then secure the retaining ring and cap. Shake the bottle until the powder is dissolved.	
Cool the formula by holding the bottom of the bottle under cold running water. Do not allow the tap water to touch the bottle cap. Test the temperature of the milk by shaking a small amount onto the back of your wrist. It should be body temperature and feel warm or cool but not hot.	
Discard any of the feed in the bottle that has not been used.	
Make up feeds 1 at a time as your baby needs them.	Advice is to make up feeds freshly as they are needed rather than to store them.
If made-up formula is stored: <ul style="list-style-type: none"> • in a fridge – use within 24 hours • in a cool bag with an ice pack – use within 4 hours • at room temperature – use within 2 hours 	

Source: Food Standards Agency, 2013, NHS,2019.

5.1 Current practices in making up powdered infant formula

At least one study has shown that it is not feasible for those who make up infant formula to easily determine the temperature of water used to reconstitute PIF in order to meet the 'above 70°C' guideline (Food Standards Agency, 2009). Following the advice to reconstitute PIF using water which had been boiled and left for 30 minutes resulted in temperatures ranging from 46°C to 74°C, because the rate of cooling depends on the volume of water boiled. Although it is specified in the guidelines that at least one litre of water should be boiled and left to cool at room temperature for 30 minutes, to achieve the minimum temperature of 70°C, a shorter waiting time might be prudent to ensure that water at the appropriate temperature is used.

The 2010 Infant Feeding Survey (McAndrew et al, 2012) reported that almost half (49%) of all mothers who had made up PIF in the last seven days followed all three recommendations of only making one feed at a time, making feeds within 30 minutes of the water boiling, and adding the water to the bottle before the powder. This is a substantial increase in the proportion of mothers following all three guidelines in 2005 (13%) but is likely to be primarily due to improved practice in relation to making up only one feed at a time rather than increased adherence to guidelines on water use.

5.2 Does the current advice ensure that the water is above 70°C when powdered infant formula is reconstituted?

Recent outbreaks of Salmonella infections linked to the intake of PIF has rekindled attention on the optimum procedures needed for the safe preparation of products. Losio et al (2018) looked at the impact of time and temperature on survival of pathogenic bacteria in PIF through artificial contamination by inoculating powdered formula with *Salmonella agona* and *Cronobacter sakazakii* and testing whether they survived when water at 70°C was used to reconstitute. They reported that using water at 70°C meant that the water was too cool once poured into the bottles to deactivate the bacteria present. Starting with water at 70°C the maximum temperatures registered in the 200ml of reconstituted PIF was between 57.5-60°C. They tested boiling 500ml of water and leaving it to cool for 10 minutes at room temperature and found that a starting temperature of mean 87°C meant that once mixed with the PIF the mean temperature was 76°C and no pathogens survived. They concluded that:

'Water at a higher temperature must be considered to prepare powdered formulas to improve food safety'

We have looked at the temperatures of water in a domestic setting, using 1 litre of water left to cool in the kettle for no more than 30 minutes (poured at 29 minutes post boiling) and found that once the water was poured in the bottle, regardless of volume, the temperature was significantly below 70°C. We repeated this several times and at periods of no more than 25 minutes, 20 minute and 15 minutes. To do these tests we followed a procedure that would be used by a caregiver, pouring the required amount of water into a bottle from the kettle in a typical ambient temperature for a household and taking the temperature before any powder was added. It is likely that the addition of powder will reduce the temperature further and therefore a water temperature of close to 70°C may not be sufficient to ensure all the powder is met with water hot enough to kill any pathogens present.

Whilst this is not laboratory data and should be repeated in a variety of settings with fully calibrated equipment, it supports the data from Losio et al (2018) that current guidance to leave the water in the kettle for no more than 30 minutes should be reviewed.

Table 6. Temperature of water poured into a feeding bottle by time water left in the kettle and volume poured into the bottle.

Time 1 litre of water left in kettle after boiling	Temperature of the water °C after pouring into the bottle (mean of multiple temperature tests).				
	210ml	180ml	150ml	120ml	90ml
No more than 30 minutes (29 minutes)	66.6	66.3	66.3	66.2	65.7
No more than 25 minutes (24 minutes)	71.1	71.0	71.0	69.1	68.2
No more than 20 minutes (19 minutes)	74.1	73.3	73.1	71.7	71.6
No more than 15 minutes (14 minutes)	76.8	76.8	76.1	76.1	75.7

Advice on energy saving

The climate emergency that has been declared by the UK Government is promoting many people to review their use of household energy. Organisations such as Which? provide advice on energy saving that includes:

‘Only fill and boil the kettle with as much water as you need’

This is included in most energy saving tips and means that many caregivers may not want to boil a litre of water to make up a relatively small amount of infant formula. Guidance should therefore also be given for smaller amounts of water as this will cool more quickly.

We recommend that the Food Standards Agency consider again the recommendations made to ensure water is at a temperature of 70°C or above when PIF is added and that any instructions are adequate for the domestic preparation of infant milks using both 1 litre volumes of water and smaller volumes. Our recommendation based on our domestic temperature tests is that a more prudent recommendation would be to boil 1 litre of water in a kettle and leave for between 15 and 20 minutes. Lasio et al (2018) recommend a time of no more than 10 minutes if 500ml of water is boiled and left in a kettle.

5.3 Using other methods for reconstituting powdered infant formula

Start4Life and Unicef UK Baby Friendly in their guide to bottle feeding (<https://www.gov.uk/government/publications/start4life-updated-guide-to-bottle-feeding/start4life-guide-to-bottle-feeding>) recommend that the safest way to make up feeds from PIF when away from home is to make the feed up freshly using a vacuum flask of boiled water. The boiling water should kill any bacteria present in the flask. The feed can then be made up in a sterilised feeding bottle using PIF pre-measured into a small, clean, dry container and the correct amount of boiled water from the vacuum flask. The Start4Life and Unicef UK Baby Friendly guidance states that vacuum flasks, if full and securely sealed, will keep the water temperature above 70°C for several hours.

We tested several typical vacuum flasks, which held three different volumes of water, over a period of between 30 minutes and three hours in a domestic setting. The flasks were warmed for one minute with boiling water before use, filled to capacity and stored at an ambient temperature of about 19°C.

Table 7 gives the average of three water temperatures when the procedures were conducted three times, with each test completed on a freshly stored batch of boiling water. These tests were not carried out in a laboratory setting and mean temperatures given represent the average of three tests undertaken. We strongly recommend these tests are repeated by the Food Standards Agency as part of work to re-assess safe preparation guidance.

Table 7. Temperature over time of different water volumes kept in a vacuum flask

Amount of water in the flask	Temperature when boiling water first added to flask °C	Temperature after 30 minutes °C	Temperature after 1 hour °C	Temperature after 2 hours °C	Temperature after 3 hours °C
Full flask: (approx. 33oz, 1000ml)	94	86	82	78	75
Full flask: (approx. 17.5oz, 500ml)	94	92	90	86	76
10oz (280ml)	93	80	74	72	66
5oz (140ml)	92	72	70	64	58

The 10oz/285ml flask of water was also tested at two hours and 30 minutes and the temperature had dropped to an average 68°C. This suggests that 10oz/285ml flask of water

should be used within two hours. A smaller volume of 5oz/145ml will only remain above 70°C for about an hour.

We found that a full, standard sized flask (approx. 17.5oz/500ml) of boiled water, securely sealed, does remain at above 70°C for about three hours.

Currently neither the Food Standards Agency, NHS or Start4life provide information about making up milks safely using other methods such as automated preparation machines, hot taps or baby kettles. To fill this gap First Steps Nutrition Trust have collated data to offer some independent advice, but independent scrutiny is needed by the Food Standards Agency and that Government public health agencies to offer advice to families.

