MICROBIOLOGICAL RISK ASSESSMENT SERIES

15

Enterobacter sakazakii (*Cronobacter* spp.) in powdered follow-up formulae

MEETING REPORT

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Declarations of Interest

Three of the 13 experts who participated in the meeting declared an interest in the topics under consideration:

Jean-Louis Cordier: He is an employee of a manufacturer of follow-up formula.

Séamus Fanning: He has an active research group in the area of *Enterobacter sakazakii* (*Cronobacter* spp.) and has received financial support from producers of follow-up formula and provided occasional diagnostic support to such producers.

Stephen Forsythe: He has an active research group in the area of *Enterobacter sakazakii* (*Cronobacter* spp.), which in the recent past received financial support from two companies producing follow-up formula.

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Foreword

Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food at both national and international level. Increasing foodborne disease incidence over recent decades seems, in many countries, to be related to an increase in disease caused by micro-organisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in patients and/or foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately, new tools that can facilitate actions seem to be on their way.

Over the past decade, risk analysis—a process consisting of risk assessment, risk management and risk communication—has emerged as a structured model for improving our food control systems with the objectives of producing safer food, reducing the number of foodborne illnesses and facilitating domestic and international trade in food. Furthermore, we are moving towards a more holistic approach to food safety, where the entire food chain needs to be considered in efforts to produce safer food.

As with any model, tools are needed for the implementation of the risk analysis paradigm. Risk assessment is the science-based component of risk analysis. Science today provides us with in-depth information on life in the world we live in. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation, and of the link between the microscopic and the macroscopic world, and how we can benefit as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing these data and information and gaining a better understanding of the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as identify the types of data necessary for estimating and optimizing mitigating interventions.

Microbiological risk assessment (MRA) can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens, including the elaboration of standards for food in international trade. However, undertaking an MRA, particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Nevertheless, foodborne illness is one of the most widespread public health problems, creating social and economic burdens as well as human suffering; it is a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at international level.

The Nutrition and Consumer Protection Division (FAO) and the Department of Food Safety, Zoonoses and Foodborne Diseases (WHO) are the lead units responsible for this initiative. The two groups have worked together to develop MRA at international level for application at both

national and international level. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen–commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and the work to date clearly indicates that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide member countries, Codex Alimentarius and other users of this material with the information they need to use risk-based tools, with the ultimate objective of ensuring that safe food is available for all consumers.

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Abbreviations

AOAC	Association of Official Analytical Chemists
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CCP	Critical Control Point
CDC	Centers for Disease Control and Prevention
CSF	Cerebrospinal fluid
DHS	Demographic Health Surveys
DNA	Deoxyribonucleic acid
f-AFLP	Fluorescent-labelled Amplified Fragment Length Polymorphism
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration [USA]
FUF	Follow-up formula
GHP	Good Hygiene Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ISO	International Organization for Standardization
MRA	Microbiological Risk Assessment
NEC	Necrotizing enterocolitis
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PIF	Powdered Infant Formula
rRNA	Ribosomal RNA (ribonucleic acid)
WHA	World Health Assembly
WHO	World Health Organization

Executive Summary

The Codex Alimentarius '*Code of Hygienic Practice for Powdered Formulae for Infants and Young Children'* was adopted with Annex I[1] and Annex III[2] at the 31st Session of the Codex Alimentarius Commission (Geneva, 30 June–4 July 2008). To finalize Annex II of the code, which establishes microbiological criteria for follow-up formulae (FUF), the 39th Session of the Codex Committee of Food Hygiene (2007) requested additional scientific advice from the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Accordingly, a technical meeting was convened (Washington DC, USA, 15–18 July 2008) with the objective of providing the scientific information to inform the decision-making process on the development of a microbiological criterion for *Enterobacter sakazakii* (*Cronobacter* spp.) for FUF intended for infants 6–12 months of age. This report documents the discussions and the outcome of that meeting. This was the third FAO/WHO meeting to address the issue of bacterial pathogens in powdered formula.

Recently, *E. sakazakii* has been reclassified as 6 species in a new genus, *Cronobacter* gen. nov. within the Enterobacteriaceae. The new species are *Cronobacter sakazakii; C. turicensis; C. malonaticus; C. muytjensii;* and *C. dublinensis;* with the sixth species indicated as genomospecies I, as currently it includes only two representative strains. All species have been linked retrospectively to clinical cases of infection in either infants or adults and therefore all species should be considered pathogenic. As the genus *Cronobacter* is synonymous with *Enterobacter sakazakii*, current identification schemes developed for *E. sakazakii* remain applicable for the *Cronobacter* genus. Furthermore, the reclassification does not require any change to the regulatory framework currently in place. To avoid any confusion arising from this taxonomic change, the designation *E. sakazakii* (*Cronobacter* spp.) is used throughout this report.

A review of documented *E. sakazakii* (*Cronobacter* spp.) infections worldwide has identified roughly 120 individually documented cases among infants and young children up to 3 years of age. Six of these cases are known to have occurred among infants 6–11 months and two cases among children in the 12–36 months age group. Of the 5 invasive (urine, blood, cerebrospinal fluid (CSF), brain tissue) cases in the 6–11 month age group, 3 had other active medical problems.

Globally, there appear to be very few surveillance data for *E. sakazakii* (*Cronobacter* spp.)-related illnesses. Although a couple of passive surveillance systems exist, no active surveillance system for *E. sakazakii* (*Cronobacter* spp.) disease has been identified. The data submitted in response to the Call for Data did not enable a detailed breakdown of numbers of cases by month for infants under 12 months, the exception being that for England and Wales, where a laboratory surveillance system identified bacterial isolates from infants <1 month and 1–11 months for the period 1992–2007, and the Philippines, where a laboratory-based antimicrobial resistance

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[1] Microbiological criteria for powdered infant formula, formula for special medical purposes and human milk fortifiers.

[2] Guidance for the establishment of monitoring programmes for *Salmonella*, *Enterobacter sakazakii* (*Cronobacter* species) and other Enterobacteriaceae in high-hygiene processing areas and in powdered formula preparation units.

surveillance system identified isolates from neonates and infants up to 12 months. These data reiterate the findings of the 2004 and 2006 meetings that amongst infants, neonates and infants less than 2 months of age are at the greatest risk of infection. While there is general agreement that immunocompromised infants are more susceptible to infection, the meeting was unable to identify a way of clearly defining the immune status of the population of concern. The meeting noted that a number of factors contribute to immune status, including the age of the infant, nutritional status, HIV status, other clinical conditions, pharmaceutical treatment, low birth weight and premature birth. The prevalence of such factors varies widely, and thus the meeting concluded that there will also be a wide variation in the prevalence of immunocompromised infants.

The meeting noted two main differences between the manufacturing processes for prepared infant formula (PIF) and follow-up formula (FUF). Firstly, due to the need for an increasingly diverse diet as infants get older, FUF may contain a wider variety of dry-mix ingredients (e.g. cocoa powder, fruit and vegetable powders or flakes, and flavours). Published data indicates that *E. sakazakii* (*Cronobacter* spp.) is likely to be present in a variety of dry ingredients unless appropriate control measures are implemented by suppliers. Such measures are essential, as there is no microbial reduction step in the FUF manufacturing process following the addition of these ingredients. Secondly, microbiological criteria, and therefore hygiene control measures, are more stringent for PIF. In some manufacturing facilities, production lines may be shared, i.e. used to manufacture both PIF and FUF. In these situations, the hygiene requirements necessary to ensure compliance with the microbiological criteria for PIF are also applied to FUF. However, in cases where FUF is produced on a dedicated line or on a line shared with other powdered products, the hygiene control measures may not be as stringent.

Very few data were available on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in products categorized as FUF for infants between 6 and 11 months. The absence of such data is most likely due to the fact that there is no mandated requirement for testing FUF for *E. sakazakii* (*Cronobacter* spp.).

Although most countries reported that FUF is marketed for infants 6 months of age or older, the available data showed that FUF is consumed by infants less than 6 months of age in both developing and developed countries, and is sometimes consumed by infants less than 1 month. However, data available on the consumption of FUF was limited. In relation to handling practices for powdered formula, the meeting found that worldwide, it likely that many caregivers to infants fail to follow the formula preparation and feeding practices recommended to reduce the risk associated with microbiological contamination of these powdered products. Educational and socioeconomic factors appear to have an impact with regard to the appropriate use of FUF.

The meeting reviewed all the available information in the context of whether or not a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) should be established for FUF, and weighed the scientific evidence for and against. Although not making an explicit recommendation on this, by presenting the available evidence the meeting sought to highlight the currently available data and how it contributes to our knowledge base and facilitates risk management decisions. In addition, the limitations of that data, particularly in relation to the narrow spectrum of the global population which it represents, are provided. In this context the analysis should provide guidance to risk managers as to whether there is any value in establishing a microbiological criterion for FUF.

MEETING REPORT

1. Introduction

International attention has been given to the safety of food for infants and young children and the prevention of potentially severe infections in infants. Since 2004, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have addressed the issue of bacterial pathogens in powdered formula, including the presence of *Enterobacter sakazakii* (*Cronobacter* spp.), through convening two joint technical meetings, in 2004 and 2006, and the development of a risk assessment model. The output of that work provided advice and guidance on mitigation strategies aimed at reducing the risk of infection in infants from consumption of powdered infant formula (PIF). It has supported the work of the Codex Alimentarius Commission (CAC) Committee on Food Hygiene (CCFH), which is responsible for developing risk management guidance in the area of food safety at the international level.

E. sakazakii (*Cronobacter* spp.) is a Gram-negative, motile, peritrichous non-spore forming, facultative anaerobic bacterium. It is an opportunistic pathogen and has been linked with serious infections in infants (FAO/WHO, 2004, 2006; Mullane et al., 2007a), notably following the consumption of PIF. Often described as an emerging pathogen, *E. sakazakii* (*Cronobacter* spp.) can cause bacteraemia and meningitis in infants and has also been isolated from infants in association with necrotizing enterocolitis (NEC). The first cases attributed to this organism occurred in 1958 in England (Urmenyi and Franklin, 1961). Since then and up to July 2008, the meeting has identified around 120 documented cases of *E. sakazakii* infection and at least 27 deaths from all parts of the world, in the published literature and in reports submitted by public health organizations and laboratories (Annex 1).

In 2007, CCFH requested additional scientific advice from FAO and WHO specifically on *E. sakazakii* (*Cronobacter* spp.) in follow-up formula (FUF) to inform the finalization of an annex to the new Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children (CAC, 2008b). The purpose of this annex is to establish microbiological criteria for FUF, and CCFH needs to make a decision on whether or not to include a criterion for *E. sakazakii* (*Cronobacter* spp.) in FUF.

In response to this request, FAO and WHO convened a meeting in Washington DC, USA, from 15 to 18 July 2008, with the objective of providing the most up-to-date scientific information to inform the decision-making process on the development of a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) in FUF. The present report documents the discussions and the outcome of that meeting.

The meeting was chaired by Dr Jeffrey Farber, Health Canada. A group of 13 experts from 7 countries participated in the meeting in their independent capacities and not as representatives of their Government, employers or institutions. They included one expert from the powdered formula manufacturing industry and two academics whose research is partly being funded by the industry. While these experts participated in the general discussion and exchange of information, they did not participate in the final adoption of the conclusions and recommendations of the meeting.

1.1 Background

The issue of pathogens, and in particular *E. sakazakii* (*Cronobacter* spp.), in PIF was brought to the attention of the 35th session (2003) of the CCFH by the 24th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) (CAC, 2003a), and by the United States of America and Canada, who introduced a risk profile for *E. sakazakii (Cronobacter* spp.*)* in PIF for consideration by the Committee (CAC, 2004). As a result, the 35th Session of the CCFH initiated the revision of the existing CAC Code of Hygienic Practices for Foods for Infants and Children (CAC, 2003b) and requested FAO and WHO to convene an expert meeting on pathogens of concern in PIF at the earliest opportunity. In response to this request, FAO and WHO convened a meeting on *E. sakazakii (Cronobacter* spp.*)* and other microorganisms in PIF, in Geneva, in February 2004, the output of which has been published in the meeting report (FAO/WHO, 2004).

The 37th Session of the Codex Committee on Food Hygiene (2005) requested FAO/WHO to expand on the scientific advice provided at the 2004 meeting. Accordingly, a technical meeting was convened on *E. sakazakii* (*Cronobacter* spp.) and *Salmonella* in PIF (FAO, Rome, 16–20 January 2006) to consider any new scientific data and to evaluate and apply a quantitative risk assessment model for *E. sakazakii* (*Cronobacter* spp.) in PIF. While noting that *E. sakazakii* (*Cronobacter* spp.) has caused invasive infection in all age groups, the meeting reiterated the findings of the 2004 expert meeting that infants appear to be the group at particular risk, but also highlighted that neonates (<28 days) and infants under 2 months of age are at greatest risk (FAO/WHO, 2006).

That meeting also reviewed a risk assessment model that had been developed to describe the factors leading to *E. sakazakii* (*Cronobacter* spp.) infection in infants and to identify potential risk mitigation strategies. The risk assessment model enables this by facilitating the comparison of different levels of product contamination and of different preparation, handling and feeding scenarios. In addition, it provides the means to evaluate microbiological criteria and sampling plans in terms of the risk reductions achieved and the percentage of product lots rejected. The risk assessment model estimates the relative risk of illness from *E. sakazakii* (*Cronobacter* spp.) posed to infants from intrinsically contaminated PIF. It does not consider contamination or recontamination from the environment or other sources post-manufacture. The model can be accessed on the Web (See www.mramodels.org).

Using the risk assessment model, the 2006 meeting concluded that some of the current preparation instructions on PIF product labels and those recommended by health authorities may lead to an increased risk of *E. sakazakii* (*Cronobacter* spp.) illnesses, and that these should be reviewed in the light of the risk assessment results. The risk assessment model was also found to be a valuable tool to assess and compare a series of criteria and sampling plans and their impact on risk reduction and on the amount of product rejected compared to a situation where no sampling plan was implemented. The meeting concluded that this tool could be applied for *E. sakazakii* (*Cronobacter* spp.) by risk managers within Codex and FAO and WHO member countries (FAO/WHO, 2006).

The findings of the 2006 technical meeting provided input to the finalization of the Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children, which was

adopted (together with Annex I[3] and Annex III[4]) at the 31st Session of the Codex Alimentarius Commission in Geneva, Switzerland, 30 June–4 July 2008 (CAC, 2008a). The 39th session of CCFH agreed to continue the work on the development of Annex II of the Code, which specifically addresses microbiological criteria for FUF. The need to establish a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) in FUF intended for infants up to 12 months of age led to a lengthy discussion in the Committee, and positions on this were clearly divided. While several delegations expressed the opinion that there was no scientific justification for a criterion for *E. sakazakii* (*Cronobacter* spp.) in this type of product, others considered that such a criterion was necessary since *E. sakazakii* (*Cronobacter* spp.) infections have been reported in infants up to 12 months of age. In the course of the discussion it was clarified that, to date, in the elaboration of scientific advice on *E. sakazakii* (*Cronobacter* spp.) in PIF, FAO and WHO had not given separate consideration to PIF versus FUF, as this had not been identified as an issue for consideration in the initial requests for scientific advice.

One of the factors that contributed to the extensive discussion on the need for microbiological criteria for FUF is the differences that exist at national level regarding the definition of PIF. While some countries adhere to the Codex definition of FUF, others do not. For example, in the USA, powdered formula products marketed for infants from birth to 11 months of age are all subject to the same regulations. Currently in the European Union, FUF refers to products for particular nutritional use by infants over the age of 4 months (trade in these products is prohibited from 31 December 2009) or for infants over the age of 6 months (trade in these products has been permitted since 1 January 2008) and are subject to different regulations to infant formula[5]. For both FUF and infant formula, it is mandatory to provide a statement on the label regarding the suitable age for use.

Given the differences of opinion on this issue within the Committee, it was agreed to submit a specific request to FAO and WHO for scientific advice to facilitate the Committee's decision on whether or not to establish a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) for FUF intended for infants up to 12 months of age.

The output of the 2006 meeting and the risk assessment model were also used by WHO and FAO in the development of guidance on the preparation, use, handling and storage of PIF so as to minimize risks where infants cannot be or are not fed breast milk (FAO/WHO, 2007). The development of such guidance was requested by World Health Assembly (WHA) Resolution 58.32 in 2005. These guidelines and a report on their adoption and implementation at national level were presented to the WHA in May 2008. The WHA was encouraged by the work of FAO, WHO and CAC on this issue, and urged Member States to implement the WHO/FAO guidelines on the safe, preparation, storage and handling of PIF, and to take action, through the implementation and monitoring of food safety measures, to reduce the risk of contamination of PIF with *E. sakazakii* (*Cronobacter* spp.) and other pathogens during both manufacture and use.

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[3] Microbiological criteria for powdered infant formula, formula for special medical purposes and human milk fortifiers.

[4] Guidance for the establishment of monitoring programmes for *Salmonella*, *Enterobacter sakazakii* (*Cronobacter* species) and other *Enterobacteriaceae* in high-hygiene processing areas and in powdered formula preparation units.

[5] Commission Directive 2006/141/EC (OJ L401, p1, 30/12/2006) of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. According to this Directive, from 31 December 2009 all labels of FUF must carry a statement to the effect that the product is suitable only for particular nutritional use by infants over the age of six months (Article 13.1b).

1.2 Scope

The scope of the work to be undertaken by FAO and WHO was defined by the series of questions that CCFH asked FAO and WHO to address. These were as follows:

- What is the number and incidence rate of confirmed *E. sakazakii* (*Cronobacter* spp.) infection in infants up to 12 months[6] (i.e. 1–11 months), presented by month, as compared to the incidence rate in all other age groups, including young children (12–36 months), older children and adults?
- Critically review all documented cases of confirmed *E. sakazakii* (*Cronobacter* spp.) infections in infants between 6 and 12 months of age, and consider specifically (i) the clinical history and outcomes, as well as (ii) the strength of the descriptive, epidemiological and/or microbiological evidence concerning the origin or source of these infections.
- Estimate the relative risk of *E. sakazakii* (*Cronobacter* spp.) infections in infants 6–12 months of age, associated with the consumption of follow-up formula, as well as any other sources as identified in the previous question?
- What is the number and incidence rate of immunocompromised infants up to 12 months, presented by month, as compared to the number and incidence rate of immunocompromised in all other age groups, including young children (12–36 months), older children and adults, and does this vary regionally?
- Taking into consideration the information generated in the above four questions, and given the application of risk management options as advocated in the Code, what is the relative risk reduction achieved by the application of microbiological criteria, as proposed in Annex 1 of the Code, to follow-up formula?
- Identify and describe active and passive surveillance systems for *E. sakazakii* (*Cronobacter* spp.) in countries.
- What is the proportion of infants less than 6 months of age that consume follow-up formula, and does this vary regionally?

In addressing these questions, the starting point was the output of the previous two expert meetings, in 2004 and 2006 (FAO/WHO, 2004, 2006). This information was considered together with additional data collected and received in response to a Call for Data issued specifically to address the questions related to FUF. While the pathogen and product of concern were clearly defined, the work to be undertaken was broader than that addressed by previous meetings, as specific consideration also had to be given to surveillance systems and immuno-compromised populations.

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[6] Although the Codex questions refer to infants between 6 and 12 months and young children 12–36 months, these age descriptors are not uniformly used in this report. Epidemiologic data and data from laboratory surveillance studies more commonly use the terms x–11 months to describe infants less than 1 year. Similarly, the descriptor x–35 months is used to describe young children less than 3 years of age. Therefore, in order to accurately reflect the available data, the terms 0–11months, 6–11 months or 12–35 months are also used in this report.

1.3 Data sources and objectives

To address these questions, FAO and WHO issued a call for relevant data via a number of different routes. Twenty-seven replies were received in response to the call. National authorities, non-governmental organizations, including academic institutions, consumer organizations and industry groups and manufacturers all submitted data and information (Submissions are listed in Annex 2). The meeting convened in July 2008 in Washington DC, USA, considered this new information, and aimed to:

- review any new epidemiological data with regard to the number and incidence rate of confirmed *E. sakazakii* (*Cronobacter* spp.) infection in infants as compared to the incidence rate in other age groups;
- review all documented cases of confirmed *E. sakazakii* (*Cronobacter* spp.) infections in infants between 6 and 11[7] months of age;
- review the available data in order to characterize the age group of 6 to 11 months in relation to their relative risk of *E. sakazakii* (*Cronobacter* spp.) infections and estimate the incidence of immunocompromised infants in those age groups;
- review available data on consumption to gain a better understanding of the population exposed to FUF; and
- analyse the available evidence in terms of whether or not a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) would lead to a reduction in risk of infections from consumption of FUF.

[7] Although the Codex questions refer to infants between 6 and 12 months and young children 12–36 months, these age descriptors are not uniformly used in this report. Epidemiologic data and data from laboratory surveillance studies more commonly use the terms x–11 months to describe infants less than 1 year. Similarly, the descriptor x–35 months is used to describe young children less than 3 years of age. Therefore, in order to accurately reflect the available data, the terms 0–11months, 6–11 months or 12–35 months are also used in this report.

2. Update on microbiological aspects

2.1 Update on the taxonomy of Enterobacter sakazakii

Enterobacter sakazakii was originally defined as a novel species in 1980 (Farmer et al., 1980). Using DNA:DNA hybridization and biochemical tests, 15 biogroups were defined, with the 16th being added more recently (Iversen et al., 2006). Existence of these divergent geno- and biogroups suggested that *E. sakazakii* may represent multiple species.

Polyphasic taxonomic approaches, including full length 16S rRNA sequencing, fluorescent-labelled Amplified Fragment Length Polymorphism (f-AFLP) analysis, as well as ribotyping together with DNA:DNA hybridization, was applied to a large collection of *E. sakazakii* isolates representing all 16 defined biotypes (Iversen et al., 2007). Based on these data, it was proposed to reclassify these strains into a new genus, *Cronobacter* gen. nov. (Iversen et al., 2008).

Cronobacter spp. is contaxic (synonymous) with *E. sakazakii.* The new genus is composed of six species, each of which could be defined based on the methods indicated above. These new species are: *Cronobacter sakazakii; C. turicensis; C. malonaticus; C. muytjensii;* and *C. dublinensis.* A sixth species was indicated as genomospecies I, but it includes only two representative strains at the present time.

The division of species for the most part follows Farmer's original 15 biogroups. Biogroups 1–4, 7, 8, 11 and 13 represent *C. sakazakii;* groups 5, 9 and 14 include *C. malonaticus; C. muytjensii* is represented by biogroup 15; *C. turicensis* is included as biogroup 16 (with the exception of two strains that appear to be a separate genomospecies and are designated as *C.* genomospecies I); and biogroups 6, 10 and 12 account for *C. dublinensis.* The last-named species can be further sub-divided into three sub-species: *C. dublinensis* subsp. *dublinensis* (biogroup 12), *C. dublinensis* subsp. *lausannensis* (biogroup 10) and *C. dublinensis* subsp. *lactardi* (biogroup 6).

Phenotypic profiling, using a range of biochemical reactions, is capable of identifying all six of the newly defined species, along with the three sub-species in the genus *Cronobacter*. These reactions include the utilization of dulcitol, lactulose, malitol, palatinose, putresine, melezitose, turanose, *myo*-inositol, *trans*-aconitate, *cis*-aconitate, 1-O-methyl α-D-glucopyranoside and 4-aminobutyrate, all as sole sources of carbon. Utilization of malonate was indicated following metabolism of malonate phenylalanine and the production of indole was determined following the addition of Kovac's reagent. In addition, analysis of the metabolic capacity of these strains was also determined with the Biotype 100 and the Biolog 'OmniLog' Phenotypic Microarray (Iversen et al., 2008).

The reclassification of *E. sakazakii* to the new genus *Cronobacter* will not require the modification of dedicated culture-based laboratory isolation and detection protocols. All currently valid laboratory methods will continue to facilitate the recognition of all of the organisms defined within the new taxonomy. Furthermore, definition of the individual species can be achieved using the phenotypic markers described above (Iversen et al., 2008).

2.2 Virulence assessment of Cronobacter species

Differences in the virulence of the *E. sakazakii* (*Cronobacter* spp.) species were noted in the previous expert meeting (FAO/WHO, 2006). Since then, further evaluation of this with respect to host inflammatory response, following intracranial inoculation of various *E. sakazakii* (*Cronobacter* spp.) strains into infant rats and rat capillary endothelial brain cells, has been undertaken (Townsend et al., 2007; Townsend, Hurrell and Forsythe, 2008). This work indicated that *E. sakazakii* (*Cronobacter* spp.) strains persisted or replicated in macrophages and showed moderate attachment and invasion of human endothelial cells. *C. turicensis* differed from *C. sakazakii* with respect to lower attachment and invasion of endothelial cells, but maintained replication in macrophages and invasion of brain endothelial cells. *C. muytjensis* strains showed moderate attachment and invasion of endothelial cells, but were less stable in macrophages and significantly less invasive of brain cells. *C. dublinensis* strains showed low attachment and high invasion of endothelial cells, and also showed significantly less brain capillary endothelial cell invasion. Further, the persistence in macrophages was only observed with *C. sakazakii* and *C. turicensis* strains. Macrophage uptake demonstrated that the ratio of the cytokines IL-10 and IL-12 secreted from macrophages is high after 24 h, suggesting a Type 2 immune response, which is inefficient in fighting intracellular infections. These findings may help explain the diversity of virulence traits among *E. sakazakii* (*Cronobacter* spp.) isolates, and an unsuccessful immune response would contribute to the opportunistic nature of this infection. Comparison of these virulence traits with known outbreak strains include high brain capillary endothelial cell invasion, macrophage replication, and most closely matches what is observed in other *E. sakazakii* (*Cronobacter* spp.) outbreak strains from Tennesse and France (Himelright et al., 2002; Caubilla-Barron et al., 2007; Townsend et al., 2007; Townsend, Hurrell and Forsythe, 2008).

Health Canada has been evaluating various animal models with a view to gaining an understanding of the dose-response relationship and virulence differences between strains. This work indicates that the neonatal gerbil model appears to be very promising due to the observed invasion of multiple organs and the brain after oral dosing. Future work will focus on the interaction with the intestinal cells of the gerbil gut, possibly being supplemented by *in vitro* work using cell lines derived from intestinal cells. Further indicators of infection that may be useful in deciphering the pathogenic potential of strains (growth in liver, brain, time to death, etc.) will be investigated. The model will be used to investigate levels of inoculation and exposure to better address the dose-response relationship, an area of information that is currently lacking. The use of, for example, germ-free gerbils will also be addressed in future endeavours.

This work indicates that there appear to be differences in virulence amongst clinical, environmental and food isolates of *E. sakazakii* (*Cronobacter* spp.). Whether this variation is relevant in human infant infections is unclear. Nevertheless, despite these studies, no data currently shows that any one of these species is not a risk to neonatal and infant health. Therefore, all six species in the genus *Cronobacter* should be considered to be pathogenic, as each one has been linked retrospectively to clinical cases of infection in either infants or adults.

2.3 Regulatory implications of taxonomic changes

Creation of a new genus simplifies the inclusion of these pathogenic organisms in the current legislation. While it is important to be aware that multiple species of *E. sakazakii* (*Cronobacter*

spp.) exist, no changes are proposed at this time to the existing regulatory framework currently in operation.

2.4 Isolation and identification

The isolation of bacteria, such as *E. sakazakii* (*Cronobacter* spp.), from dried foods requires a series of steps to resuscitate stressed cells that would otherwise not be cultured, and therefore not be recorded. Methods for the specific detection of *E. sakazakii* (*Cronobacter* spp.) from powdered formula have improved in recent years, and are increasingly being used. This includes the Technical Standard ISO/TS 22964 (2006) method from the International Organization for Standardization (ISO). Therefore, our ability to monitor the bacterium is improving, compared with earlier studies that used methods with less specificity and selectivity. Methods available for monitoring *E. sakazakii* (*Cronobacter* spp.) have recently been reviewed by Fanning and Forsythe (2007). Culturing methods have improved with the development of chromogenic agars, which help to distinguish *E. sakazakii* (*Cronobacter* spp.) from other Enterobacteriaceae that may additionally be present in powdered formula. Confirmed identification is feasible with improved phenotyping databases, and DNA-sequence-based methods. International standardization of improved methods by the ISO and FDA-AOAC is currently underway, and the results are expected in the near future. As stated above (Section 2.1), all currently validated laboratory methods will continue to facilitate the recognition of all species defined within the new taxonomy.

The detection of bacteria from normally sterile sites (e.g. blood, CSF) is less complex than their detection and isolation from powdered formula, as the organisms would not be stressed, and are unlikely to be in a mixed population. Nevertheless, accurate clinical identification of isolates as *E. sakazakii* (*Cronobacter* spp.) has been restricted by the use of methods that have not been specifically validated for the organism.

Molecular sub-typing protocols for *E. sakazakii* (*Cronobacter* spp.) are also being developed and used to characterize the organism. (Block et al., 2002; Drudy et al., 2006; Mullane et al., 2007b, 2008; Healy et al., 2008). Such tools have numerous applications, such as in the analysis of clinical samples, in outbreak investigations and in environmental surveillance, and an increasing number of reports describe their application and utility as part of an overall hazard analysis critical control point (HACCP) programme. With regard to environmental surveillance, these approaches could provide valuable data, which could contribute to a better understanding of the microbial ecology of the local and general environment and facilitate the definition of 'hot-spots' of contamination and transmission routes (Mullane et al., 2007a,b, 2008).

PFGE is regarded as a 'gold-standard' sub-typing protocol. However, currently, no standardized method exists for sub-typing *E. sakazakii* (*Cronobacter* spp.). An *ad hoc* group within the PulseNet system, consisting of five internationally recognized laboratories, is currently working on the development of a standardized PFGE protocol to sub-type *E. sakazakii* (*Cronobacter* spp.).

2.5 Conclusions

Since 2006, significant work has been undertaken to further our understanding of *E. sakazakii* (*Cronobacter* spp.). One of the outcomes has been the reclassification of *E. sakazakii* strains into a new genus, *Cronobacter* gen. nov., with six species. *Cronobacter* spp. is synonymous with *E. sakazakii* and throughout this report the term *E. sakazakii* (*Cronobacter* spp.) will be used in recognition of this reclassification.

Work has also progressed on the virulence of these organisms, and recent studies indicate that there appear to be differences in virulence amongst clinical, environmental and food isolates of *E. sakazakii* (*Cronobacter* spp.) but there is no indication that any one of these species is not a risk to neonatal and infant health. Therefore, all six species in the genus *Cronobacter* should be considered to be pathogenic. However, further research will be needed before a dose-response relationship can be established for these organisms.

The ability to monitor powdered formula for *E. sakazakii* (*Cronobacter* spp.) has improved in recent years. However, analysis of clinical samples does not seem to have progressed at the same rate. The reclassification of the organism will not require the modification of dedicated culture-based laboratory isolation and detection protocols and currently valid laboratory methods will continue to facilitate the recognition of all of the organisms defined within the new taxonomy. International standardization of improved methods is also currently underway and should contribute to more comparable data sets in the future. Molecular sub-typing protocols are also being developed for *E. sakazakii* (*Cronobacter* spp.), and are considered to have an application in environmental surveillance and outbreak investigations. However, currently, no standardized method exists for sub-typing *E. sakazakii* (*Cronobacter* spp.), although a number of laboratories are working on this.

Finally, while the meeting noted that it was important to be aware that multiple species of *E. sakazakii* (*Cronobacter* spp.) exist, the new taxonomy was not considered to affect the existing regulatory framework, and concluded that the current Codex microbiological criteria for *E. sakazakii* (*Cronobacter* spp.) remain a valid means to support efforts to reduce the risk to neonatal and infant health.

3. Epidemiology and public health

3.1 Current data sources

The burden-of-illness pyramid is a model for understanding disease reporting. This shows the chain of events that must occur for an episode of illness in the population to be registered in surveillance. The bottom of the pyramid (Figure 1) depicts a portion of the general population that is exposed to an organism. Some of these may become ill, and some of these may seek medical care. A specimen for laboratory analysis may be obtained from some of those who seek medical care. The laboratory seeks to detect and identify the causative organism and thereby confirm the case. It is this laboratory-confirmed case that is reported to a public health agency.