Formula preparation machines

Formula preparation machines are marketed as being a sterile and convenient method of preparing formula feeds at the correct temperature for consumption, within minutes. Whilst the scale of use of these is not known, recent research at Swansea University suggests that just over 50% of families are using preparation machines (personal communication, Amy Brown).

In the UK, the most popular formula preparation machine available at high street retailers is the Tommee Tippee Perfect Prep™ Machine. This machine claims to “*prepare a fresh bottle at just the right serving temperature within 2 minutes*”. The machine uses a two-step process to prepare the feed. In the first step the machine dispenses a ‘hot shot’ of water directly into the bottle. The user then has two minutes to add the PIF, place the holding cap on the bottle, shake to mix and return the bottle to the machine. In step two, cold water is added by the machine to make up the selected feed volume to a comfortable temperature to feed immediately.

Whilst research into the safety and efficacy of the Perfect Prep™ Machine has been carried out by the manufacturer, this is not currently in the public domain and the manufacturer has declined to release it citing business competition reasons. Mayborn Group Ltd, who produce Tommy Tippee brand products, have said:

“Our Perfect Prep product has been tested by an independent laboratory that validated that the ‘hot shot’ of water addressed the (E.Sakazakii) species of concern. The laboratory used was Intertek Testing Services (UK) Limited. The filter we use is not a standard water filter, such as the ones you might find in a Britta system – it’s an antibacterial filter. We have independently validated the removal of bacteria that may be present in water, and we have done this test in extreme circumstances, dosing the water with significantly higher levels of bacteria than typically found in water supplies, so we can be truly confident of the filter efficiency. Validation was carried out by Intertek Testing Services (UK) Limited.”

However, unpublished university-based research which investigated the efficacy and temperature profile of the Tommee Tippee Perfect Prep™ Machine using PIF inoculated with known amounts of *Cronobacter sakazakii* has suggested that, whilst the machine’s hot shot of water dispensed onto a small volume of powder was able to eradicate more than 95% of the bacteria, it failed to reduce their numbers to an undetectable level. Whilst the machine produced water for the ‘hot shot’ at a temperature higher than the 70°C stipulated in current guidelines, the temperature fell to around 60°C after two minutes. Furthermore, when PIF was added at 30,

60 and 90 seconds after the 'hot shot', the temperatures in the bottle were only maintained for around five seconds before they fell again to between 52.5°C and 55.5°C.

This research showed that, depending on when the PIF is added, the water temperature may be too low to effectively eradicate all bacteria present. The volume of the initial hot shot of water used for a 4oz feed is about one fluid ounce, and it is questionable whether this small volume of water can adequately make contact at the right temperature with the amount of PIF added. The research suggests that this volume of water is insufficient to maintain a temperature of greater than 70°C for the duration of the two-minute window recommended for the addition of PIF. This data has not been published in a peer-reviewed journal and therefore can only be considered as contributory evidence at the present time (First Steps Nutrition Trust, personal communication).

The Food Standards Agency made the following comment when asked about the safety of these formula machines in 2014:

"The issues we have with it are, although it states it dispenses a 'hot shot' at 70°C to kill bacteria that potentially could be in the powder, the reality (if you watch the TT advert) is that the amount of hot water used is very small, and once this is dispensed into a cold bottle/cold powder the heat will be quickly lost (more so than when preparing a full bottle with cooled, boiled water to >70°C), so we would be interested to see whether TT have done any validation to see what temperatures the hot shot/powder combo actually reaches (and whether this is enough to destroy any bacteria). The other issue, is that the rest of the bottle is then topped up with cold water, which TT state is filtered to remove impurities. Again we would be interested to know whether it has been validated that the TT filter removes potential bacteria in the tap water (as this won't previously have been boiled). At present the Food Standards Agency would still advocate the use of our Best Practice Guidance, to use cooled, boiled water at >70°C to make up infant formula."

(Email communication between Francesca Entwistle (Unicef UK Baby Friendly) and Lorna Rowswell at FSA. February 2014)

The Food Standards Agency have not issued further information, but the Food Safety Authority in Ireland recently made the following statement on its website:

'The FSAI does not recommend the use of automatic machines to prepare bottles of powdered infant formula because there is insufficient data available to verify the safety and efficacy of these machines. The FSAI continues to recommend the use of cold tap water that has been boiled once and then cooled for no longer than 30 minutes to >70°C to prepare feeds from powdered infant formula'

https://www.fsai.ie/faq/bottle_feeding_safely.html

Hot taps

Many kitchens are now fitted with hot taps where either 'boiling' or cold water is dispensed directly. Recent survey data from Swansea University suggests that about 13% of families are using hot taps to make up PIF (personal communication, Amy Brown).

Current advice to leave water to cool for no more than 30 minutes is based on one litre of water being boiled and left in the kettle, with the aim that the water is still at a temperature of 70°C or above when the powdered infant formula is added. If smaller volumes of water are used cooling times will be significantly shorter.

Using a hot tap water will be dispensed directly into a bottle if being used for making up infant formula. We have tested temperatures using one type of hot tap in a domestic kitchen as a guide for those supporting families who make up formula this way.

Table 8 shows different volumes of water where the temperature was tested after the water had been added to the bottle and after five, 10 or 15 minutes. An 8oz (240ml) baby bottle was used and water added in ounces as recommended for making up feeds for different ages of babies. The temperatures were all taken twice, and the mean temperature has been provided. We put the lid on the bottle after adding the water and it is likely that leaving the bottle uncovered will impact on the temperature.

Table 8. Temperature of water taken from a domestic hot tap added to bottles in different amounts.

Amount of water	Temperature immediately after water added °C	Temperature 5 minutes after water added °C	Temperature 10 minutes after water added °C	Temperature 15 minutes after water added °C
2oz (60ml)	79.1	60.7		
3oz (90ml)	84.6	69.0		
4oz (120ml)	88.5	72.0	65.5	
5oz (150ml)	89.5	74.3	69.8	
6oz (180ml)	90.0	77.7	70.5	66.0
7oz (210ml)	90.4	78.1	73.8	68.3
8oz (240ml)	91.2	80.0	75.0	70.2

These results are based on a small number of tests in a domestic kitchen and it is likely that other hot taps might provide water at different temperatures and the time taken to fill the bottle to the correct level might vary. Care is needed when filling the bottles not to scald, particularly if you bend down to the right level to see the bottle markings to add the correct amount of water. Our conclusion from this is that if using a hot tap to fill the bottle, the powdered infant formula should probably be added:

- Immediately if volumes of 2-3oz (60-90ml) are being made up
- After no more than 5 minutes for volumes of 4oz-5oz (120ml-180ml)
- After no more than 10 minutes for volumes 6oz-8oz (210ml-240ml)

Again, however, we believe that advice should be provided by public health departments and the NHS following a comprehensive review of water temperatures when using this method of reconstitution.

Baby kettles

Baby kettles are now available on the market which claim to keep water at the correct temperature after being boiled to allow families to make up infant formula without the need for cooling before the powder is added. These kettles appear to keep water at a temperature of 70°C for three hours. It is worth noting that this is no longer than 500ml of boiled water can be kept in a thermos flask and still remain above 70°C which may be a cheaper option for families

There is a potential risk that the water in these kettles may be repeatedly boiled (e.g. set to re-boil after three hours) to maintain the temperature, concentrating elements in the water. Fresh water should always be used in the kettle. The water is also likely to cool below 70°C being poured into the bottle and is therefore likely to be less than 70°C when the powder is added. We have not tested these kettles but suggest caution if families are using these to make up infant formula.