Figure 1.Burden-of-illness pyramid.

Surveillance systems for communicable diseases can be based upon one or more reporting mechanisms. Active surveillance systems involve public health authorities soliciting information at regular intervals from sources such as community or hospital laboratories or clinicians. It is the most sensitive type of surveillance and therefore produces the most accurate case counts and rates. Active surveillance systems are rare because of their resource demands. An alternative is passive surveillance, which can be either mandatory or voluntary. In mandatory passive surveillance systems, clinical laboratories, clinicians or other professionals are required by statute to report cases to public health authorities. In general, this type of surveillance is used for 'reportable' or 'notifiable' infections. Based on studies in several countries on a range of diseases, it is accepted that this type of reporting is less complete than active surveillance. Voluntary passive surveillance does not require sources to report cases and typically does not involve formal channels for reporting illnesses. Instead, voluntary passive

reporting relies upon the initiative of the treating clinician, laboratory, patient or advocate, to relay information about a case to public health authorities. This type of surveillance is the least sensitive. Although it can be useful in indicating trends, it cannot be used to calculate accurate rates of illness

No active surveillance system for *E. sakazakii* (*Cronobacter* spp.) disease has been identified based on the available information on surveillance systems. *E. sakazakii* (*Cronobacter* spp.) infection was identified as a notifiable condition in Brazil and Hungary. Invasive *E. sakazakii* (*Cronobacter* spp.) disease, based on clinical and laboratory criteria, is also notifiable in New Zealand. In the United States of America, invasive infection among children less than 12 months of age has been notifiable using a mandatory, passive system in one state only, Minnesota, since 2005. Because *E. sakazakii* (*Cronobacter* spp.) disease is rare and there are relatively small populations in most of the places where *E. sakazakii* (*Cronobacter* spp.) disease is now notifiable (less than 5 million people in each of New Zealand and Minnesota, less than 10 million in Hungary) many years of surveillance will be required to establish a reliable estimate of incidence for these populations. Further, it is unknown how their incidences compare with that in other jurisdictions.

Most countries reported having a foodborne disease surveillance system and/or an outbreak reporting system that would encompass *E. sakazakii* (*Cronobacter* spp.) infection. However, it is noteworthy that instances were reported where cases were identified by outbreak or voluntary passive reporting, but not by the national foodborne disease reporting system. Unpublished *E. sakazakii* (*Cronobacter* spp.) cases have been identified through voluntary passive reporting. This type of case ascertainment is used by jurisdictions for *E. sakazakii* (*Cronobacter* spp.) infection; cases reported may represent an indeterminate fraction of actual cases. Further, with voluntary reporting, it is possible that deaths or severe disease such as meningitis are more commonly reported than milder disease. Existing data suggest that very young infants are at a greater risk of severe disease and death from infection with this organism; it might be that a differential proportion of cases among this age group are reported through the voluntary system than for those cases among older infants and toddlers. Some countries have laboratory-based surveillance systems focusing on, for example, nosocomial infections, bacteraemias or antimicrobial resistance, which include *E. sakazakii* (*Cronobacter* spp.) in the organisms under surveillance. However, these data are rarely sufficient to examine exposure factors.

Additionally, outbreaks of any disease are easiest to detect when cases cluster both geographically and temporally. Outbreaks of *E. sakazakii* (*Cronobacter* spp.) disease have been detected most commonly among newborn and very young infants in hospital nurseries and neonatal intensive care units. In these settings, relatively large numbers of infants are at risk of exposure to a common disease vehicle, and clinicians quickly notice when more than one infant develops infection due to the same pathogen. These factors—relatively large numbers of both exposed and unexposed, and ill and well infants—simplify cluster investigation and epidemiologic implication of a source. Groups of older infants are rarely housed together in the same facility or exposed to a common *E. sakazakii* (*Cronobacter* spp.) source (for example fed from the same can of powdered formula). Therefore, because exposure is believed to be rare and reporting is voluntary, community-based outbreaks among older infants are less likely to be detected than hospital-based outbreaks among very young infants.

No estimates have been made of the ‘multipliers’ that could be applied at the various stages in the *E. sakazakii* (*Cronobacter* spp.) burden-of-illness pyramid to estimate the actual burden of disease, or indeed for comparable diseases, such as listeriosis. Such estimates are likely to be country specific. It is also possible that the number of case reports of *E. sakazakii* (*Cronobacter*

spp.) published in the literature will decrease when it is no longer considered an emerging infection.

A limiting factor in the diagnosis of *E. sakazakii* (*Cronobacter* spp.) infections and their sources is the ability or capacity of clinical, food and environmental laboratories to identify the organism. Although good progress has been made with the methodology, as is the case with most emerging pathogens there is a need for increased awareness and capacity building.

3.2 Review of cases of E. sakazakii *infections*

Documented (not necessarily published) cases of *E. sakazakii* (*Cronobacter* spp.) infection in infants and young children from 1961 to 2008 have been collated and tabulated by various authors and groups (Data submissions from France, International Formula Council (IFC), International Dietary Foods Industries (ISDI), Nestlé, UCD, USA (CDC), Iversen and Forsyth, 2003). This information has been collated and an overview of all cases is presented in Annex 1. Collectively, there are approximately 120 of these recorded cases among infants and young children less than 3 years of age. Eight cases are known to have occurred among children 6–35 months old (Table 1). Laboratory surveillance data from two countries, the UK and the Philippines, indicate an additional 85 infections among infants and young children in these two countries alone.

Six cases occurred among infants 6–11 months old. Of these, 5 cases were invasive (isolated from blood, CSF, brain tissue or urine[8]). Three of the 5 case-patients had other active medical problems (one received feeds via continuous infusion through a gastrostomy tube; one had severe combined immunodeficiency syndrome; one had recently undergone surgical correction for vesico-ureteral reflux). Of the remaining two infants with invasive disease onset between 6–11 months of age, one had a history of premature birth (33 weeks estimated gestational age) and the other had no known medical problems. Outcomes are known for 3 of the 5 patients with invasive disease: all survived. All 5 of the case-patients with invasive disease consumed PIF, and PIF associated with 3 of these case-patients was tested. PIF associated with one case did not yield *E. sakazakii* (*Cronobacter* spp.), but both the prepared formula within the hospital and the blender used to prepare the formula yielded *E. sakazakii* (*Cronobacter* spp.). Approximately 100 g of PIF associated with the second case was available for testing; this did not yield *E. sakazakii* (*Cronobacter* spp.). Investigators of the third case were not certain they had obtained the correct lot of powdered formula; however, the lot they tested did not yield *E. sakazakii* (*Cronobacter* spp.). No other source of *E. sakazakii* (*Cronobacter* spp.) was identified for any of the 5 cases with invasive disease in this age group[9].

Two cases occurred among toddlers 12–35 months old. Both cases were invasive. Both case-patients had ongoing medical problems. One had a posterior fossa dermoid cyst, which also

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[8] A trustworthy culture of urine (e.g. from a suprapubic aspiration or a catheterization performed by an expert) was considered to be technically indicative of invasive disease, since urine is supposed to be sterile while it remains inside the body. While recognizing that urine isolates are not always considered to be indicative of invasive disease (e.g due to poorly performed cultures), they were included here as there was adequate information about where or how the cultures were performed in the documented cases to indicate a high likelihood that they were performed correctly. Also, in contrast to other age groups except the elderly, urinary tract infections are a serious infection and major source of bloodstream infections in infants.

[9] These five cases all occurred in the USA, where FUF is not marketed for infants. Thus, all cases had consumed PIF.

yielded *Corynebacterium aquaticum* and *Enterobacter cloacae*, and the other had Kasabach-Merrit syndrome and had recently received chemotherapy. The outcome is known for one case-patient; this child survived. One of the case-patients consumed powdered formula marketed for infants 9–24 months old. The opened can of powdered formula associated with this case yielded *E. sakazakii* (*Cronobacter* spp.) with a PFGE pattern indistinguishable from the clinical isolate. Sealed cans of powdered formula and environmental samples from the case-patient's home and childcare setting did not yield *E. sakazakii* (*Cronobacter* spp.). The other case-patient is not known to have consumed powdered formula products, and no source of infection was identified for this patient.

Given that all well described cases among children 6–35 months old have occurred sporadically rather than as part of an outbreak, epidemiological methods could not be used to implicate a source of infection. Instead, investigators were required to rely on microbiological testing of suspect vehicles. Such testing is predicated on the retention by the infant's caretakers of possible vehicles, in sufficient quantities to enable valid testing, under appropriate storage conditions. Frequently, by the time cases come to the attention of investigators, items the child was exposed to have been cleaned, used up, or thrown away. When powdered formula is suspected as a vehicle, the product lot number must also be known and investigators must be able to access large quantities of factory-sealed product to enable testing sufficient for investigative purposes. Further, the organism may occur in clumps rather than as an evenly distributed contaminant within powdered formula products. Therefore, results of tests of product other than that already consumed by the ill child might not reflect contamination of the product the ill child was exposed to. All of these factors combine to make identification of the infection vehicle extremely difficult among sporadic cases. Indeed, even among very large outbreaks of other foodborne disease, implication of a vehicle through microbiological methods is infrequently successful.

3.3. Numbers and incidence of infection

The number of well documented cases of *E. sakazakii* (*Cronobacter* spp.) infections in infants worldwide has increased in recent years but remains very low compared to many other infectious diseases. Although there are reports in the literature of *E. sakazakii* (*Cronobacter* spp.) infections in older age groups, including adults, these have been less well documented in the literature than infections in infants, and no systematic review of cases in children and adults appears to have been undertaken.

The response to the Call for Data revealed a very mixed picture in terms of the extent of surveillance for *E. sakazakii* (*Cronobacter* spp.) infections and the numbers and incidence of infections in neonates, other infants, children and adults. Overall, there are too few data sets available to give a definitive picture of the likely number of infections attributable to this organism, or variation in incidence from country to country or between regions. The difficulty in drawing conclusions from passive surveillance data has already been discussed in Section 3.1.

Data available for bacteraemia due to *Enterobacter* spp. in England and Wales and Northern Ireland include data for *E. sakazakii* (*Cronobacter* spp.). *E. sakazakii* (*Cronobacter* spp.) comprised 2.4% of bacteraemia reports for *Enterobacter* spp. between 2003 and 2007 (HPA, 2007). Although there are caveats on the interpretation of data from the laboratory (see Section 3.1) the bacteraemia dataset does provide a basis for a more detailed investigation of the information collected on *E. sakazakii* (*Cronobacter* spp.).

Table 2 shows the laboratory reports for *Enterobacter sakazakii* (*Cronobacter* spp.) infections in the UK 1999–2007 according to different age groups. Clinical or epidemiological data are not routinely collected as part of the surveillance system. Of the 570 laboratory-reported infections in this period, 15 were from infants (<1 year) and 16 from children aged 1–4 years. The data from England and Wales between 1992 and 2007 (Table 3) includes laboratory reports of isolations from neonates (13 reports) and from infants aged 1–11 months (18 reports). No distinct trend over time is seen for any of the age groups.

From the data submitted by other countries, Hungary indicated that *E. sakazakii* (*Cronobacter* spp.) has been isolated from human samples 25–29 times between 2002 and 2006, with 5 reports in children <1 year and 1 in those aged 6–12 months in 2003. Tunisia reported 26 *E. sakazakii* (*Cronobacter* spp.) isolates for 2006 and 2007, although the ages of these cases were not specified. Furthermore, in these datasets no clinical history was provided. It is unclear whether the isolations were from bacteraemias (indicative of invasive infection) and there are indications in some cases suggesting colonization of other sites (e.g. sores, secretions). This makes it difficult to compare themwith the data from bacteraemias in the UK, notwithstanding the differences in surveillance systems operating in different countries.

Table 1. Details on recorded cases of *E. sakazakii* (*Cronobacter* spp.) isolated from any source among children aged 6–35 months old.

Age months	Year	Location	Patient location at onset for *E. sakazaki*	culture positive	Other known medical conditions	Outcome	Part of known cluster or outbreak	Received powdered formula	Notes about powdered formula testing	Other infor-mation	Reference
6	1990	USA (MD*)	Hospital	Blood	Jejunal atresia corrected in neonatal period; gastrostomy (fed by continuous infusion via enteric tube)	Recov-ered	No	Yes (type of product un-known)	Dry powdered formula did not yield E. sakazakii; formula prepared within hospital and blender used to prepare formula yielded E. sakazakii	Had concomitant Leuconostoc mesenteroides bacteraemia	Noriega et al., 1990
6	2003	Hungary	Un-known	"gastric, sore and nasal se-cretion"	Unknown	Unknown	No	Unknown			Julia Cseh, Hungarian Food Safety Office (submitted in response to Call for Data)
8	2003	USA (CA*)	Com-munity	Blood	none	Recov-ered	No	Yes (PIF with iron)	Family submitted ~100 g of PIF from open can; this sample did not yield *E. sakazakii*. CDC does not have information about whether FDA tested product from sealed cans of same lot	Environmental samples from home did not yield *E. sakazakii*	Bowen and Braden, 2006
8	2005	USA (MN*)	Un-known	Urine	Undisclosed anomaly of chromosome 17; vesicoureteral reflux surgically repaired at 8 months of age	Unknown	no	Yes (PIF with DHA and ARA**)	Unknown		CDC, unpublished data
10	2003	USA (MN*)	Un-known	Blood	Premature (33 wk EGA)	Unknown	No	Yes (PIF designed for pre-mature or low birth weight infants)	Unknown		CDC, unpublished data

Age months	Year	Location	Patient location at onset	culture positive for *E. sakazaki*	Other known medical conditions	Outcome	Part of known cluster or outbreak	Received powdered formula	Notes about powdered formula testing	Other information	Reference
10	2004	USA (UT*)	Long-term care facility for children	Blood	severe combined immunodeficiency disorder	Recovered	No	Yes (PIF for infants and young children especially for those with food allergies)	Opened can being used at time fevers developed was tested at CDC and did not yield E. sakazakii. CDC does not have information about whether product from sealed cans of same lot were tested by manufacturer or FDA	Infant was fed powdered infant formula exclusively	Bowen and Braden, 2006
13	2007	USA (MI*)	Community	Blood	Kasabach-Merrit syndrome; recent chemotherapy	Unknown	No	Yes (PIF designed for infants and toddlers of 9–24 months)	Opened can yielded *E. sakazakii* isolate powdered formulaGE pattern indistinguishable from patient isolate. FDA tested 2 lots associated with patient and did not find *E. sakazakii*	Environmental samples from patient home and daycare provider did not yield *E. sakazakii*	CDC, unpublished data
20	1996	Canada	Community	Brain tissue	Posterior fossa dermoid cyst	Recovered	No	No		Brain abscess tissue also yielded *Corynebacterium, aquaticum* and *Enterobacter cloacae*	Tekkok et al., 1996

*Key to USA States: CA = California; MI = Michigan; MD = Maryland; MN = Minnesota; UT = Utah. Notes: ‡ wk = week; EGA = estimated gestational age.
** DHA = docosahexaenoic acid; ARA = arachidonic acid.

Table 2. *Enterobacter sakazakii* (*Cronobacter* spp.) laboratory-confirmed reports by age group, United Kingdom (England, Scotland, Wales and Northern Ireland) 1999–2007.

Age Group	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total
<1 year*	0	0	1	3	5	1	3	2	0	15
14 years	2	3	4	2	1	0	0	3	1	16
59 years	1	1	0	0	2	0	0	0	0	4
1014 years	1	2	0	1	0	2	1	0	0	7
1544 years	8	9	7	6	13	10	10	10	6	79
4564 years	9	17	16	27	22	19	13	29	11	163
6574 years	11	13	13	12	23	21	6	9	11	119
75+ years	16	5	19	14	15	23	23	22	15	152
Unknown	1	1	6	4	2	0	1*	0	0	15
Total	49	51	66	69	83	76	57	75	44	570

NOTES: * Includes data for infant less than 1 month old.
DATA SOURCES: Health Protection Agency (HPA); Health Protection Scotland (HPS); Communicable Disease Surveillance Centre Northern Ireland (CDSC(NI).