6.0 Conclusion

We believe that it remains essential that the reconstitution of PIF should be done with water at a temperature of 70°C or more to kill any bacteria present, and that real risks to health remain should infants be given PIF made up with water at lower temperatures. We do not believe that there is sufficient evidence of benefit from the addition of probiotics to powdered infant milks to justify relaxing temperature regulations when making up these milks. We, however, strongly recommend the Food Standards Agency, the Department of Health and Social Care and other health departments in the UK to review any benefit and risk associated with the addition of probiotics to infant milks as a matter of urgency.

We also believe that a review of the current advice to caregivers in the UK is needed to ensure that water is indeed at 70°C or above following instructions given by public health departments. Our conclusion is that the waiting time in the kettle needs to be reviewed and that times should be given for both one litre and for 500ml since many people may want to reduce the amount of water they boil to save energy. We would also like to see independent government review and testing of all methods of reconstitution including preparation machines, hot taps and baby kettles.

7.0 References

- Agostoni C, Buonocore G, Carnielli, V et al for the ESPGHAN Committee on Nutrition (2010) Enteral Nutrient Supply for Preterm Infants: Commentary From the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. *Journal of Paediatric Gastroenterology and Nutrition*. **50**, 85-91.
- AlFaleh K and Anabrees J (2014) Probiotics for prevention of necrotizing enterocolitis in preterm Infants. *Evidence Based Child Health*, **9**, 584-671.
- Al-Holy M, Lin M, Abu-Ghoush M, Al-Qadiri H, Rasco B. (2009). Thermal Resistance, Survival and Inactivation of *Enterobacter Sakazakii* (*Cronobacter* spp.) in Powdered and Reconstituted Infant Formula. *Journal of Food Safety*. **29**, 287-301.
- Almajed FS and Forsythe SJ (2016) *Cronobacter sakazakii* clinical isolates overcome host barriers and evade the immune response. *Microbial Pathogenesis*, **90**, 55-63.
- Arku B, Mullane N, Fox E, Fanning S, Jordan K. (2008). *Enterobacter sakazakii* survives spray drying. *International Journal of Dairy Technology* **61**, 102-108.
- Assad M, Elliott M, Abraham J (2016). Decreased cost and improved feeding tolerance in VLBW infants fed an exclusive human milk diet. *Journal of Perinatology*. **36**, 216-220.
- Awaishah S, Al-Nabulsi A, Osaili T, Ibrahim S, Holley R (2013). Inhibition of *Cronobacter sakazakii* by Heat Labile Bacteriocins Produced by Probiotic LAB Isolated from Healthy Infants. *Journal of Food Science*. **78**, M1416-M1420.
- Beuchat L, Kim H, Gurtler J, Lin L, Ryu J, Richards G. (2009.) *Cronobacter sakazakii* in foods and factors affecting its survival, growth and inactivation. *International Journal of Food Microbiology*. **136**, 204-213.
- Biering G, Karlsson S, Clark N et al (1989) Three Cases of Neonatal Meningitis Caused by *Enterobacter sakazakii* in Powdered Milk. *Journal of Clinical Microbiology* **27**, 2054-2056.
- Bowen A and Braden C (2006) Invasive *Enterobacter sakazakii* Disease in Infants. *Emerging Infectious Diseases* **12**, 1185-1189.
- Braegger C, Chmielewska A, Decsi T, et al (2011). Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, **52**, 238-250.
- Breeuwer P, Lardeau A, Peterz M, Jooston MA (2003). Desiccation and heat tolerance of *Enterobacter sakazakii*. *J Applied Microbiology*, **95**, 967-973.
- Brouard C, Espié E, Weill F-X et al (2007) Two Consecutive Large Outbreaks of Salmonella enterica Serotype Agona Infections in Infants Linked to the Consumption of Powdered Infant Formula. *The Pediatric Infectious Disease Journal*, **26**, 148-152.
- Burdette J and Santos C (2000) *Enterobacter sakazakii* brain abscess in the neonate: the importance of neuroradiologic imaging. *Pediatric Radiology* **30**, 33-34.
- Cacho, N, Parker L, Neu J. (2017) Necrotizing Enterocolitis and Human Milk Feeding: A Systematic Review. *Clinics in Perinatology*. **44**, 49-67
- Cahill S, Wachsmuth I, de Lordes Costarrica M, Embarel P. (2008) Powdered Infant Formula as a Source of Salmonella Infection in Infants. *Food Safety*, **46**, 268-273
- Campbell D and Soboleva T (2015) *Reconstituting Powdered Infant Formula - A Review*. Ministry of Health, New Zealand.

Caubilla-Barron J and Forsythe S (2007). Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae*. *Journal of Food Protection* **70**, 2111-2117

Caubilla-Barron J, Hurrell E, Townsend S, Cheetham P, Loc-Carrillo C, Fayet O, et al. (2007) Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *J Clin Microbiol*, **45**,3979–85. doi:10.1128/JCM.01075-07

Centers for Disease Control and Prevention (CDC) (2019). *Cronobacter infections and infants*. Access at: <https://www.cdc.gov/features/cronobacter/index.htm>

Centers for Disease Control and Prevention (CDC) (2016) *National Notifiable Infectious Diseases and Conditions: United States*. Available at: <https://wonder.cdc.gov/nndss/static/2016/annual/2016-table4.html>

Centers for Disease Control and Prevention (CDC) (2015) *Serotypes and the Importance of Serotyping Salmonella*. Available at: <https://www.cdc.gov/salmonella/reportspubs/salmonella-atlas/serotyping-importance.html>

Centres for Disease Control and Prevention (CDC) (2008) *Cronobacter Species Isolation in Two Infants -- New Mexico, 2008. Morbidity and Mortality Weekly Report*. Available at: <https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5842a3.htm>
Last accessed February 2018.

Centers for Disease Control and Prevention (CDC) (2002) *Enterobacter sakazakii Infections Associated With the Use of Powdered Infant Formula—Tennessee, 2001 JAMA* **287**(17) 2204-2205.
doi:10.1001/jama.287.17.2204

Centers for Disease Control and Prevention (CDC) (1993). *Salmonella Serotype Tennessee in Powdered Milk Products and Infant Formula -- Canada and United States, 1993*. Available at: <https://www.cdc.gov/mmwr/preview/mmwrhtml/00021081.htm>

CAC (2008). *Codex Alimentarius Commission Code of Hygienic Practice for Powdered Formulae for Infants and Young Children (CAC/RCP 66-2008)* Available at: www.fao.org/input/download/standards/11026/CXP_066e.pdf

CAC (1979) *Codex Alimentarius Committee Recommended International Code of Hygienic Practice for Foods for Infants and Children (CAC/RCP 21-1979)*. Available at : ftp://ftp.ksph.kz/food_legislation/docs_for_training_in_almaty_e_/ghp_infant_formula/ghp_infant_formula_e.pdf

Chap J, Jackson P, Siquera R et al (2009) International survey of *Cronobacter sakazakii* and other *Cronobacter spp.* in follow up formulas and infant foods. *International Journal of Food Microbiology* **136**, 185--188 .

Chaves CEV, Brandão MLL, Paniago AMMP (2018). Fatal *Cronobacter sakazakii* Sequence Type 494 Meningitis in a Newborn, Brazil. *Emerg Infec Dis*, **24**, 1458-1460.

Cheng LH, Crim SM, Cole RC, Shane AL et al (2013) Epidemiology of infant salmonellosis in the United States, 1996-2008: A Foodborne Diseases Active Surveillance Network Study. *J Ped Infectious Disease Society*, **2**, 232-239.

Dancer, GI, Mah JH, MS, Rhee, IG. Hwang, Kang. DH (2009). Resistance of *Enterobacter sakazakii* (*Cronobacter spp.*) to environmental stresses. *Journal of Applied Microbiology*, **107**, 1606–1614.

Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF (2002) Probiotics Feeding in Prevention of Urinary Tract Infection, Bacterial Sepsis and Necrotizing Enterocolitis in Preterm Infants. *Neonatology*, **82**, 103-108.

Deshpande G, Rao S, Patole S, Bulsara M (2010) Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates. *Pediatrics*, **125**, 921-930.

Edelson-Mammel S, Porteous M, Buchanan, R (2006) Acid Resistance of Twelve Strains of *Enterobacter sakazakii*, and the impact of Habituating the Cells to an Acid Environment. *Food Microbiology and Safety*, **71**, 201-207.

Edelson-Mammel S, and Buchanan, R (2004). Thermal Inactivation of *Enterobacter sakazakii* in Rehydrated Infant Formula. *Journal of Food Protection*, **67**, 60-63.

El-Gamal M, El Dairouty R, Okda A et al. (2013) Incidence and Interrelation of *Cronobacter sakazakii* and Other Foodborne Bacteria in Some Milk Products and Infant Formula Milks in Cairo and Giza Area. *World Applied Sciences Journal*, **26**, 129-1141.

El-Sharoud W, O'Brien S, Negrodo C, Iversen C, Fanning S, Healy B. (2009). Characterization of *Cronobacter* recovered from dried milk and related products. *BMC Microbiology* **9** 24

Estuningsih S, Kress C, Hassan AA, O, et al. (2006), *Enterobacteriaceae* in Dehydrated Powdered Infant Formula Manufactured in Indonesia and Malaysia, *Journal for Food Protection*, **69**, 3013-3017.

Euler A, Byrne W, Cousins L, Ament M, Leake R, Walsh J. (1977) Increased serum gastrin concentrations and gastric acid hyposecretion in the immediate newborn period. *Gastroenterology*, **72**, 1271-1273.

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) 2019. *Rapid outbreak assessment* <https://www.ecdc.europa.eu/en/publications-data/rapid-outbreak-assessment-multi-country-outbreak-salmonella-poona-infections>

European Food Safety Authority, EFSA, (2014). Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA Journal*, **12** (7), 3760. Available at <http://www.efsa.europa.eu/en/efsajournal/doc/3760.pdf>

FAO/WHO (2001). Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including milk powder with live lactic acid bacteria. FAO/WHO. Geneva.

FAO/WHO (2004). *Enterobacter sakazakii* and other microorganisms in powdered infant formula. Meeting report. Geneva, Switzerland, 2-5 February 2004. Microbiological Risk Assessment Series, No.6 Available at: <http://www.fao.org/3/a-y5502e.pdf>

FAO/WHO (2006). *Enterobacter sakazakii* and *Salmonella* in powdered infant formula. Meeting Report. Joint FAO/WHO Technical Meeting on *Enterobacter sakazakii* and *Salmonella* in Powdered Infant Formula, Rome, Italy, 16-20 January 2006. [FAO/WHO] *Microbiological Risk Assessment Series*, No. 10.

FAO/WHO (2008). *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formulae and *Salmonella* in powdered infant formula. Meeting report. *Microbiological Risk Assessment Series*, No. 15. Rome. Available at: http://www.who.int/foodsafety/publications/micro/MRA_followup.pdf

Fàbrega A and Vila J. (2013). *Salmonella enterica* Serovar Typhimurium Skills To Succeed in the Host: Virulence and Regulation. *Clinical Microbiology Reviews*, **26**, 308-341

Farmer III, JJ. (2015) My 40-Year History with *Cronobacter/Enterobacter sakazakii* – Lessons Learned, Myths Debunked, and Recommendations. *Frontiers in Pediatrics*, **3**, 84, | <https://doi.org/10.3389/fped.2015.00084>

Fei P, Jiang Y, Jiang Y et al. (2017). Prevalence, Molecular Characterization, and Antibiotic Susceptibility of *Cronobacter sakazakii* Isolates from Powdered Infant Formula Collected from Chinese Retail Markets. *Frontiers in Microbiology*, **8**, 2026 Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5651101/>

Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) FDA and CDC Update: *Investigation of Cronobacter bacteria illness in infants* (2011) Available at: https://www.cdc.gov/media/releases/2011/s1230_Cronobacter.html

Food Safety Authority Ireland, (2011). *Salmonella species*. Microbial Factsheet Series Issue No .1. Available at: www.fsai.ie

Food Safety Authority Ireland, (2007). *Microbiological safety of dried infant formulae and dried dietary foods for special medical purposes, intended for infants below 6 months of age*. https://www.fsai.ie/uploadedFiles/Monitoring_and_Enforcement/Monitoring/Surveillance/micro_2006_infant_for.pdf

Food Standards Agency (2005). *Guidance for Health Professionals on Safe Preparation, Storage and Handling of Powdered Infant Formula*. Food Standards Agency. London.

Food Standards Agency (2009). *Bacteriocidal Preparation of Powdered Infant Formula*. Available at: http://www.foodbase.org.uk/admin/tools/reportdocuments/395-1-697_b13010.pdf

Food Standards Agency (2013) *Advisory Committee on the Microbiological Safety of Food: Safe Preparation of Powdered Infant Formula ACM/1108*. Available at: https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm1108.pdf

Friedemann, M. (2009). Epidemiology of invasive neonatal *Cronobacter (Enterobacter sakazakii)* infections. *European Journal of Clinical Microbiology and Infectious Diseases*, **28**, 1297–1304.

Gallagher P and Ball W. (1991) Cerebral infarctions due to CNS infection with *Enterobacter sakazakii*. *Pediatric Radiology* **21** (2) pp 135-136

Grishin A, Papillon S, Bell B, Wang J, Ford H (2013). The Role of the Intestinal Microbiota in the Pathogenesis of Necrotizing Enterocolitis. *Seminars in Pediatric Surgery* **22**. 69-75

Grummer-Strawn L, Rollins N (2015) Summarising the health effects of breastfeeding. *Acta Paediatrica*, Special issue December 2015, 1-2.

Gurtler, J. B., and L. R. Beuchat (2007). Growth of *Enterobacter sakazakii* in reconstituted infant formula as affected by composition and temperature. *Journal of Food Protection* **70**, 2095–2103.

Harmsen H, Wildeboer-Veloo A, Raangs G et al. (2000). Analysis of Intestinal Flora Development in Breast-Fed and Formula-Fed Infants by Using Molecular Identification and Detection Methods. *Journal of Pediatric Gastroenterology and Nutrition* **30**, 61-67

Hassam JS, Naser WE (2018) Incidence of *Cronobacter sakazakii* in Iraqi infants with neonatal sepsis. *Indian J Public Health*, **9**, 942-947

Heuvelink A,E, Zwartkruis-Nahuis, JTM, van der AH, Wit, B, van Oosterom, R, de Boer, E. (2003). Handhavingsactie *Enterobacter sakazakii* in zuigelingenvoeding. Projectnummer: OT 0210 8 p. Available at: <http://agris.fao.org/agris-search/search.do?recordID=NL2004717206>

Himelright I, Harris E, Lorch V, Anderson M. (2002). *Enterobacter sakazakii* infections associated with the use of powdered infant formula – Tennessee, 2001. *Journal of the American Medical Association*. **287**, 2204–2205.

Hoque A, Ahmed T, Shahidullah M, et al. (2010). Isolation and molecular identification of *Cronobacter spp.* from powdered infant formula (PIF) in Bangladesh. *International Journal of Food Microbiology*; **142** 375-378.

Hormann E (2010) Reducing the Risk for Formula-Fed Infants: Examining the Guidelines. *Birth*, **37**,72-76

Hunter C, Petrosyan M, Ford H, Prasadarao N. (2008) *Enterobacter sakazakii*: An Emerging Pathogen in Infants and Neonates. *Surgical Infections*, **9**, 533-539

- Hurrell E, Kucerova E, Loughlin M, Caubilla-Barron J, Forsythe S (2009) Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella* serovars and other *Enterobacteriaceae*. *International Journal of Food Microbiology*, **136**, 227-231
- Hyman P, Clarke D, Everett S et al. (1985) Gastric acid secretory function in preterm infants. *The Journal of Pediatrics*, **106**, 467-471
- Iversen C and Forsythe S (2004) Isolation of *Enterobacter sakazakii* and other *Enterobacteriaceae* from powdered infant formula milk and related products. *Food Microbiology*, **21**, 771-777.
- Iversen, C, Lane M, Forsythe SJ. (2004). The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. *Letters in Applied Microbiology*, **38**, 378–382.
- Iversen C and Forsythe S (2003) Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*, **14**, 443-454.
- Jackson E, Parra-Flores J, Fernández-Escartín E et al (2015) Reevaluation of a Suspected *Cronobacter sakazakii* Outbreak in Mexico. *Journal of Food Protection*, **78**, 1191-1196
- Jaffaar M, Shebli M, Mussa A et al (2015) Detection of *Enterobacter sakazakii* from Commercial Children Dry Milk. *Journal of Environmental Protection*, **6**, 1170-1175.
- Jaradat Z, Al Mousa W, Elbetieha A, Al Nabulsi A, Tall B. (2014) *Cronobacter* spp. – opportunistic food-borne pathogens. A review of their virulence and environmental-adaptive traits. *Journal of Medical Microbiology*, **63**, 1023–1037
- Jacobs SE, Tobin JM, Opie GF, Donath S et al (2013). Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial. *Pediatrics*, **132**, 1055-1062
- Jason J. (2012). Prevention of Invasive *Cronobacter* Infections in Young Infants Fed Powdered Infant Formulas. *Pediatrics*, **130**, e1076-e1084
- Jones G, Pardos de la Gandara M, Herrera-Leon L, Herrera-Leon S, Varela-Martinez C et al (2019) Outbreak of *Salmonella enterica* serotype Poona in infants linked to persistent *Salmonella* contamination in an infant formula manufacturing facility France, August 2018-February 2019. *Euro Surveill*, **24**, 1-7.
- Joseph S, Hariri S, Masood, Forsythe S (2013) Sialic acid utilization by *Cronobacter sakazakii*. *Microbial Informatics and Experimentation* **3**, <https://doi.org/10.1186/2042-5783-3-3>
- Jourdan N, Le Hello S, Delmas G et al (2008) Nationwide Outbreak of *Salmonella enterica* Serotype Give Infections in Infants in France, Linked to Infant Milk Formula, September, 2008. *Eurosurveillance* **13** (39).
- Jourdan-da Silva N, Fabre L, Robinson E et al (2018) Ongoing nationwide outbreak of *Salmonella Agona* associated with internationally distributed infant milk products, France, December 2017. *Eurosurveillance* **23** (2)
- Kandhai, M, Heuvelink A, Reij M, Beumer R, Dijk R, van Tilburg J, van Schothorst M, and Gorris L. (2010). A study into the occurrence of *Cronobacter* spp. in The Netherlands between 2001 and 2005. *Food Control* **21**, 1127–1136
- Kalyantanda G, Shumyak L, Archibald L K. (2015) *Cronobacter* species contamination of powdered infant formula and the implications for neonatal health. *Frontiers in Pediatrics*, July 2015. <https://doi.org/10.3389/fped.2015.00056>
- Karkaneh, M, Fraser L, Jou H, Vohra S (2019). Effectiveness of probiotics in infantile colic: A rapid review. *Pediatrics and Child Health*, pxz007, <https://doi.org/10.1093/pch/pxz007>
- Kent R, Fitzgerald G, Hill C et al (2015). Novel Approaches to Improve the Intrinsic Microbiological Safety of Infant Milk Formula. *Nutrients*. **7**, 1217-1244

- Kim H and Beuchat L (2005) Survival and Growth of *Enterobacter sakazakii* on fresh-cut fruits and vegetables and in unpasteurized juices as affected by storage temperature. *Journal of Food Protection*, **68**, 2541-2552.
- Kim H, Ryu J, Beuchat L. (2006) Attachment and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. *Applied Environmental Microbiology* **73**, pp5845-5856.
- Kim H, Ryu J, Beuchat L. (2007) Effectiveness of disinfectants in killing *Enterobacter sakazakii* in suspension, dried on the surface of stainless steel, and in a biofilm. *Applied and Environmental Microbiology*, **73**, 1256-1265.
- Kim S, Oh S, Lee Y, et al (2011) Microbial contamination of food products consumed by infants and babies in Korea. *Letters in Applied Microbiology*; **53**, 532-538.
- Kosloske A. (1984). Pathogenesis and prevention of necrotizing enterocolitis: a hypothesis based on personal observation and a review of the literature. *Pediatrics*, **74**, 1086-1092.
- Kucerova, E, Joseph S and Forsythe S (2011). *Cronobacter*: diversity and ubiquity. *Quality Assurance and Safety of Foods and Crops*. **3** (3): 104–122. doi:10.1111/j.1757-837X.2011.00104.x.
- Lai, K.K., (2001). *Enterobacter sakazakii* Infections among Neonates, Infants, Children, and Adults: Case Reports and a Review of the Literature. *Medicine*, **80**, 113 – 122.
- Lehner A, Riedel K, Rattei T et al (2006) Molecular characterization of the α -glucosidase activity in *Enterobacter sakazakii* reveals the presence of a putative gene cluster for palatinose metabolism. *Systematic and Applied Microbiology*, **29**, 609-625.
- Li Z, Ge W, Li K, Gan J, Zhang Y, Zhang Q, Luo R, Chen L, Liang Y, Wang Q, Xi M, Xia X, Wang X, Yang B. (2016) Prevalence and Characterization of *Cronobacter sakazakii* in Retail Milk-Based Infant and Baby Foods in Shaanxi, China. *Foodborne Pathogens and Disease*, **13**, 221-227.
- Lin PW, Nasr TR, Berardinelli AJ, Kumar A, Neish As (2008) The Probiotic *Lactobacillus GG* may Augment Intestinal Host Defense by Regulating Apoptosis and Promoting Cytoprotective Responses in the Developing Murine Gut. *Pediatric Research*, **64**, 511-516.
- Lu Y, Liu P, Li, C, Sha M, Fang Jet al (2019) Prevalence and Genetic Diversity of *Cronobacter* Species Isolated From Four Infant Formula Production Factories in China. *Frontiers in Microbiology* <https://doi.org/10.3389/fmicb.2019.01938>
- Lucas A and Cole T (1990). Breastmilk and neonatal necrotising enterocolitis. *The Lancet*. **336** (8730) 1519-1523.
- Losio MN, Pavoni E, Finazzi G, Agostoi C et al (2018). Preparation of powdered infant formula: could product safety be improved? *JPGN*, **67**, 543-546.
- Maçi R, Bijo B, Xinxo A, Shehu F and Memoçi H. (2015) Prevalence of Salmonella spp. in Imported Powdered Infant Formula (PIF). *Albanian Journal of Agricultural Sciences*. **14**, 236-240
- Maffei D and Schanler R (2017) Human milk is the feeding strategy to prevent necrotizing enterocolitis! *Seminars in Perinatology*. **41**, 36-40.
- Maldonado J, Cañabate F, Sempere L, et al (2012). Human milk probiotic *Lactobacillus fermentum* CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract Infections in infants. *Journal of Pediatric Gastroenterology and Nutrition*, **54**, 55-61.
- McAndrew F, Thompson J, Fellows L, Large A, Speed M, Renfrew M (2012). *Infant Feeding Survey 2010*. Available at: <https://data.gov.uk/dataset/c941b6d8-bfd1-4ca8-9687-73b1a8f1b59a/infant-feeding-survey-2010>