Table 3. *Enterobacter sakazakii* (*Cronobacter* spp.) laboratory-confirmed reports by age group for England and Wales 1999–2007

Age Group	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**Total**
<1 month	0	3	0	1	2	3	0	0	0	1	1	0	0	3	0	0	14
1–11 months	1	1	1	3	2	2	1	0	0	0	2	2	1	0	2	0	18
1–4 years	0	2	4	2	2	3	2	2	2	3	2	0	0	0	2	1	27
5–9 years	0	0	1	0	3	3	0	1	1	0	0	2	0	0	0	0	11
10–14 years	0	0	0	0	0	0	1	1	2	0	1	0	2	1	0	0	8
15–44 years	5	1	4	5	6	15	6	7	6	7	5	12	10	9	10	6	114
45–64 years	4	1	8	15	8	26	21	8	16	14	20	20	17	10	25	9	222
65–74 years	1	6	17	8	20	18	14	8	11	9	9	17	20	6	7	8	179
75+ years	5	6	7	6	15	19	12	14	4	17	10	14	21	20	20	12	202
Unknown	2	2	0	0	2	2	3	1	1	5	4	2	0	0	0	0	22
Total	18	22	42	40	60	91	60	42	43	56	54	69	71	49	66	36	819

SOURCE: Labbase 2–11/03/2008: Health Protection Agency Centre for Infections Environmental and Enteric Diseases Department. Note that the database is dynamic and, as such, data are subject to change.

The Philippines reported information collected from the Research Institute of Tropical Medicine's Antimicrobial Resistance Surveillance Program within the Department of Health between 1998 and 2007. This dataset indicated a total of 237 cases in the period 1998 to 2007 (Table 4) and provides information on the site of isolation and the age of the patient (Table 5). Of these cases, 18 were in infants less than 1 month old and 5 in infants 1–2 months old. Very few cases were reported for older infants. However, 9 cases were reported in young children 12–35 months (Table 5). As with the UK data, as these come from a laboratory-based surveillance system there is no information available on the symptoms, outcome or whether or not the infants and young children had consumed PIF or FUF.

Table 4. *Enterobacter sakazakii* (*Cronobacter* spp.) laboratory-confirmed isolates by age group for 11 regions in the Philippines.

Age Group	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total
<1 month	1	1	4	3	2	2	2	3	0	0	18
1–2 months	0	1	1	0	0	0	1	2	0	0	5
3–4 months	0	0	0	1	0	0	0	0	0	0	1
5–6 months	0	0	0	0	0	1	0	0	0	0	1
7–8 months	0	0	0	1	0	0	0	0	0	0	1
9–10 months	0	0	0	0	0	0	0	0	0	0	0
11–12 months	0	0	0	0	0	0	0	0	1	0	1
1–4 years	0	0	0	1	2	1	0	3	3	1	11
5–10 years	0	0	0	2	0	2	1	6	0	0	11
>10 years	0	1	5	29	18	34	23	19	17	17	163
Unknown	0	0	0	5	1	3	8	3	3	2	25
Total	1	3	10	42	23	43	35	36	24	20	237

Table 5. Site of isolation of *Enterobacter sakazakii* (*Cronobacter* spp.) in infants and young children up to 3 years of age in the Philippines

Age Group	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total
<1 month	UC (1)	U (1)	W (2) B (1) O (1)	B (2) UC (1)	UC (1) W (1)	S (1) B (1)	B (2)	B (3)	—	—	18
1–2 months	—	E (1)	B (1)	—	—	—	B (1)	B (2)	—	—	5
3–4 months	—	—	—	B (1)	—	—	—	—	—	—	1
5–6 months	—	—	—	0	—	U (1)	—	—	—	—	1
7–8 months	—	—	—	W (1)	—	—	—	—	—	—	1
9–10 months	—	—	—	—	—	—	—	—	—	—	—
11–12 months	—	—	—	—	—	—	—	—	W (1)	—	1
13–35 months	—	—	—	—	U (1)	U (1)	—	B (1) W (1) E (1)	B (2) U (1)	U (1)	9

KEY to site of isolation: B = Blood; E = Ear; O = Other; S = Sputum; U = Urine; UC = Umbilical cord; W = Wound.

3.3.1 Incidence of *E. sakazakii* (*Cronobacter* spp.) infection

The only data available to make any estimation of incidence of *E. sakazakii* (*Cronobacter* spp. infection is that of England and Wales and the Philippines. While these data were used to provide some figures on incidence, there are a number of a caveats of which one must be aware, and which, as noted in Section 3.1, reduces the accuracy of such estimates. Thus, it is important to remember that the incidence data presented below are based on laboratory isolations. While it has been confirmed that the figures available relate to individual cases, there may be more cases than these. For example, in providing data the Philippines noted that this may not be representative of all *E. sakazakii* (*Cronobacter* spp.) cases as only sentinel hospitals provide data to the laboratory surveillance programme. Furthermore, there are not sentinel hospitals in all regions of the country. In addition the data from England and Wales represents bacteraemia cases only, while that from the Philippines represents a range of *E. sakazakii* (*Cronobacter* spp.) infections, thus making comparisons difficult.

Data from England and Wales are available for infants under 1 month and from 1–11 months for 1992–2007. Based on these data, an estimated annual incidence rate for neonates was 17.60 per million population over the period 1992–2007. For infants aged 1–11 months, the estimated incidence rate was 2.06 per million population, among children 1–4 years it was 0.70 per million, and for those 5–9 years it was 0.22 per million population (Figure 2).

Taken together, the data for infants and young children from England and Wales 1992–2007 is indicative of a decreasing incidence rate with age. This is by a factor of 8.5 between <1 month and 1–11 months; by a factor of about 3 between 1–11 months and 1–4 years; and by a factor of 3 between 1–4 years and 5–9 years. The trend is illustrated in Figure 2.

Data from the Philippines were obtained from 11 sentinel hospitals serving approximately 63% of the country, and include isolates from any human source between 1998 and 2007. Because regional age-stratified data were not available during the writing of this report, we used population data from the entire 2000 census to estimate incidence rates by age. These estimates are likely to underestimate the actual incidence of *E. sakazakii* isolation in this population by 37% or more. However, using these data, the annual incidence rate in the Philippines between 1998 and 2007 of *E. sakazakii* isolation from any site for infants less than 12 months was 1.4 per million population; among children 12–23 months it was 0.05 per million population; among children 24–35 months it was 0.25 per million; among children 36–48 months it was 0.16 per million; and among children 5–9 years it was 0.11 per million.

A similar pattern is seen in data from the Philippines 1998–2007 (Figure 3) to that from England and Wales. Although the caveats about incidence estimation described above apply, the degree of underestimation should be similar across age groups and, therefore, comparisons of rates between age groups should be meaningful. Thus, the incidence of isolation of *E. sakazakii* from any site decreased by a factor of 10 between infants and children 1–4 years old, and did not substantially decrease further among children 5–9 years old. More detailed information about isolation site was available for children <48 months old. Using these data, the incidence of invasive cases (blood or urine isolates; no CSF isolates were reported) decreased by a factor of 4 between infants <1 year and children 24–35 months old, and decreased minimally between 24–35 months and 36–48 months. No invasive cases were reported in the 12–23 month age group for this period.

Infections with *Salmonella* and *Campylobacter* have also been reported to show a decrease in incidence rates between infants and young children (Acheson and Lubin, 2008; Koehler et al., 2006). The incidence rate for *Salmonella* infections in young children (1–4 years) in FoodNet 1996–1998 was threefold lower than that for infants (Koehler et al., 2006).

Considering the UK data as a whole for 1999–2007, and with neonates included in the infant category, then the highest incidence rate was in those aged 75 years or more (3.75 per million population), followed by the 65–74 years age group (2.65 per million population). For infants (<12 months) the rate was 2.45 and for young children (1–4 years) it was 0.65.

Two special studies used to estimate incidence of invasive *E. sakazakii* (*Cronobacter* spp.) disease were presented in the previous meeting report (FAO/WHO, 2006). CDC queried FoodNet sites within the USA for invasive cases among infants in 2002. They identified 4 cases for an estimated incidence of 1 invasive case among 100 000 infants per year. Stoll et al. (2004) looked for cases among a network of 19 neonatal intensive care units in the USA, and estimated 9.4 invasive cases per 100 000 very-low-birth-weight infants during the study period.

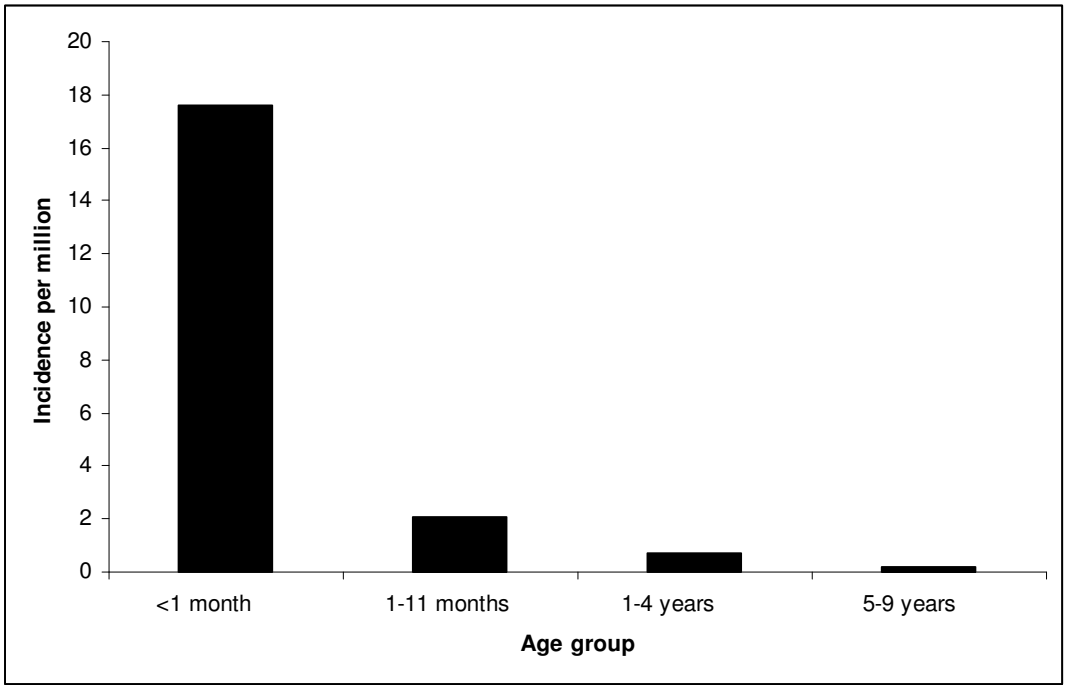

Figure 2. Crude annual incidence rate per million population for *E. sakazakii* (*Cronobacter* spp.) infections by age group in England and Wales 1992–2007.
SOURCE: Health Protection Agency for laboratory reports.

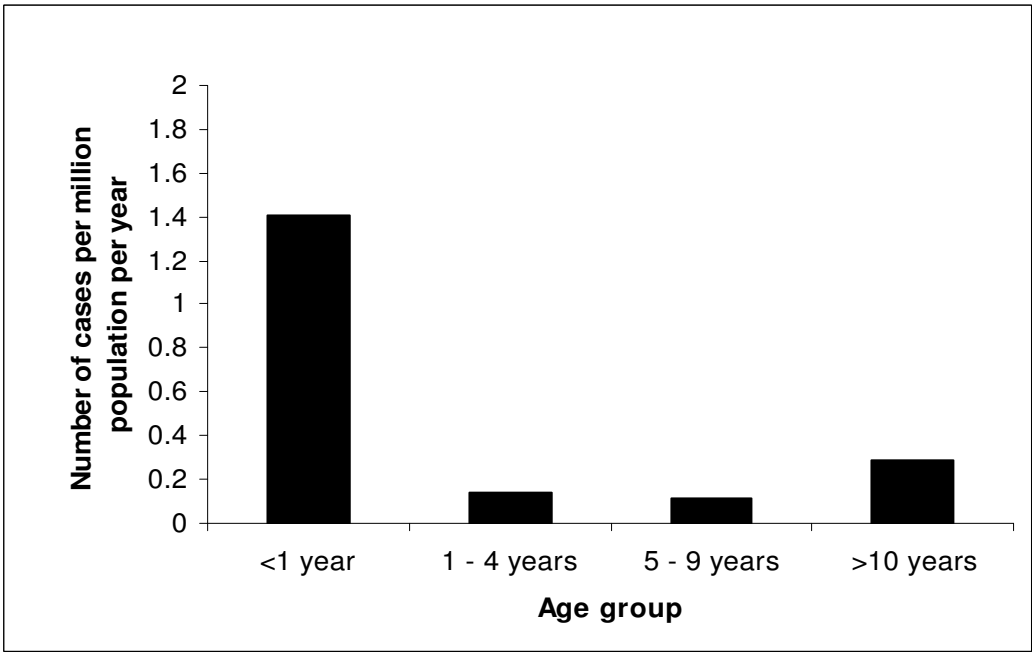

Figure 3. Crude annual incidence rate per million population for *E. sakazakii* (*Cronobacter* spp.) infections by age group in the Philippines 1998–2007.

3.4 Immune status of population of concern

The meeting was unable to identify a way of clearly defining the immune status of the population of concern and this was also reflected in the lack of data provided in response to the Call for Data. There does not seem to be a commonly understood definition for the term "immunocompromised", which can be due to the effects of disease, pharmaceutical treatment or diet, among other factors.

In developed countries, individuals may have an underlying condition that suppresses their T-cell mediated immunity, thereby potentially increasing their susceptibility to a range of diseases, including opportunistic infections. In the USA, 4% of the entire population is considered immunocompromised (defined as transplant patients, people who are HIV positive, those receiving chemotherapy or other immunosuppressive treatments, or people with chronic diseases) (FDA, 2007). Further, an unclear proportion of infants and toddlers may be temporarily immunocompromised at any time due to the effects of acute illness, injury, stress or pharmaceutical treatments, such as steroids for asthma exacerbations. In addition, there has been a significant increase in the prescription of gastric acid-suppressing medications for gastro-oesophageal reflux in infants and toddlers in industrialized settings (Khoshoo et al., 2007; Savino and Castagno, 2008). While not traditionally considered immuno-suppressing, such medications impair one of the first lines of defence humans have against ingested pathogens. Infants, in addition, are considered to have lower levels of gastric acid, making them more vulnerable to infections.

Age is an important factor in relation to immune status of infants. Neonates and young infants have a transitory immunodeficiency affecting a broad number of immune functions, and that increases the risk of infection. Reduced bactericidal properties, developmental immaturity, decreased proliferative responses and dysregulation of cytokine networks are all contributing factors. Neonatal innate immunity exhibits reduced production of mucus, acid, immunoglobulin and gut motility necessary for adequate responses to pathogenic material and to prevent bacterial adherence. Infants between 2 and 6 months of age become IgG deficient due to catabolism of maternal IgG. IgM expression is genetically constrained until 2 months of age. Further, developmental regulation of carbohydrate expression on cell surfaces may account for certain pathogen tissue tropisms and age specificity influencing host susceptibility. Macrophages' chemotaxic, phagocytic and bacteriocidal activities are reduced. Diminished antigen presentation and cytokine production may be attributed to a decrease in T-cell production in the neonate. Neonatal bone-marrow-derived neutrophiles are quickly depleted during infection and several bacteriocidal deficiencies in both production and function have been well documented. Studies have shown that intracellular survival contributes to disease pathogenesis in neonatal animal models. Several meningitic pathogens, including *E. sakazakii* (*Cronobacter* spp.) have been shown to persist and replicate within macrophages (Townsend et al. 2007, 2008). Neonates also show a bias toward Th2 immune responses that are less active against bacterial intracellular infections, demonstrate a weak response toward lipopolysaccharide, and preclude the induction of an appropriate Th1 response. This could contribute to the significant increase in sepsis observed in very-low-birth-weight and premature infants.

In data from the United Kingdom, of the 88 cases of *E. sakazakii* (*Cronobacter* spp.) infection reported in Scotland between 1998 and 2007 (age <1 to 90 years), 8 (9%) were known to be immunocompromised (1 pancreatic cancer, 1 ovarian cancer, 3 liver cancer, 2 chronic renal failure, 1 post-operative coronary bypass graft). The immune status of the infants <1 year old was not known.