- Mardaneh J and Dallal M (2017) Study of *Cronobacter sakazakii* Strains Isolated from Powdered Milk Infant Formula by Phenotypic and Molecular Methods in Iran. *Archives of Pediatric Infectious Diseases* **5** (1):e38867. doi: 10.5812/pedinfect.38)
- Mihatsch W, Braegger C, Desci T et al. (2012) Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. *Clinical Nutrition* **31**, 6-15.
- Mullane N, Drudy D, Whyte P et al (2006). *Enterobacter sakazakii*: biological properties and significance in dried infant milk formula (IMF) powder. *International Journal of Dairy Technology* **59**, 102-111.
- Muytjens H, Roelofs-Willems H, Jaspars G (1988) Quality of powdered substitutes for breast milk with regard to members of the family *Enterobacteriaceae*. *Journal of Clinical Microbiology*, **26**, 743-746.
- Muytjens H, Zanen H, Sonderkamp H et al. (1983) Analysis of Eight Cases of Neonatal Meningitis and Sepsis Due to *Enterobacter Sakazakii*. *Journal of Clinical Microbiology*. **18**, 115-120.
- National Centre for Epidemiology (Centro Nacional de Epidemiologia /Instituto de Salud Carlos III) (2011) Outbreak of gastroenteritis by *Salmonella poona* in several autonomous communities in 2010-2011. *Boletín Epidemiológico Semanal*, **19**, 176-185.
- Nazarowec-White M, Farber J (1997) Incidence, Survival and Growth of *Enterobacter sakazakii* in Infant Formula. *International Journal of Food Microbiology*; **34**, 103-113.
- Neu J. (2014) Probiotics and Necrotizing Enterocolitis. *Clinical Perinatology* **41**, 967-978.
- Neu, J (2005). Neonatal Necrotizing Enterocolitis: An Update. *Acta Paediatrica Suppl* **94**, 100-105.
- NHS (2019). *Formula milk: common questions*. Access at <https://www.nhs.uk/conditions/pregnancy-and-baby/infant-formula-questions/>
- Norberg S, Stanton C, Ross R, Hill C, Fitzgerald G, Cotter P (2012). *Cronobacter* spp. in Powdered Infant Formula. *Journal of Food Protection* **75**, 607-620.
- Norwegian Scientific Committee on Food and Environment (2014) *Assessment of infant formula and follow-on formula supplemented with Lactobacillus fermentum CECT 5716*
<https://vkm.no/english/riskassessments/allpublications/assessmentofinfantformulaandfollowonformulasupplementedwithlactobacillusfermentumcect5716.4.27ef9ca915e07938c3b295ae.html>
- Norwegian Scientific Committee on Food and Environment (2016) *Health risk assessment of a food supplement containing Lactobacillus reuteri Protectis*.
<https://vkm.no/english/riskassessments/allpublications/healthriskassessmentofafoodsupplementcontaininglactobacillusreuteriprotectis.4.2375207615dac0245aee54b6.html>
- NSW Food Authority (2011) *Microbiological quality of powdered infant formula 2011*, Australian New Zealand Food Standards Code NS/FA/CP048/1106: 1-17. Available at:
http://www.foodauthority.nsw.gov.au/_Documents/scienceandtechnical/Microbiological_quality_powdered_formula.pdf
- NZFSA, (2009). *Infant Formula and Cronobacter sakazakii Survey Report*. NZFSA Survey Report No 2/09 www.foodsafety.govt.nz/elibrary/industry/Infant_FormulaProvides_Information.pdf
- ONS (2019) *Births in England and Wales, 2018*. Available at:
<https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2018>
- Oh SW, Chen PC, Kang DH (2007) Biofilm formation by *Enterobacter sakazakii* grown in artificial broth and infant milk formula on plastic surface. *J Rapid Methods and Automation in Microbiology*, **15**, 311-319.

- Oonaka K, Furuhashi K, Hara M and Fukuyama M (2010) Powder Infant Formula Milk Contaminated with *Enterobacter sakazakii*. *Japanese Journal of Infectious Diseases*. **63**,103-107.
- Osaili T and Forsythe S. (2009) Desiccation resistance and persistence of *Cronobacter* species in infant formula. *International Journal of Food Microbiology*. **136**, 214-220
- Osaili T, Shaker,R, Ayyash M, Holley R. (2008) Effect of *Bifidobacterium Breve* on the Growth of *Enterobacter Sakazakii* in Rehydrated Infant Milk Formula. *Journal of Food Safety*, **28**, 34-46.
- Palcich G, de Moraes Gillio C, Aragon-Alegro L, et al (2009) *Enterobacter sakazakii* in Dried Infant Formulas and Milk Kitchens of Maternity Wards in São Paulo, Brazil. *Journal for Food Protection*; **72**, 37-42.
- Park J, Soek W, Choi B et al (2004) *Salmonella enterica* Serovar London Infections Associated with Consumption of Infant Formula. *Yonsei Medical Journal* **45**, 43-48.
- Parra-Flores J et al. (2015) Risk of Cronobacter Sakazakii contamination in powdered milk for infant nutrition. *Revista Chilena de Nutricion* **42** 83-89 Available at: https://scielo.conicyt.cl/scielo.php?script=sci_abstract&pid=S0717-75182015000100011&lng=es&nrm=iso&tlng=en
- Patrick M, Mahon B, Greene S et al (2014) Incidence of *Cronobacter* spp. Infections, United States, 2003-2009. *Emerging Infectious Diseases*, **20**, 1520-1523.
- Peter, C.S., Feuerhahn, M., Bohnhorst, B., Schalausd, M., Ziesing, S., von der Hardt, H., Poets, C.F., (1999). Necrotising enterocolitis: is there a relationship to specific pathogens? *Eur. J. Pediatr.* **158**, 67-70.
- Quin C Estaki M et al (2018) Probiotic supplementation and associated infant gut microbiome and health: a cautionary retrospective clinical comparison. *Sci Rep*, **8**, 8283, accessed at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5974413/>
- Rashidat EA, Olugbo EA, Ifeanyi SS, et al, (2013) Isolation and PCR detection of *Cronobacter sakazakii* from powdered infant formulae retailed in Nigeria. *American Journal of Food and Nutrition*; **3**,182-187.
- Richards, G, Gurtler J, and Beuchat L. (2005). Survival and growth of *Enterobacter sakazakii* in infant rice cereal reconstituted with water, milk, liquid infant formula, or apple juice. *Journal of Applied Microbiology*. **99**, 844–850.
- Rodriguez-Urrego J, Herrera-León S, Echeita-Sarriondia A et al (2010) Nationwide outbreak of Salmonella serotype Kedougou associated with infant formula, Spain, 2008. *Eurosurveillance* **15** (22) Available at: <https://www.eurosurveillance.org/docserver/fulltext/eurosurveillance/15/22/art19582-en.pdf?expires=1518538339&id=id&accname=guest&checksum=8E510147B056AC907D9A9976F4C1887C>
- Rowe B, Begg N, Hutchinson D et al. (1987) *Salmonella ealing* Infections Associated with Consumption of Infant Dried Milk. *The Lancet* **330** (8564) 900-903.
- Samuels N, van de Graff RA, de Jonge RCJ, Reiss UKM, Vermuelien MJ (2017). Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. *BMC Pediatrics*, **17**, Article number: 105.
- Schmid, M, C. Iversen, I. Gontia, R. Stephan, A. Hofmann, A. Hartmann, B. Jha, L. Eberl, Riedel K, Lehner A. (2009). Evidence for a plant-associated natural habitat for *Cronobacter* spp. *Research in Microbiology*. **160**, 608–614.
- Scottish Government. (2018) Scottish Maternal and Infant Nutrition Survey 2017. Available at: www.isdscotland.org
- Shaker R, Osaili T, Al-Omary W, et al (2007) Isolation of *Enterobacter sakazakii* and other *Enterobacter* spp. from food and food production environments. *Food Control*; **18**,1241-45.

Silano M, Paganin P, Davanzo R (2016). Time for the 70C water precautionary option in the home dilution of powdered infant formula. *Italian J Pediatrics*, **42**:17. DOI 10.1186/s13052-0156-02289-9

Siqueira Santos S, da Silva N, Amstalden Junqueira V et al (2013) Screening for *Cronobacter* Species in Powdered and Reconstituted Infant Formulas and from Equipment Used in Formula Preparation in Maternity Hospitals. *Annals of Nutrition and Metabolism*, **63**, 62-68

Skirrow B (1987) A demographic survey of campylobacter, salmonella and shigella infections in England: A Public Health Laboratory Service Survey. *Epidemiology and Infection*, **99**, 647-657.

Soto A, Martin V, Jiménez E et al (2014) Lactobacilli and Bifidobacteria in Human Breast Milk: Influence of Antibiotherapy and Other Host and Clinical Factors. *Journal of Pediatric Gastroenterology and Nutrition*. **59**, 78-88

Strydom A, Cawthorn D, Cameron M, Witthuhn R (2012) Species of *Cronobacter* – A review of recent advances in the genus and their significance in infant formula milk. *International Dairy Journal*. **27**, 3-12.

Strysko J, Cope JR, Martin H, Tarr C, Hise K, Collier S, Bowen A (2020) Food safety and invasive *Cronobacter* infections during early infancy 1961-2018. *Emerging Infectious Diseases*, **26**, 857-865.

Szajewska A, Skorka A, Piescik-Lech M (2015) Fermented infant formulas without live bacteria: a systematic review. *European J Pediatrics*, **174**, 1413-1420.

Teitelbaum J, Walker W (2002). Nutritional impact of pre-and probiotics as protective gastrointestinal organisms. *Annual Review of Nutrition*, **22**, 107-138

Threlfall E, Ward L, Hampton M et al (1998). Molecular fingerprinting defines a strain of *Salmonella enterica* serotype Anatum responsible for an international outbreak associated with formula-dried milk. *Epidemiology and Infection*. **121**, 289-293

Townsend, S and Forsythe, S.J. (2008) *The neonatal intestinal microbial flora, immunity, and infections*. Chapter 3 in J. Farber and S.J. Forsythe (Eds) *Enterobacter sakazakii*. ASM Press.

Townsend S, Hurrell E, Gonzalez-Gomez I, Lowe J, Frye J, Forsythe S, et al. *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. (2007) *Microbiology* **153** (Pt 10):3538–47. doi:10.1099/mic.0.2007/009316-0

Underwood M (2017) Impact of probiotics on necrotizing enterocolitis. *Seminars in Perinatology*, **41**, 41-51

Usera M, Rodriguez A, Echeita A, Cano R. (1996) Multiple Analysis of a Foodborne Outbreak caused by Infant Formula Contaminated by an Atypical *Salmonella virchow* Strain. *European Journal of Clinical Microbiology and Infectious Diseases*, **17**, 551-555

Van Acker J, De Smet F, Muyldermans G et al. (2001) Outbreak of Necrotizing Enterocolitis Associated with *Enterobacter sakazakii* in Powdered Milk Formula. *Journal of Clinical Microbiology*, **39**, 293-297.

van den Akker CHP, van Goudoever JB, Szajewska H, Embleton, ND; Hojsak I, Reid, D, Shamir, R for the ESPGHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis *Journal of Pediatric Gastroenterology and Nutrition*, **67**, 103–122

Victora C, Bahl R, Barros A. et al. for The Lancet Breastfeeding Series Group. (2016) Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *The Lancet*. **387**, 475–490

Walsh, D., C. Molloy, C. Iversen, J. Carroll, C. Cagney, S. Fanning, and G. Duffy. (2011). Survival characteristics of environmental and clinically derived strains of *Cronobacter sakazakii* in infant milk formula (IMF) and ingredients. *J. Appl. Microbiol.* 110:697–703

Weinberger M, Solnik-Isaac H, Shachar D et al. (2006) *Salmonella enterica* serovar Virchow: epidemiology, resistance patterns and molecular characterisation of an invasive *Salmonella* serotype in Israel. *Clinical Microbiology and Infection*, **12**, 999-1005.

Wilkinson TA, Scott KA, Carroll AE (2019) Mixed message on formula mixing. *Pediatrics*, **143**. DOI: 10.1542/peds.2018-25255

Willis J and Robinson J (1988) *Enterobacter sakazakii* meningitis in neonates. *The Pediatric Infectious Disease Journal*, **7**, 196-199

WHO (2007). *How to prepare formula for bottle feeding at home*. Available at: https://www.who.int/foodsafety/publications/micro/PIF_Bottle_en.pdf

Xin G, Yinping D, Shaofei Y et al (2018) Contamination and characterization of multiple pathogens in powdered formula at retail collected between 2014 and 2015 in China. *Food Control*, **87**,40-45..

Yang B, Zhao H, Cui S, Wang Y, Xia X, Xi M, Wang X, Meng J, Ge W. (2014) Prevalence and characterization of *Salmonella enterica* in dried milk-related infant foods in Shaanxi, China. *Journal of Dairy Science* **97**, 6754-6760

Zhu S, Schnell S, Fischer M (2013). Growth inhibition of *Cronobacter* spp. strains in reconstituted powdered infant formula acidified with organic acids supported by natural stomach acidity. *Food Microbiology*, **35**, 121-128

Zink D (2003) *FDA Field Survey of powdered infant formula manufacturing*. U.S. Food and Drug Administration Food Advisory Committee Mtg. March 18-19 available at: <https://wayback.archive-it.org/org-1137/20170404081809/https://www.fda.gov/ohrms/dockets/ac/03/slides/3939s1.htm>

Appendix 1: Reported bacterial contamination of powdered infant milk samples[†]

Reference	Year of data collection	Location	Organisms	Samples tested (n)	Positive samples (%)
Lu et al 2019	2013-2014	China	<i>Cronobacter spp</i>	6111 (from 8 different factories)	2.3%
Xin et al 2018	2014-2015	China	<i>Cronobacter spp.</i> <i>Bacillus cereus</i> <i>Clostridium sporogenes</i>	119	3.4% 36.1% 9.2%
Fei et al 2017	2015-2017	China	<i>C. sakazakii</i>	2020	2.8%
Li et al, 2016	2010-2012	China	<i>C. sakazakii</i>	705	16.9%
Mardaneh and Dallal (2017)	2014-2015	Iran	<i>C. sakazakii</i>	125	7.2%
Jaffaar et al, 2015	2014	Iraq	<i>C. sakazakii</i>	39	10.3%
Yang et al 2014		China	<i>Salmonella spp.</i>	246	2.0%
Parra-Flores et al, 2015	2013-2014	Chile	<i>Cronobacter sakazakii</i>	72	2.7%
Siqueira Santos et al 2013		Brazil	<i>Cronobacter spp.</i> <i>C. sakazakii</i>	42	29% 5%
Maçi et al, 2015	2013-2015	Albania	<i>Salmonella spp.</i>	69	1.4%
Rashidat et al 2013	2013	Nigeria	<i>Cronobacter spp</i>	154	2%
El-Gamal et al, 2013	N/K	Egypt	<i>C. sakazakii</i>	50	24%
Hoque et al 2010	2010	Bangladesh	<i>Cronobacter spp</i>	32	3%
NSWFA 2011	2009-10	Australia IF	<i>Salmonella spp</i> <i>C. sakazakii</i> <i>Enterobacteriaceae</i>	57	0 0 3.5%
NZFSA 2009	2009	New Zealand	<i>C. sakazakii</i>	34	0
Chap et al, 2009		Brazil Indonesia Jordan Korea Malaysia Portugal UK	<i>C.sakazakii</i>	91	3%
Oonaka et al. (2010)	2006-2008	Japan	<i>E. sakazakii</i>	149	6%
Palcich et al 2009	2006	Brazil	<i>E. sakazakii</i>	186	0.5%
FSAI 2007	2006	Ireland	<i>E. sakazakii</i> <i>Salmonella</i>	719	0 0