In 2006, an estimated 1 514 086 pregnant women were infected with HIV in all low- and middle-income countries (UNICEF, 2007). Without treatment, approximately 35% of the infants resulting from these pregnancies would become infected with HIV. As part of the strategy to prevent transmission of HIV from mother to child, when replacement feeding is acceptable, feasible, affordable, sustainable and safe, WHO recommends avoidance of all breastfeeding by HIV-infected women (WHO, 2006). In most industrialized countries, women with HIV are encouraged to feed their children infant formula rather than breast milk. Programmes for the prevention of mother-to-child transmission of HIV in some developing countries, such as Botswana, where 37% of pregnant women are infected with HIV (CDC, 2008), have begun supplying PIF to mothers with HIV. The expansion of such programmes could have a significant impact on the proportions of vulnerable children fed powdered formula products early in life.

Relatively little data is available on immunocompromised infants and children in the under three year age group or in older age groups from countries in the developing world. However, a high burden of infections, including major illnesses, both enteric and respiratory in nature, result in a cycle of infection and malnutrition leading to impaired immune function (Figure 4). These factors result in children being underweight, stunted or wasted (Table 5). Up to about 30% of children in developing countries can be of low birth weight, with a less than optimal thymic function resulting in a lowered T-cell function, the effects of which are seen in later life.

Malnutrition is the major cause of immunodeficiency leading to immunocompromised infants and children. Weight is one indicator of malnourishment, and data from the State of the World's Children 2008 (UNICEF, 2008) shows that approximately 25% of the worlds under-fives are moderately or severely underweight (Table 6). Malnutrition related to micronutrient deficiency, e.g. zinc deficiency, can aggravate the situation, leading to a state of acquired secondary immunodeficiency. The large burden of infections at the mucosal surfaces of the gut can lead to intestinal enteropathy, making children more susceptible to diseases. A reduction of the innate and adaptive responses can occur. These factors have a major impact on children, but also affect adults, especially the elderly, who can be as susceptible as children to malnutrition and micronutrient deficiency leading to an immunocompromised state. Table 5 presents some of the parameters that can be used as indicators of immune status in infants and young children, and their prevalence in different regions of the world.

There was little information available on consumption of powdered formula among the malnourished populations. Data from some countries indicates that, among such populations, powdered formula is not an economically feasible option. Thus, as with HIV, exposure to such products may be the result of an intervention programme.

3.5 Conclusions

There are no active surveillance systems for *E. sakazakii* (*Cronobacter* spp.) disease, based on the information reviewed and considered by the meeting. *E. sakazakii* (*Cronobacter* spp.) infection is a notifiable condition in two countries, and invasive *E. sakazakii* (*Cronobacter* spp.) disease is notifiable using a mandatory, passive system in another country and one state in the USA. Such a limited number of systems cannot be expected to provide an overview of *E. sakazakii* (*Cronobacter* spp.) disease from the global perspective. Given the limited scope and recent implementation of such systems, many years of surveillance will be required to establish a reliable estimate of incidence for these populations. Although national foodborne disease surveillance systems exist in many countries they have not identified cases of *E. sakazakii* (*Cronobacter* spp.) infections to date. Most *E. sakazakii* (*Cronobacter* spp.)

infections were identified by hospital outbreak investigations or voluntary passive reporting. Thus, it can be concluded that existing surveillance systems may not be capturing potential cases.

Only a small number of documented cases were found for the 6–11-month age group, and even less for the 12–35-month age group. A number of the case patients had underlying medical conditions. This indicates that *E. sakazakii* (*Cronobacter* spp.) infections can occur in older infants and young children. However, it is more difficult to address the extent to which it can occur and the source of the infection. All well described cases among children 6–35 months old were identified as sporadic cases rather than part of a point-source hospital outbreak. Thus, epidemiological methods could not be used to implicate a source of infection, and investigators were required to rely on microbiological testing of suspect vehicles. Testing may not be a sensitive approach, even within the setting of a large foodborne outbreak, especially when low-level, sporadic contamination is suspected.

Data from laboratory surveillance seems to suggest a decrease in *E. sakazakii* (*Cronobacter* spp.) with increasing age. However, such a system provides very little information about the case patient. Voluntary reporting systems, in general, may have a bias toward the more severe infections and may only capture those data. It is considered plausible that older infants experience a milder disease, further reducing the potential of capturing those cases through one of the existing surveillance systems. The meeting did use available information to estimate the incidence of *E sakazakii* (*Cronobacter* spp.) infection in two countries. Although these calculations may provide useful information on trends, the meeting acknowledged the severe limitations of the data.

The immune status of a population is not easy to assess, and the apparent lack of a universally agreed definition complicated discussion of this issue. Nevertheless, the meeting identified a range of factors contributing to immunodeficiency and noted the broad prevalence of some of these factors. The prevalence of immunocompromising conditions may be relatively low in developed countries (e.g. 4% in the USA), but the picture seems very different in developing countries, where the prevalence of such factors can be up to 40%. This indicates the prevalence of immunocompromised infants and children could also vary accordingly, and highlights the need for caution in the extrapolation of data from developed countries to developing countries.

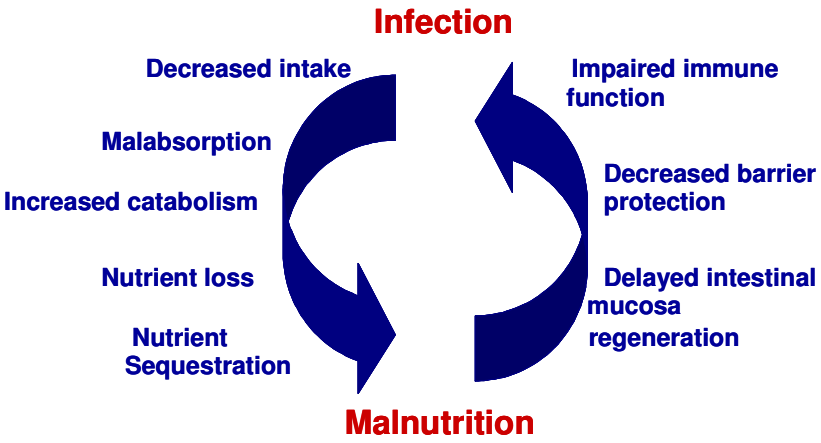

Figure 4. Infection and malnutrition cycle leading to impaired immune function.

Table 6. Malnutrition, infection and other parameters as indicators for gauging immunocompromised status of children.

Country groupings	Sub-Saharan Africa	Eastern and Southern Africa	West and Central Africa	Middle East and North Africa	South Asia	East Asia and Pacific	Latin America and Caribbean	CEE / CIS	Industrialized countries	World
% of infants with low birth weight*	14	14	14	16	29	6	9	6	7	15
% exclusively breastfed (<6 months)*	30	39	21	28	45	43	-	19	-	38
% breastfed with complimentary food (6–9 months)*	67	71	63	57	55	45	-	44	-	56
% of under-fives suffering from underweight*	28	28	28	17	42	14	7	5	-	25
% of under-fives suffering from wasting*	9	7	10	8	18	-	2	2	-	11
% of under-fives suffering from stunting*	38	41	36	25	46	16	16	12	-	31
HIV prevalence among pregnant women (15–24 years) in capital city (thousands)* #	9.7	13.5	4	-	-	-	-	-	-	-
Estimated no of children (0–14 years) living with HIV, 2005 (thousands)*#	2000	1400	650	33	130	50	54	9	13	2300
% under-fives with suspected pneumonia taken to an appropriate health care provider*#	40	44	36	66	62	64 (excl. China)	-	57	-	56 (excl. China)
% under-fives with diarrhoea receiving oral rehydration treatment and continued feeding*#	30	32	29	38	35	61 (excl. China)	-	-	-	38 (excl. China)
% Pre-school-age children with anaemia**	64.6				47.7 (Asia) 28 (Oceania)		39.5		6 (N. America) 1.5 (Europe)	47.4

NOTES: * Data taken from Statistical Tables 2, 3 and 4 of the State of the World's Children 2008 (UNICEF, 2008) (countries included in the above groupings are listed on page 148 of that report).
** Data taken from Worldwide prevalence of anaemia 1993–2005; WHO Global database on anaemia (WHO, 2008)
# Data for HIV, pneumonia and diarrhoea included as markers of disease burden due to different infections that may lead to an immunocompromised status.

4. Production of follow-up formula

4.1 Differences between production systems for powdered infant formula and follow-up-formula

4.1.1 Introduction

The manufacture of PIF has been described and discussed in the reports of the first two FAO/WHO expert meetings on *E. sakazakii* and other pathogens in PIF (FAO/WHO, 2004, 2006). Additional details on the manufacturing processes can be found in Cordier (2007).

Codex Alimentarius defines follow-up formula as "a food intended for use as a liquid part of the weaning diet for the infant from the 6th month on and for young children", with infants defined as "a person not more than 12 months of age" and young children as "persons from the age of more than 12 months up to the age of three years (36 months)" (CAC, 2008b). FUF is further defined as a "food prepared from the milk of cows or other animals and/or other constituents of animal and/or plant origin, which have proved to be suitable for infants from the 6th month on and for young children" (CAC, 1987). Thus FUF are products consumed by infants between 6 and 12 months and young children between 12 and 36 months. However, as noted in the introduction to this report, national or regional authorities may adhere to this definition or may define FUF differently.

FUF are manufactured in many countries throughout the world. The annual production quantities of FUF are summarized in Table 7.

Table 7. Annual global production quantities of FUF (×1000 tonne) 2003–2006.

Year	2003	2004	2005	2006	2007*
World production (×1000 tonne)	257.9	269.8	285.4	305.7	329.0

Notes: *extrapolated from mid-year estimates.
SOURCE: Packaged food data, Euromonitor International 2008 (Copyright and Database right)

4.1.2 Processing technologies

FUF are manufactured using processes that are almost identical to those used for PIF, as well as any other type of powdered dairy products for consumers >36 months of age, such as dairy-based beverages, fortified milk powders, and products used in medical nutrition. These processes can be classified into: (1) Wet-mix processes; (2) Dry-mix processes; or (3) combined processes, and are described in more detail in Cordier (2007). Important aspects of the wet-mix and dry-mix processes are briefly considered below.

4.1.3 Wet-mix processes

A heat-treatment step is applied to the wet-mix. This is equivalent to the heat process applied to other infant formula and is managed as a critical control point (CCP) in the manufacturing process. Processing conditions may vary slightly depending on the manufacturer and the particular product, but these differences are insignificant with respect to the inactivation of bacterial pathogens such as *Salmonella* and *E. sakazakii* (*Cronobacter* spp.). These heat treatments are validated to ensure the safety of the final products and achieve a minimum 10-log unit reduction for vegetative microorganisms, including *Salmonella*, *E. sakazakii* (*Cronobacter*

spp.) and other Enterobacteriaceae. It is not uncommon for these processes to be capable of a theoretical 50–80 log unit reduction for these vegetative microorganisms.

Other processing steps, either before or after the heat-treatment, are equivalent for both PIF and FUF. Details are discussed in the reports of the first two FAO/WHO expert meetings (FAO/WHO, 2004, 2006).

4.1.4 Dry-mix processes

As with the wet-mix process described above, there are no significant differences for dry-mix processes between the manufacture of PIF or FUF. All processing steps are unit operations that are common and widely applied in the manufacture of any type of powdered dairy-based product.

The addition of dry-mix ingredients is performed either continuously or on a single batch basis after the heat-treatment. The microbiological quality of these ingredients is critical, because no further killing step is applied in the manufacturing process. Dry-mix ingredients must therefore strictly meet the same microbiological requirements as the finished products to ensure that the finished product complies with existing microbiological criteria for both safety and hygiene.

The most significant difference between PIF and FUF lies in the fact that FUF may contain a wider variety of dry-mix ingredients. This is a consequence of the need for a more diversified diet for infants aged 6 to 11 months, and particularly for young children (12–35 months). Such dry-mix ingredients include, but are not limited to, cocoa powder, fruit and vegetable powders or flakes, and flavours. These ingredients are not normally used in PIF.

A number of these ingredients are manufactured using completely different food processing technologies and, as a consequence, using different hygiene control measures. The microbiological quality of these ingredients may not necessarily meet the most stringent requirements typically applied to infant formula. As can be seen from different peer-reviewed publications (Friedemann, 2007; Iversen and Forsythe, 2004; Kandhai et al., 2004), *E. sakazakii* (*Cronobacter* spp.) is a ubiquitous microorganism that can occasionally be found in such ingredients. In general, more stringent hygiene control measures would be required to reduce the risk of contamination by this pathogen if these ingredients were required to meet the microbiological criteria for PIF. No new data on the prevalence and levels of *E. sakazakii* (*Cronobacter* spp.) in raw materials are available on dry-mix ingredients used for the manufacture of infant formula since the previous FAO/WHO expert meetings. However, published data indicates that *E. sakazakii* (*Cronobacter* spp.) is likely to be present in a variety of dry ingredients unless appropriate measures, as outlined in FAO/WHO (2004, 2006) are implemented at the supplier level.

4.1.5 Product portfolio

Because the manufacturing processes for PIF and FUF are nearly identical, production lines may be used to manufacture any type of product falling within the scope of the Codex Alimentarius Recommended Code of Practice General Principles of Hygiene (CAC, 2004b), or even other powdered dairy products for consumers beyond 36 months.

Depending on the design of the manufacturing facility and the product portfolio, the production lines may be dedicated, i.e. used to manufacture a single category of product (PIF or FUF), or shared, i.e. used to manufacture multiple products that may have different microbiological requirements (PIF and FUF).

4.1.6 Hygiene control measures

The concepts and approach of the hygiene control measures (GHP/GMP and HACCP) in the manufacture of these categories of products are outlined in the previous FAO/WHO reports (2004 and 2006) and also discussed in detail by Cordier (2007).

The stringency of the hygiene control measures required for a particular processing line will depend on the microbiological criteria for products manufactured on that line. The current Codex criteria for PIF require the absence of *E. sakazakii* (*Cronobacter* spp.) in each of 30 samples of 10 g of PIF (i.e. 2-class plan with n=30, m=absence in 10 g, c=0), while there are currently no criteria for *E. sakazakii* (*Cronobacter* spp.) in FUF. In the case of a dedicated line, the requirements are obvious. In the case of a shared line, the product with the most stringent criteria will determine the overall hygiene control measures required, as well as the verification procedures for the processing environment and processing lines (see Annex III of the Code of Hygienic Practices (CAC, 2008b). While examples of different scenarios are provided below, others also exist:

A. **Dedicated line**. Manufacture of PIF for consumption by infants 0 to 12 months of age.

B. **Shared line**. Manufacture of FUF on lines on which PIF is also manufactured.

For scenario A, stringent hygiene requirements are applied to ensure the compliance of PIF with end product criteria for pathogenic microorganisms (i.e. *E. sakazakii* (*Cronobacter* spp.) and *Salmonella* spp.) and hygiene indicators (i.e. Enterobacteriaceae and mesophilic aerobic bacteria) as laid down in Annex I of the revised Code of Hygienic Practices (CAC, 2008b). Such requirements have already been adopted in a number of countries.

For scenario B, the most stringent hygiene requirements are applied, i.e. those which ensure compliance of PIF with the end-product criteria as outlined above. Testing of environmental samples, line samples as well as raw materials is performed to ensure that these hygiene measures are effective and consistently met, thus ensuring that the line is able, at all times, to deliver infant formula complying with established criteria. However, the FUF may not be tested for *E. sakazakii* (*Cronobacter* spp.) if criteria for this pathogen have not been considered in existing regulations.

4.2 Prevalence of E. sakazakii *(*Cronobacter *spp.) in FUF*

Data are scarce on the p revalence of *E. sakazakii* (*Cronobacter* spp.) in products categorized as FUF for infants between 6 and 11 months. The absence of such data is probably due to the fact that few regulatory authorities have established criteria for *E. sakazakii* (*Cronobacter* spp.) for these products, and hence the absence of specific testing. From the data submitted in response to the FAO/WHO Call for Data, two countries provided information on analytical data on products tested. Estonia reported isolation of *E. sakazakii* (*Cronobacter* spp.) from two batches of FUF (each batch was about 6000 kg). The powder was tested using the ISO:TS 22964 (ISO, 2006) method and one of 30 ten-gram samples tested positive in one batch and two samples tested positive in the other batch. Information on the total number of batches tested was not available. Japan indicated that one manufacturer reported a contamination rate of 3% for powdered formula (no age range defined) in 2007. In Japan, FUF is marketed for infants 9 months and older.

Two recent surveys for *E. sakazakii* (*Cronobacter* spp.) in FUF have been undertaken: one by University College Dublin (UCD, Ireland) and the other by Nottingham Trent University in the United Kingdom, and the results were provided in response to the FAO/WHO Call for Data

(Annex 2). The survey undertaken by UCD focused on 31 different infant food products covering 18 brands from 7 manufacturers, and including milk-based (n=22) and soy-based FUF (n=1). No *E. sakazakii* (*Cronobacter* spp.) were found in any of the 23 milk- or soy-based FUF. The international survey for *E. sakazakii* (*Cronobacter* spp.) in FUF organized by Nottingham Trent University involved laboratories in Brazil, UK, Portugal, Malaysia, Indonesia and Republic of Korea, and analysed FUF purchased from local markets. *E. sakazakii* (*Cronobacter* spp.) was isolated from 1/106 (about 1%) of the FUF brands tested. Other Enterobacteriaceae and *Acinetobacter,* which correspond with Category B organisms ('organisms causality plausible, but not yet demonstrated') based on the output of the previous FAO/WHO expert meetings (FAO/WHO, 2004, 2006) were also isolated from FUF.

When more stringent hygiene requirements are implemented in PIF manufacturing lines to control Enterobacteriaceae, there is a significant reduction in the prevalence of these organisms over a period of time. Although there is no direct mathematical correlation between a reduction in Enterobacteriaceae and a reduction in *E. sakazakii* (*Cronobacter* spp.), the implementation of such measures nevertheless contributes to a reduction in *E. sakazakii* (*Cronobacter* spp.). Reductions of *E. sakazakii* (*Cronobacter* spp.) ranging from about 10^{-4} to 10^{-6} cfu/g have been calculated for some factories based on data from 2004 (Cordier, 2007). These calculations have been made using the same approach as outlined in the 2006 FAO/WHO report, considering the total number of positive batches over one year (positive batches were not released for sale or consumption). Although there are few data for *E. sakazakii* (*Cronobacter* spp.) in FUF, similar reductions might be expected for FUF manufactured on lines shared with PIF, or for FUF manufactured on dedicated lines if similar stringent hygiene requirements are applied. Such requirements are reflected by the existence of stringent criteria for Enterobacteriaceae in the finished product (absence in 10 g samples), as is already implemented by the EC.

In the case of production lines where more lenient or no specific hygiene requirements exist with respect to Enterobacteriaceae, such as on lines that are shared between FUF for infants >6 months and FUF for young children >12 months, the mean prevalence and level of *E. sakazakii* (*Cronobacter* spp.) in the product can be expected to be higher. The mean level of organisms in contaminated product may be of the order of 10^{-3} as presented in the FAO/WHO report (2006) or illustrated by data gathered from different factories in 2002 before the implementation of stringent hygiene measures (Cordier, 2007).

4.3 Conclusions

PIF and FUF are powdered products manufactured in an almost identical manner. One difference relates to the broader range of ingredients used in FUF, reflecting the increasingly diverse diet with the increasing age of infants and young children. These ingredients are produced using different technologies and under different hygiene control measures, and therefore with varying levels of microbial contamination. Thus, one of the challenges for manufactures of FUF is ensuring that these diverse ingredients that are used in the manufacture of FUF meet the same hygiene and microbiological standards as those required for the finished product. A second difference is that the stringency of hygiene control measures and microbiological criteria applied to the manufacture of PIF may be different from those for FUF. These requirements are determined by the particular processing line and are the same only when PIF and FUF are produced on the same line.

Little information is available of the prevalence and concentration of *E. sakazkaii* (*Cronobacter* spp.) in FUF. While two surveys of FUF in the market place have been undertaken, the lack of data from more long-term studies seems to reflect the absence of

requirements to test FUF for *E. sakazkaii* (*Cronobacter* spp.). Thus, in reality, there is not adequate data available to make a comparison between PIF and FUF in terms of microbiological quality. This ultimately means that any assessment of the impact of microbiological criteria on the prevalence of contaminated FUF in the market place, and thus ultimately the risk of illness associated with this product, is going to be based on a set of assumptions regarding the levels of contamination.

5. Consumption of follow-up formula

5.1 Data sources

The availability, preparation and use of FUF were assessed primarily using data submitted in response to the FAO/WHO Call for Data. A summary of these data is provided in Table 8. While this included data from countries in different geographical regions and at different stages of development, the database still covers relatively few countries. It is also important to recognize other limitations with these data:

- A number of submissions were based on survey data; however, some of these surveys were not representative of the overall population in that country. For example, Ghana surveyed 60 working mothers from Ministries of Education, Communications, Health, Road and Transport, and Trade, Industry and President's special initiative. This sample may be biased towards middle to upper socio- economic classes. Likewise in Guatemala, 300 mothers from the capital of the country were surveyed. This is not an accurate representation of the country, as consumption of FUF in rural areas is reported to be minimal due to economic constraints.
- Data could not be compared between countries because of the way it was reported, as some countries reported consumption of FUF for infants less than or equal to 6 months, while others reported consumption at 6 months.

As this database covers relatively few countries, the meeting also considered other data sources, such as the Demographic and Health Surveys (DHS) (http://www.measuredhs.com). One particular survey addressed the types of foods received by infants and children in different age groups. However, the surveys refer only to infant formula, and it is not clear whether there was any distinction made between PIF and FUF or powdered and liquid formulas. Nevertheless it was considered that this type of data could contribute to our knowledge on the potential number of infants that consume FUF. An advantage of the DHS surveys is that they were designed to be nationally and regionally representative of the countries.

5.2 Consumption of FUF

5.2.1 Consumption of FUF by age and region

Most countries reported that FUF is marketed for infants 6 months of age or older. This is in line with the Codex Alimentarius definition of FUF, namely a food intended for use as a liquid part of the weaning diet from the 6th month on and for young children. However, as noted earlier, national or regional authorities may adhere to this definition or may define FUF differently. In the EU, PIF and FUF have been regulated since 1991. Recently, this legislation has been updated to change the introductory age from 4 months to 6 months onwards[10]. Trade in FUF complying with the new Directive (from 6 months onwards) has been permitted since 1 January 2008, while the trade in FUF complying with the old Directive (i.e. from 4 months) is

[10] Commission Directive 2006/141/EC (OJ L401, p1, 30/12/2006) of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. According to this Directive, from 31 December 2009 all labels of FUF must carry a statement to the effect that the product is suitable only for particular nutritional use by infants over the age of six months (Article 13.1b).

Table 8. Summary of consumption data submitted in response to the FAO/WHO Call for Data

Country	Age group for which FUF is marketed (m = months)	Consumption				Notes
		Age at which consumption of FUF actually begins (m=months)	Proportion of infants consuming FUF			
			<6 months	6–12 months	1–3 years	
Argentina	2 age groups: Infants: 6–12 m Young children: 12–36 m	NA	NA	NA	NA	
Austria	Not stated	10% of infants at 3 m consume FUF	60.4% of infants at 6 m consume FUF	48.9% of infants at 12 m consume FUF.	NA	Data taken from an Austrian Report "Infant nutrition today: 2006: The quality of infrastructure and counselling services at birth clinics in Austria: Infant Nutrition in the first year of life"
Brazil	2 age groups: Infants: 6–12 m Young children: 12–36 m	6 months (as established by national legislation)	NA	NA	NA	
Estonia	2 age groups: 0–12 m 6–12 m	NA	NA	NA	NA	
France	According to Codex recommendations (i.e. from the 6th month onwards)	<4 m *	NA	NA	NA	Data was not provided on the proportion of infants consuming FUF; however, data was provided in graphical format on the contribution of different types of baby foods to the total calorific intake of infants and young children 0–36 months of age (presented in Figure 8).

Country	Age group for which FUF is marketed (m = months)	Consumption				Notes
		Age at which consumption of FUF actually begins (m=months)	Proportion of infants consuming FUF			
			<6 months	6–12 months	1–3 years	
Ghana	6 m and older	4 months (3.3% of survey respondents who fed their children FUF started at 4 months)	33.3% (33.3% of survey respondents who fed their children FUF started at or before 6 months)	16.7% (16.7% of survey respondents who fed their children FUF started between 7 and 12 months)	1.7% (1.7% of survey respondents who fed their children FUF started at 14 months)	Data presented are from a survey of 60 working mothers from Ministries of Education, Communications, Health, Road and Transport, and Trade, Industry and President s special initiative with children <10 years old. About 28% of respondents used only infant formula, 12% used only FUF and 38% used both.
Guatemala	6–36 m	6 months	<10% of infants <6 months in urban areas consume FUF. Very few infants <6 months from rural areas consume FUF.	65% of infants in this age group consume FUF	20% of children >1 year consume FUF	The data presented are from a survey undertaken by Abbott on the use of follow-up formula among 300 mothers in the capital city. These data therefore are not representative of consumption in the overall country.
Ireland	6 m	5.5 m (average age of introduction of FUF)	11% of infants at 5 months have consumed FUF.	60% of infants at 6 months have consumed FUF.	NA	Data were provided by Nutricia.
Korea (Rep. of)	6 m forward	6 m	NA	NA	NA	
Luxemburg	4 m forward	May be as young as 2 m	Approximately 58% of infants at 4 months of age have consumed FUF (see note)	NA	NA	At 4 months only 42% of babies are exclusively breastfed, so it has been assumed that 58% are by then formula fed to some extend, with the vast majority fed FUF (extract from response to Call for Data). In Luxembourg many parents (supported by doctors views), feel that FUF increases satiety in infants and feed it even earlier hoping to minimize sleep disturbance during the nights.

Country	Age group for which FUF is marketed (m = months)	Consumption				Notes
		Age at which consumption of FUF actually begins (m=months)	Proportion of infants consuming FUF			
			<6 months	6–12 months	1–3 years	
Malta	4 m forward (by law)	NA	NA	NA	NA	
Nicaragua	0- 60 m	6 m (according to Ministry of Health Regulations)	NA	NA	NA	67% of infants <2 years are breast fed
New Zealand	6-12 m	NA	NA	NA	NA	
Norway	4 m	4 m	NA	NA	NA	Only imported FUF is available in Norway: as it is only imported by 2 small companies, consumption is thought to be low. However, an infant formula which is targeted at infants 4 months of age or older is available on the market.
Philippines	6-36 m	4.4 m (mean age)	In 2003 39.2% of infants < 12 months were fed with milk formula alone or in combination with other foods. The age distribution was as follows: 27.9% 0-5 m; 35.7% 6-11 m; 46.2% 12-23 m			Data presented are from the 6th National Nutrition Survey (2003) on Infant Feeding Practices in the Philippines (Food and Nutrition Research Institute- Department of Science and Technology).
Switzerland	Before 1 April 2008: after 4 months After 1 April 2008: after 6 months♦	NA	NA	NA	NA	

Country	Age group for which FUF is marketed (m = months)	Consumption				Notes
		Age at which consumption of FUF actually begins (m=months)	Proportion of infants consuming FUF			
			<6 months	6–12 months	1–3 years	
UK	6 m forward	2% of mothers surveyed said they had given their baby FUF by 4 weeks	34% of mothers surveyed said they had first given their baby FUF by 6 months	51% of mothers surveyed said they had first given their baby FUF by 9 months	NA	Data presented are from the 2005 Infant Feeding Survey (Bolling et al., 2007) A total of 9416 mothers were surveyed.
		17% of all mothers who had fed FUF to their babies started when the baby was younger than 3 months.	74% of mothers who fed FUF to their infants started when the infant was 6 months or younger.	23% of mothers who fed FUF to their infants started when the infant was between 7 and 12 months.	NA	Data presented are from a telephone survey of 1000 new mothers and pregnant women conducted by independent research company MORI and commissioned by The National Childbirth Trust Charity and UNICEF-UK (2005). 1000 mothers were surveyed and of these 272 had fed FUF to their infants.

Notes: NA = Not available. Responses were variable, e.g., no response to these specific questions, response was “no data” or “cannot respond to question”.

♣ This information was extracted from a graph submitted by France on the contribution of different types of baby foods to the total calorific intake of infants and young children 0–36 months of age

♦ With a revision of the Ordinance on Foods for Special Dietary Purposes (in force since 1 April 2008) the age of introduction has been changed to ‘after 6 months’.

prohibited after 31 December 2009. Thus the introductory age of FUF currently marketed in the EU may be either 4 months or 6 months onwards. Based on this it would be expected that consumption of FUF would begin at 4 months in some European countries.

Analysis of the available data indicated that marketing recommendations regarding the introductory age to FUF were not always followed, with a number of countries reporting 'early use', i.e. consumption at an age earlier than that recommended (e.g. France, Ghana, Ireland, Luxembourg, Philippines and UK). For example, in the UK, FUF is marketed for infants 6 months of age or older; however, a survey undertaken in 2005 by an independent research company, MORI, and commissioned by The National Childbirth Trust and UNICEF, indicated that of the 272 caregivers who had used FUF, 17% had introduced it into their infants diet by 3 months (MORI, 2005). A further survey undertaken in the UK in 2005 reported that 2% of caregivers had introduced FUF into their infants diets by 28 days and 4% by 8 weeks (Bolling et al., 2007). This is relevant as neonates (less or equal to 28 days) and infants less than 2 months of age have been identified as those infants at greatest risk of *E. sakazakii* (*Cronobacter* spp.) infection. Reasons identified for the "early use" of FUF are discussed in Section 5.3.

Data on the consumption of FUF by age groups 6–12 months and 1–3 years are also presented in Table 8. Although it is difficult to make comparisons between consumption patterns in different countries, it is clear that a large proportion of infants in the 6–12month age group are consuming FUF, e.g. 65% in Guatemala, and in Ireland 60% of infants at 6 months have consumed FUF. Very little information was submitted on the consumption of FUF by young children (1–3 years), although there are indications of decrease in FUF consumption with increasing age. In the USA, consumption of infant formula (USA regulations do not distinguish between PIF and FUF in the 6–11 month age group) decreases from 67.3% at 6 months to 36.4% at 11 months (Table 9).

Given the limited data available on the consumption of FUF, data on infant formula from DHS surveys were also reviewed as an indicator of the extent to which formula products might be used in different regions of the world. Consumption of infant formula was considered to indicate a potential market for FUF. Data on the percentage infant formula consumption among different age groups (from 0 to 35 month) from DHS surveys undertaken between 2000 and 2007 was reviewed from countries in sub-Saharan Africa, south and southeast Asia, and Latin America and the Caribbean. Consumption of infant formula was highest among 4 to 5 month olds in Latin America and the Caribbean, and 10–11 months in sub-Saharan Africa and south and southeast Asia (Figure 5). In these three regions, consumption was still greater than 5% in young children 30–35 months. These data indicate variations in consumption in infant formula at a regional level. An even greater variation in consumption exists between countries (Figure 6). Since consumption of the product of concern is an important factor in terms of exposure, it was considered appropriate to use these data to highlight the variation in consumption of these product types around the world. As with data on the prevalence of indicators of immune status, this highlights the need for caution in extrapolation of data from one country to another.

5.2.2 Amount or volume of FUF consumed

Data from the Infant Feeding Practices Study II, conducted in the USA and collected in 2005–2007, show the amount of formula consumed by healthy infants up to 12 months of age per feeding and per day (Tables 9 and 10). The information was not collected for FUF as a separate category because USA regulations do not distinguish FUF and PIF for infants. At month 1, most infants (80%) consume less than 148 ml/feeding; however at month 6 and month 12 most infants (50.4% and 41% respectively) consume 148–177.5 ml/feeding (Table 10). The median

amount consumed per day increased from 673 to 887 ml/day between month 1 and 6; however, it decreased to 486 ml/day at month 12. This may be explained by the introduction of other foods into infants' diets at this age.

Table 9. Percentage of infants fed any formula and, among those given at least one feeding per day of formula, the median volume consumed per day, the sample size for each age, and the percentage of infants of each age who consumed each category of volume of formula per day.