Kim et al, 2011	2005	Korea	<i>Cronobacter</i> spp. <i>Enterobacteriaceae</i>	75	5% 18.7%
Shaker et al 2007	Unknown	Jordan	<i>E. sakazakii</i>	8	25%
Estuningsih et al 2006	Unknown	Indonesia >4 mo	<i>Enterobacteriaceae</i> <i>E. sakazakii</i>	74	47% 13.5%
Iversen and Forsythe 2004	2003	UK	<i>Enterobacteriaceae</i> <i>E. sakazakii</i> <i>Salmonella</i>	82	11% 2.4% 0
Zink 2003	2002	USA NS	<i>E. sakazakii</i>	22	22.7%
Heuvelink et al, 2003	2002	The Netherlands	<i>E. sakazakii</i> <i>Enterobacteriaceae</i>	101	2% 4%
Nazarowec-White and Farber 1997	Unknown	Canada	<i>E. sakazakii</i>	120	6.7%
Muytjens et al 1988	Unknown	Samples from 35 countries	<i>Enterobacteriaceae</i> <i>E. sakazakii</i> <i>Salmonella</i>	141	52.5 14.2% 0

†The *Cronobacter* genus were formerly known as the single species *Enterobacter sakazakii*, and surveys before ~2007 used this name. While it is therefore unclear which species were being described, studies have indicated that the majority of isolated strains are usually *Cronobacter sakazakii* (Forsythe, 2010, Sonbol et al, 2013, Akineden et al, 2017).

Appendix 2: Examples of infant milk recalls due to risk of bacterial contamination

Year	Country	Manufacturer	Brand/Product	Microorganism/ microbial toxin
2019	Global recall of Spanish manufactured products	Lactalis, Sodilac	Modilac Expert Rice HA, Modilac HA, Picot AR, Blemil 1, Blemil 2.	<i>Salmonella poona</i>
2019	Canada	Loblaw Companies Ltd.	Parents Choice infant formula for babies sensitive to lactose	<i>Cronobacter spp.</i>
2019	Canada	Costco Wholesale Canada Ltd.	Kirkland infant formula for babies sensitive to lactose	<i>Cronobacter spp.</i>
2018	France and Belgium	Premiobio	Premilait 0-6m	<i>Cronobacter sakazakii</i>
2018	Chile	Nestle	PreNan	<i>Staphylococcus aureus</i>
2018	Singapore (for export to Malaysia)	Dumex	Mamil Gold Infant Milk Formula Stage 1	<i>Cronobacter sakazakii</i>
2017	France (global recall as exported to 83 countries)	Lactalis	Milumel Bio, Picot SL	Samonella
2017	Chile	Abbott	Pediasure	<i>Cronobacter sakazakii</i>
2017	Dominican Republic	Nutriben	Nutriben AC Digest	<i>Cronobacter spp.</i>
2016	Hong Kong	Holle	Organic infant formula 1	<i>Cronobacter sakazakii</i>
2015	Argentina	SanCor	SanCor Baby 2	<i>Cronobacter sakazakii</i>
2014	Chile	Abbott	Pediasure	<i>Cronobacter spp.</i>
2013	Malaysia	Danone	Dumex Dupro Step 2, Mamex Cherish Step 1, Mamex Explore Step 2, Bebelac Step 2, Aptamil 2, Karicare	<i>Cronobacter spp.</i>
2013	China	Fonterra, Danone, Abbott and others	All baby milk products made in New Zealand as contamination found in whey protein used in multiple products.	<i>Clostridium botulinum</i>
2013	Thailand	Danone	Dupro Step 2, Hi-Q Step 1 & 2, Hi-Q Super Gold Step 1 & 2	<i>Cronobacter spp.</i>
2013	Vietnam	Danone and Abbott	Dumex Gold Step 2, Karicare Infant Stage 1, Karicare Gold Plus 2, Similac Gain Plus Eye-Q	<i>Cronobacter spp.</i>
2012	Germany	Milupa	Aptamil Pre	<i>Cronobacter spp.</i>

2012	Russia	Fasska, Belgium	Damil	Salmonella
2011	USA	Mead Johnson	Enfamil Newborn	<i>Cronobacter spp.</i>
2010	Dubai	Hero	Hero Follow-on Milk	<i>Cronobacter spp.</i>
2009	France	Vitagermine	Babynat Organic Infant Milk	<i>Cronobacter spp.</i>
2009	Taiwan	Wei Chuan	Infant Formula	<i>Cronobacter spp.</i>
2008	China	Sanlu	Sanlu	<i>Cronobacter spp.</i>
2008	Spain	Sanutri	Natur 1, Confort 1, Confort 2	Salmonella
2008	France	Novolac	Novolac AR Digest	Salmonella
2007	Luxembourg and Austria	Hipp	Hypoallergene Anfangsnahrung HA1	<i>Cronobacter spp.</i>
2007	Germany	Milupa	Pre Aptamil HA	<i>Cronobacter spp.</i>
2007	Slovenia and Croatia	Humana	Babylove Dauermilch Tested	<i>Cronobacter spp.</i>
2007	Argentina	Nutricia	Nutrilon Prematuros	<i>Cronobacter spp.</i>
2006	Cyprus	Jotis	Sanilac 1	<i>Cronobacter spp.</i>
2006	South Korea	Namyang	Namyang Infant Milk	<i>Cronobacter spp.</i>
2006	Argentina	Nestlé	NAN 1	<i>Cronobacter spp.</i>
2005	Luxembourg	Babymil	Cereabib 1 &2	<i>Cronobacter spp.</i>
2005	Argentina	Mead Johnson	Enfamil AR	<i>Cronobacter spp.</i>
2005	France	Picot	Picot	Salmonella
2005	France	Danone – used Picot manufacturing facilities for products for export.	Bledilait 2, Gallia 2, Nursie 2, Alma 2, Gallia 2	Salmonella
2005	Brazil	Numico/Milupa distributor: Produtos Nutricionais Ltda	Aptamil infant formula	<i>Cronobacter spp.</i>
2004	France, Hong Kong, UK, Brazil, Gambia, Gabon and others	Mead Johnson	Pregestimil	<i>Cronobacter spp.</i>
2003	USA	Mead Johnson	Enfacare LIPIL Infant Formula	<i>Cronobacter spp.</i>
2002	China	Wyeth	Nursoy Promise	<i>Cronobacter spp.</i>
2002	USA	Mead Johnson	Portagen	<i>Cronobacter spp.</i>
2002	USA	Wyeth	Baby Basics, Kozy Kids, CVS, Hill Country Fare, HEB Baby, American Fare Little Ones, Home Best, Safeway Select, Healthy Baby, Walgreens, Parent's Choice	<i>Cronobacter spp.</i>
2001	UK	Wyeth	SMA Gold, SMA White	Botulism

Data from IBFAN and other documented reports.