	Fed any formula (%)*	Median ml/day**	N	<296 ml/day (%)**	299–591 ml/day (%)**	594–887 ml/day (%)**	890–1183 ml/day (%)**	1186–1479 ml/day (%)**	>1479 ml/day (%)**
Month 1	57.2	673	923	20.5	16.5	39.4	14.6	6.7	2.3
Month 2	61.1	813	1149	14.2	13.1	37.5	23.2	8.4	3.6
Month 3	60.5	828	1211	10.8	11.6	34.9	27.2	11.0	4.5
Month 4	62.9	828	1156	9.2	10.2	34.9	29.5	11.6	4.6
Month 5	64.7	828	1248	8.0	11.1	37.4	28.8	10.1	4.6
Month 6	67.3	887	1231	8.3	11.2	35.6	28.4	10.9	5.6
Month 7	68.9	813	1250	7.5	12.6	46.0	23.7	6.2	3.8
Month 9	70.8	732	1251	6.2	17.4	50.4	19.1	4.7	2.2
Month 10	70.9	665	1182	7.7	21.2	49.8	14.3	5.0	2.0
Month 12	36.4	486	554	18.9	37.2	33.6	7.8	1.4	1.1

NOTES: *Among all infants. **Among infants given at least one feeding of formula per day.
SOURCE: Infant Feeding Practices Study II, USA, 2005-2007. Note that the sample is longitudinal, and therefore the same infants are the subjects at each age.

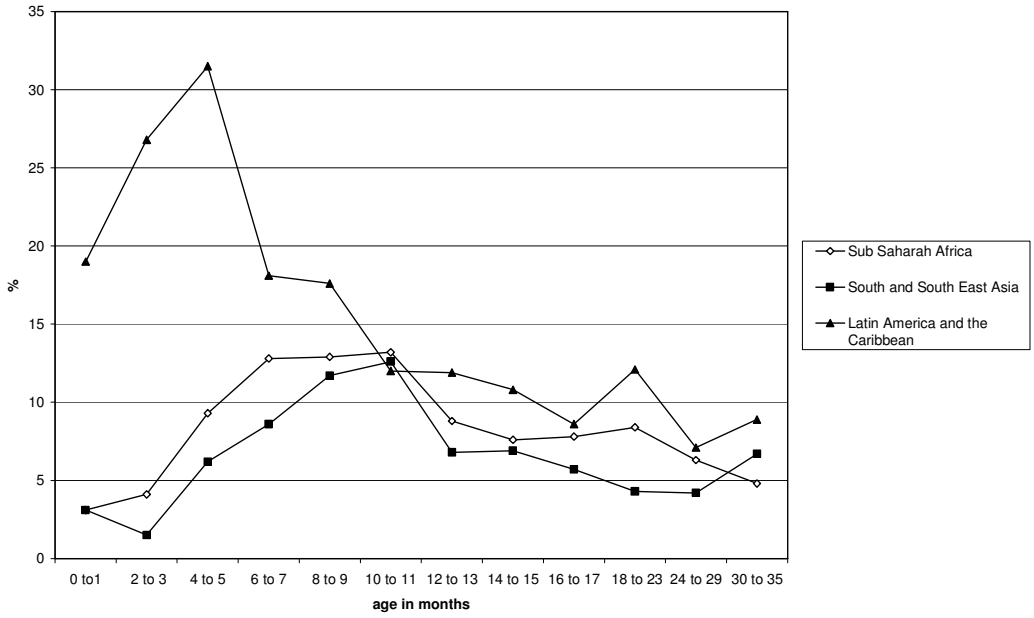

Figure 5. Median percentage of infants and young children consuming infant formula in sub-Saharan Africa, south and southeast Asia, and Latin American and the Caribbean based on the most recent survey data from countries in the three regions between 2000 and 2007.
Source of data: DHS (http://www.measuredhs.com/)

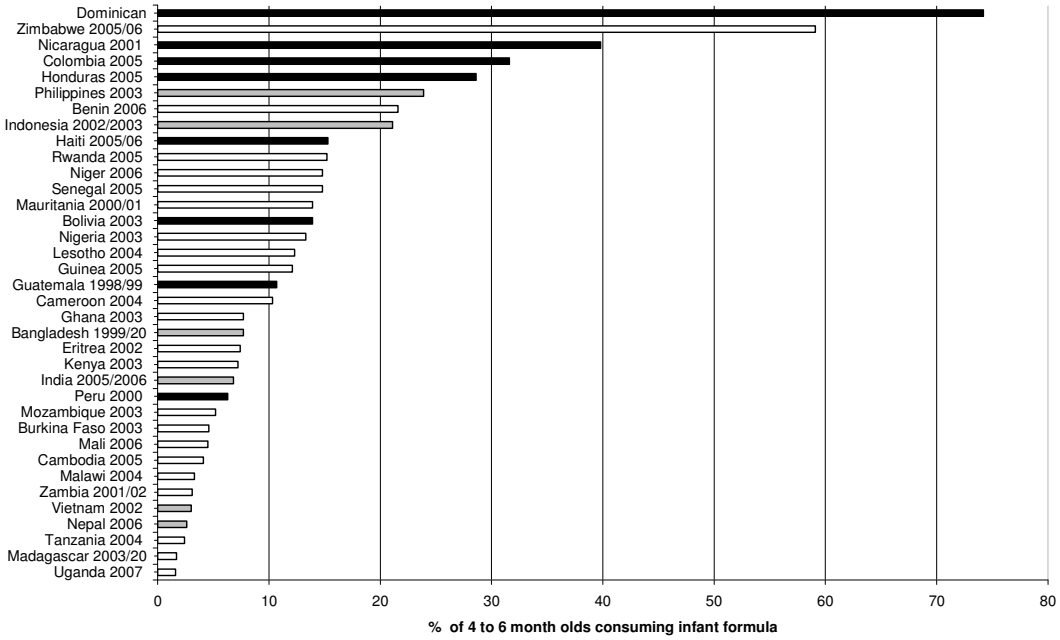

Figure 6. Consumption of infant formula among 4–6 month olds in surveyed countries in sub-Saharan Africa (white bars), south and southeast Asia (grey bars), and Latin America and the Caribbean (black bars).
Source of data: DHS (http://www.measuredhs.com/)

Table 10. Percentage of infants of each age who consumed each category of volume of formula* per feeding.

	Month 1	Month 3	Month 6	Month 7	Month 9	Month 10	Month 12
<148 ml	80.0	40.7	20.7	18.4	16.1	16.0	20.4
148 to 177.5 ml	17.2	46.8	50.4	48.4	49.8	44.8	41.0
207 to 237 ml	2.1	11.6	13.6	31.2	32.3	37.0	34.9
> 237 ml	0.7	0.8	15.4	2.0	1.8	2.2	3.7

NOTES: *The survey included infant formula marketed in both liquid and powdered formats; 88% of caregivers fed their infants with powdered infant formula.
SOURCE: Infant Feeding Practices Study II, USA, 2005-2007.

5.2.3 Consumption of other foods

The exposure of infants to other foods is worth considering as these may also be a potential source of microbial pathogens. In the USA, it has been shown that almost half of 4-month-old infants had consumed solid foods (2101 four-month-old infants were investigated), despite recommendations that complementary foods should not be fed to infants aged 4-months or younger[11]. At 6 months of age (n=2046 infants) more than 80% of infants consumed solid foods

[11] The American Academy of Pediatrics (AAP) Committee on Nutrition recommends that infants begin consuming foods in addition to breast milk or formula between the ages of 4 and 6 months, while the AAP Section on Breastfeeding recommends delaying the introduction of complementary foods until infants are 6 months of age.

on a daily basis (99.7% consumed either breast milk or formula[12], 80.7% consumed cereal, 22% consumed any meat/meat substitute products, 7.3% consumed other milk products and 4.8% consumed fatty/sugared foods). Further data on this study (i.e., consumption statistics of various foods from birth to 1 year) are presented in Figure 7 (Grummer-Strawn, Scanlon & Fein, in press).

Data from France for 2005 on the types of 'baby foods' consumed by infants (0–36 months) is presented in Figure 8. It clearly depicts the diversity of foods introduced at a young age into infants' diets. For example, at 4 months of age infants are exposed to infant formula, follow-up formula, dairy beverages (usually UHT), infant cereals, dairy desserts (usually yoghurt or fresh cheese) as well as processed vegetables and fruits.

In terms of other parts of the world, data from the DHS surveys indicate a broad range of foods consumed by infants from a young age (Figure 9).

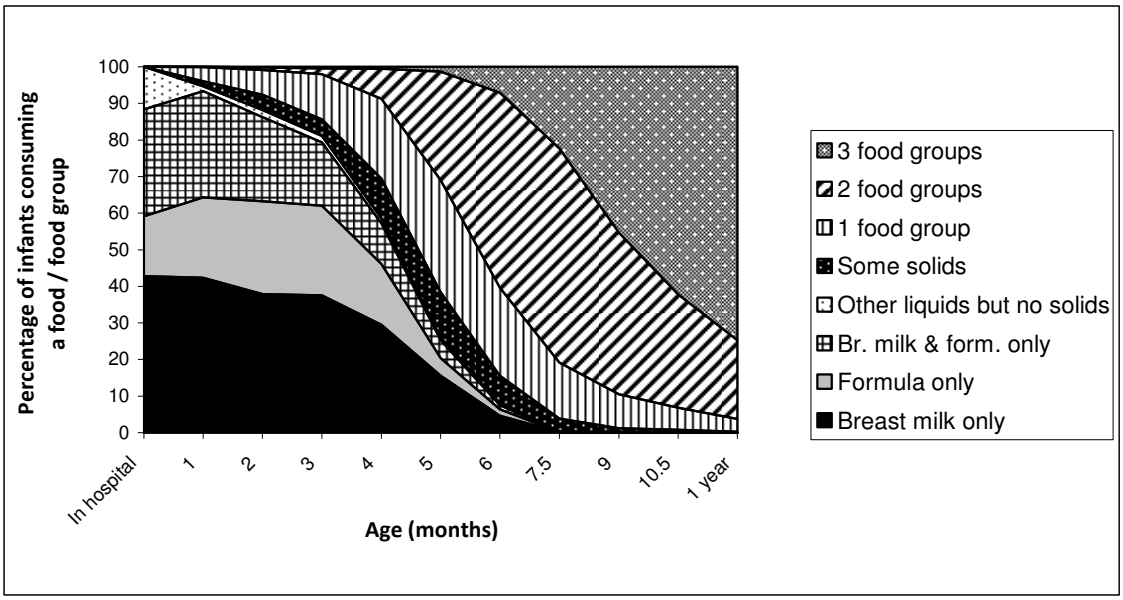

Figure 7. US data on type of infant feeding by age (Grummer-Strawn , Scanlon and Fein, in press)
Notes: * The number of groups of solid foods consumed by infants indicates the number of food groups (cereal, meat and meat substitutes, and fruits and vegetables) from which infants consumed at least one serving/day. All other categories are based on consumption at least once during the previous week among infants who did not consume any food group on a daily basis.

[12] The survey included infant formula marketed in both liquid and powdered formats; 88% of caregivers fed their infants with powdered infant formula.

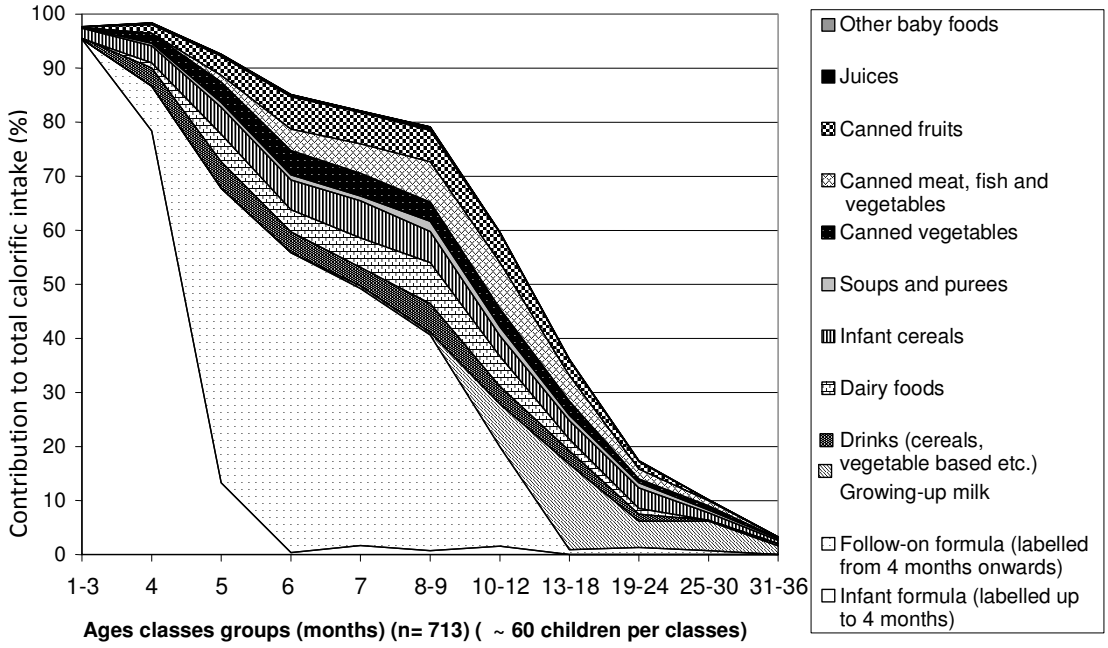

Figure 8. Data from France on the contribution of different types of baby foods to the total calorific intake of infants and young children 1–36 months of age. Note that all the foods included in this survey were intended for infants and/or young children.

Source: © SFAE/ Fantino 2005 study / Université of Bourgogne - Pr M. FANTINO for the Syndicat Français des aliments de l'enfance (March 05).

5.3 Consumer attitudes towards FUF

Consumer attitudes towards FUF are also important to consider, as well as their rationale for using FUF rather than other feeding options. While a number of sources reported on consumer attitudes, the most comprehensive source was the 2005 Infant Feeding Survey in the UK (Bolling et al., 2007). The survey reported reasons cited by caregivers for feeding FUF to their baby:

- 23% said they had used it with a previous child.
- 22% said they had been advised to use the formula by a doctor or health visitor.
- 20% said they believed it provided their baby with more nutrients.
- 18% said the baby was still hungry after being fed infant formula.

This survey (Bolling et al., 2007) also mentioned that some consumers believed that FUF took longer to digest and therefore was "especially suitable" for hungrier babies, although there was no evidence for this claim. In another data source it was reported that many parents in Luxembourg (supported by doctors) used FUF to increase the satiety of infants, and used it earlier than recommended on the label to reduce sleep disturbances during the night.

The 2005 Infant Feeding Survey (Bolling et al., 2007) in the UK also evaluated the impact of educational levels and socioeconomic status of the caregiver on the age at which FUF was first

introduced into the infant diet. The survey found that caregivers from 'routine and manual occupation groups' and 'those who had never worked' were more likely to have given their baby FUF at an earlier stage than those 'from managerial and professional occupations'. A similar pattern was evident by education level, as 'mothers with the lowest education level' were more likely to introduce FUF at an earlier age compared with 'mothers with higher levels of education'. These findings indicate that the socioeconomic class and educational level of the mother may be important factors regarding the appropriate use of FUF. While the findings of these UK studies cannot be extrapolated to other countries or regions, they nonetheless provide some important information about the knowledge and attitudes of consumers in choosing to purchase follow-up formula.

5.4 Feeding and handling practices

The 2006 expert meeting noted that the hygienic and handling practices of caregivers may influence risk of illness from PIF. Recent information about these practices is available from three countries: UK, USA and Italy.

To assess the extent to which caregivers followed recommendations of the UK Food Standards Agency and the UK Department of Health (FSA/DH, 2007) regarding preparation and storage of PIF, caregivers of 4–10-week-old infants were asked a series of questions about how they had prepared formula, both in and out of the home, in the previous seven days (Bolling et al., 2007). Only 13% of all caregivers who had prepared PIF (n=8309) followed all three of the following recommendations, namely 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. Furthermore, 34% of caregivers who had made up powdered formula to feed their baby away from the home were not following recommendations, either because they were not keeping pre-prepared feeds chilled or because they were using cold water to make up feeds when out (Bolling et al., 2007). However, focus groups also found that little information about formula preparation and storage is given by health care professionals. Caregivers obtain this type of information from family, friends and formula packages (Bolling et al., 2007). Although this survey provides data regarding the handling and storage of PIF, it is likely that similar practices are undertaken for FUF, as the preparation and storage recommendations for both products are similar.

The USA survey (Infant Feeding Practices Study II) conducted in 2005–2007 obtained information from formula-feeding caregivers when their infants were 2, 5, 7 and 9 months old, with sample sizes ranging from 1200 to 1400 mothers, depending on infant age (Labiner-Wolfe, Fein, and Shealy, in press). Caregivers who used powdered formula were not analysed separately from all formula users; however, 80-93% of infants who consumed any formula were fed powdered formula. In the USA, FUF is regulated in the same manner as PIF, and distinctions between the two products were not made. Handling instructions do not differ between the two products, and therefore no differences by product are likely to have occurred. This survey found that neither hygiene nor chilling practices varied by infant age; caregivers were not more careful with young infants. More than half of the caregivers (55%) said that they did not always wash their hands before preparing infant formula. About 4 to 6% of caregivers said they sometimes used bottle nipples again without cleaning them in any way, and about a third of caregivers sometimes only rinsed bottle nipples with water between uses. Only 4 to 6% of caregivers said they keep prepared formula at room temperature for more than two hours and then feed it to their infant. However, 18 to 20% put their infant to bed at night with a bottle of something other than water. These bottles may have contained reconstituted formula and it is possible that this was consumed after the bottles had been left at room temperature for several

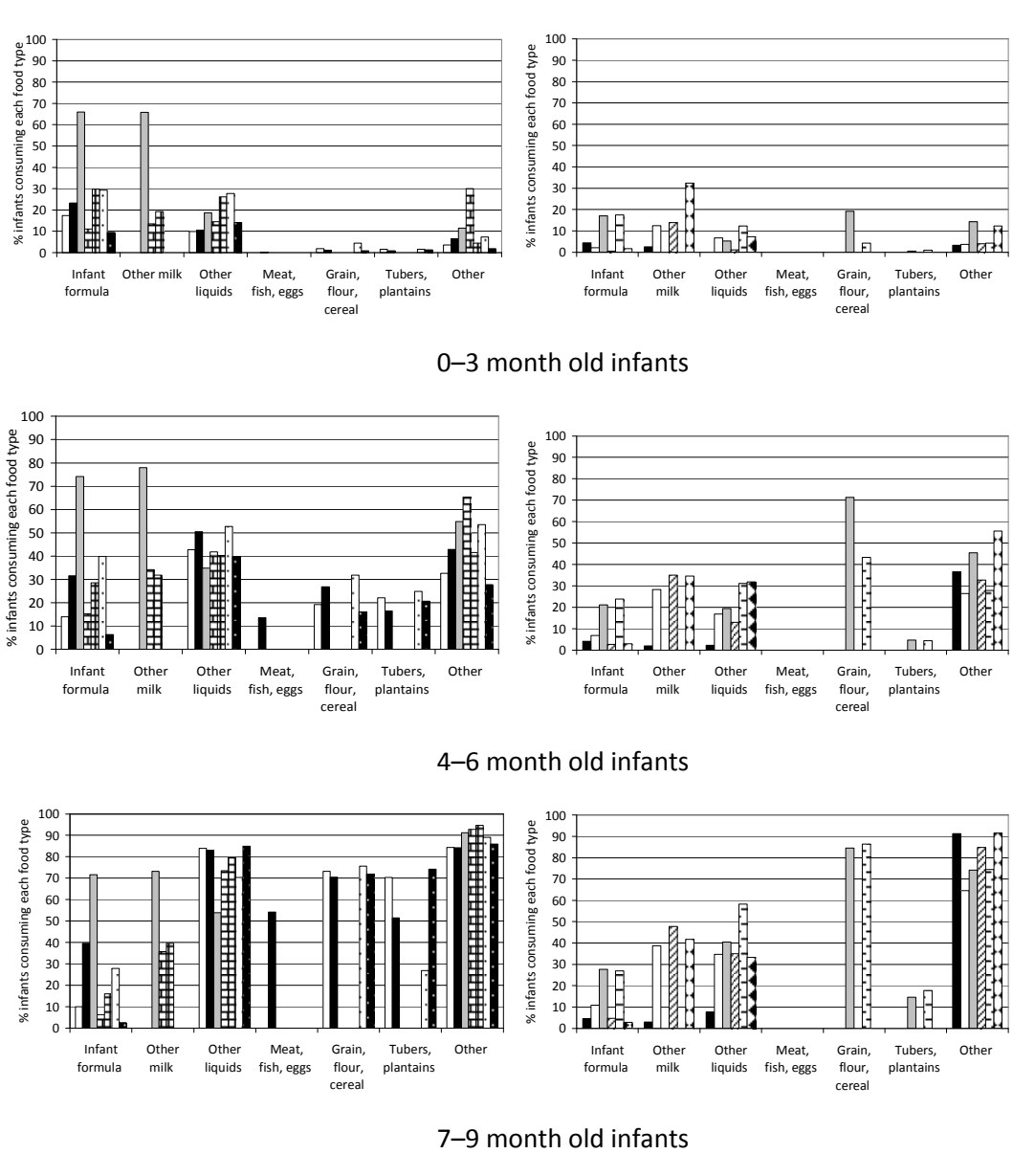

Figure 9. Consumption of different foods by infants aged 0–3 months, 4–6 months and 7–9 months in selected countries in Latin America and the Caribbean, and south and southeast Asia.
Source of data: DHS (http://www.measuredhs.com/)

hours. The study also showed that among caregivers of two-month-old infants, 88% had not received instruction on formula preparation, and 82% had not received instruction on formula storage from a health care professional

An Italian study conducted in one city, Trieste, sampled 124 caregivers of infants 1–11 months old. Results show that 18% of respondents reported they do not always wash their hands before preparing formula, and 52% do not always wash bottles between uses. Considering four of the WHO recommendations regarding preparation of PIF—(a) sterilize the bottle at each feed; (b) wash hands with warm water and soap before preparation; (c) use water of at least 70°C and add PIF to the water; and (d) use prepared formula immediately and discard any left over—only 11% reported they use all four practices to prepare and feed formula safely. However, 62% of the caregivers interviewed had received instruction from a health care professional about preparation and the remainder followed instructions found on the formula package.

There was no recent information available on handling and preparation practices in developing countries. However, in terms of the provision of instructions and information, one report (Ghana) indicated that the principle source of information on FUF was product labelling (45%), together with health services (20%), retailers (5%) and friends and relatives (6%). Such a reliance on product labels for information highlights the importance of this information being accurate and easy to understand. This may require labelling and product information in local languages and the inclusion of instructions in pictorial formats.

In summary, a substantial percentage of caregivers in developed countries do not use basic hygiene and the recommended procedures within their country for safely preparing, storing and feeding infant formula. It is likely that infant caregivers in developing countries, where hygiene and cooling require greater effort, do not have safer practices than those in developed countries. In addition, health care professionals in some developed countries may not be a source of information about infant formula preparation and storage for most parents.

5.5 Conclusions

Despite the limited data, there is information to indicate that FUF is consumed by infants less than six months of age in developed countries, and that it is consumed by a small proportion of infants at greatest risk of *E. sakazakii* (*Cronobacter* spp.) infection, i.e. neonates and infants less than two months of age. Caregivers give FUF to infants younger than recommended in national legislation and on labels for a number of reasons, e.g. a belief that it is more nutritious and/or satisfying for the infant, to prevent sleepless nights, and previous experience with such products. Socioeconomic and educational factors also play a role. Conversely, data on the consumption of other foods suggests that a small proportion of neonates and young infants may be exposed to a wide range of foods early in life. This seems to be the case in both developed and developing countries and in different regions of the world. In addition, recent studies on the preparation, handling and use of powdered formula indicate that there is still quite a lot of work to be done to educate caregivers of infants on the most appropriate means to handle these non-sterile products. While such guidance exists, two of the studies indicate that this information is not reaching those who need it, i.e. the caregivers of infants. If caregivers are not receiving this practical information on the use of powdered formula, it is not surprising that products such as FUF are being fed to infants younger than intended by national legislation and international recommendations.

6. Guidance for risk managers

6.1 Scope of the analysis

At the time of this expert meeting, the CAC had adopted the Code of Hygienic Practice for Powdered Formulae for Infants and Young Children (CAC/RCP 66-2008) (CAC, 2008b), including Annex 1, which stipulates a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) in PIF with the following 2-class sampling plan: n=30, m=absence in 10 g and c=0.

CCFH has specifically asked for scientific advice pertaining to the potential application of a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) for follow-up formula. The analysis presented in this section is intended to support that decision-making process. The analysis presented does not imply that other sampling plan options should be excluded from consideration by CCFH or that the choice of an intermediate sampling plan by CCFH would be inappropriate (e.g. 15 samples of 5 g, 10 samples of 10 g, 30 samples of 5 g or any other combination that is clearly less stringent than the plan which requires absence in 30 samples of 10 g).

Ultimately, the choice of a sampling plan option for FUF may depend on the relative risk associated with consumption of this class of product compared to PIF, and the level of risk reduction that would be associated with the implementation of the plan. In comparing FUF to PIF, the meeting assumed the relative stringency of any hygiene measure established by the risk managers would be in proportion to the relative risk posed by consumption of the product. Further, risk managers would need to combine an evaluation of differences in the level of risk with the certainty that differences are known to exist. Only a credible difference in the risk level of FUF and PIF consumption would justify different risk management measures in otherwise similar products and consumer groups.

6.2 Analysis of the evidence

The meeting reviewed all the available information in the context of whether or not a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) should be established for FUF, and weighed the scientific evidence for and against. Based on this, the meeting concluded that there was not a clearly defined scientific justification either for or against the establishment of such an international microbiological criterion. However, by presenting the available evidence, the meeting sought to present the data that is currently available and highlight both how it contributes to our knowledge base and could be used for determining alternative risk management options. Furthermore, the limitations of those data, particularly in relation to the narrow spectrum of the global population which they represent, is described.

6.2.1 Review of documented cases and the strength of evidence that formula was involved

Category of Evidence

- Six cases among infants 6–12 months have been reasonably well described.

Five of the six cases are known to have consumed PIF. In one case, hospital-prepared PIF and the blender used only to prepare PIF within the hospital yielded *E. sakazakii* (*Cronobacter* spp.).

One of two well documented cases involving toddlers 12–35 months old who had consumed powdered formula; the opened can of powdered formula recently consumed by the case-patient yielded *E. sakazakii* (*Cronobacter* spp.) with a PFGE pattern indistinguishable from the clinical isolate. No other sources of infection were identified, despite extensive environmental testing in some cases.

Caveats

In no case were the factory-sealed containers of powdered formula lots associated with the cases found to contain *E. sakazakii* (*Cronobacter* spp.).

Environmental testing was not undertaken in all cases.

Conclusion

- Given the challenges in obtaining and testing powdered formula products linked to cases, there is evidence that powdered formula has been a vehicle of *E. sakazakii* (*Cronobacter* spp.) infections among older infants and toddlers.

6.2.2 Relative prevalence of E. sakazakii (Cronobacter spp.) cases in 0–6 versus 6–12 month age groups

Category of Evidence

Although the meeting found some examples, overall, few cases of *E. sakazakii* (*Cronobacter* spp.) infections are observed in consumers in the 6–12 month age range.

Very little can be said about the overall rate of *E. sakazakii* (*Cronobacter* spp.) in the population, as data is only available from a few countries.

The observed differences in reporting between <6 months and >6 months may reflect a difference in severity of disease rather than difference in the incidence of all illness in the two age groups, since severity of disease is one of the key, well established sources of under-reporting bias in infectious disease surveillance systems.

The observed differences in reporting may also reflect the controlled hospital environment where clustered newborns may be exposed to a contaminant, but also where surveillance systems exist, and infection control personnel respond and report illnesses.

Caveats

Foodborne disease surveillance systems for the general population in general may not be sufficiently sensitive to capture potential cases.

The lack of knowledge of the factors that lead to incomplete reporting make it impossible to rule out an age-related difference in reporting bias.

While data was presented from several developed countries, it is not directly comparable to populations of infants in other countries, especially those where malnutrition or a higher disease burden may result in a higher prevalence of immunecompromised infants of 6–12 months.

Conclusion

- The level of uncertainty in the overall rate of *E. sakazakii* (*Cronobacter* spp.) infections does not imply an equivalent incidence of *E. sakazakii* (*Cronobacter* spp.) infections in these two age groups, and the meeting did recognize that the limited data indicate that the risk of

infection and thus the incidence of *E. sakazakii* (*Cronobacter* spp.) illness appears to decrease with age.

- Nevertheless, given this uncertainty, the overall difference in risk between the two age groups was not quantifiable.

6.2.3 Consumption of FUF by infants aged 0–6 months

Category of evidence

Clear evidence was provided that FUF is consumed by infants in the 0–6 month age group.

There is evidence to indicate that FUF is even consumed by the group at risk, i.e. neonates and infants <2 months.

Feeding infants less than 6 months with FUF appeared to be correlated with literacy and socioeconomic status, as well as beliefs that the product improves satiety and assists in sleep.

Caveats

The available data to support this came primarily from industrialized countries, and the level of consumption of FUF by infants aged 0–6 months might be more or less in countries of different developmental status.

Data on the consumption of other foods indicates that a certain proportion of infants in the 0–6 month age group appear to be exposed to a range of foods.

Conclusion

- FUF is consumed by infants less than 6 months and in at least some countries it is consumed by a small proportion of the group of infants at greatest risk, i.e. <2 months.

6.2.4 Similarity of production of PIF and FUF, and prevalence of *E. sakazakii* (*Cronobacter* spp.) in FUF

Category of Evidence

Production processes for PIF and FUF are almost identical.

The main differences that can have an impact on risk are the addition of a more diverse range of ingredients, which may be produced using different technologies and hygiene standards, and in some cases more lenient hygiene regimes, compared with powdered formula.

There is little data on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in FUF, and therefore it is difficult to compare with PIF, although assumptions can be made based on the data that is available for PIF such that if the FUF is produced under a similar hygienic regime to PIF, the prevalence and level of contamination will also be similar.

Caveats

The lack of data on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in FUF is probably related to the lack of requirements for testing FUF for this pathogen.

Any assumptions on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in FUF would need to take into consideration whether the product was manufactures on a dedicated line or a production line shared with other products that might have greater or lesser hygiene requirements.

Conclusion

- From a technological perspective it should be feasible to produce FUF under the same hygiene standards as applied to PIF, although particular challenges exist in relation to sourcing of high quality ingredients.
- However, it was recognized that the technological capacity alone is not a basis for risk-based decision-making.
- The existing risk assessment model can be used to look at the impact of specific microbiological criteria on the risk associated with *E. sakazakii* (*Cronobacter* spp.) in FUF, being mindful of the fact that in the absence of data, inputs on the level and prevalence of contamination will have to be assumed.

6.2.5 Suitability of 6 months[13] as a threshold to differentiate risk groups from a food safety perspective

Category of Evidence

- The age of 6 months has no specific scientific meaning and constitutes an arbitrary threshold with respect to the susceptibility of an infant to foodborne hazards.
- The immune status of an infant is highly variable for a great variety of reasons, only one of which is the set of changes that tend to occur as a function of time.
- Given the prevalence of conditions that contribute to immune deficiency in infants and young children under five years, the age of an infant alone is a poor predictor of susceptibility to infection and the expected severity of outcomes.
- Infants have been defined as the group at risk, with neonates and infants under 2 months of age being "at greatest risk" based on data suggesting that the sharpest decline in risk appears to be after the first two months of life.
- The level of risk appears to be lower in older infants and the illness to be less severe.

Caveats

While the level of risk appears to be lower in older infants, this is based on data from a few developed countries. In addition, it may reflect a difficulty in detecting sporadic cases, which seem to be more common in older infants, as well as a reporting bias towards more severe illnesses, which may not be as common in older infants.

Conclusion

- It is currently not possible to quantify the difference in risk that may exist between younger and older infants.
- There is currently no scientific basis to support a threshold of 6 months to differentiate between susceptible populations of infants.

[13] The 6 month threshold is being considered as, based on Codex guidelines, FUF may be consumed from the 6th month of life as part of a weaning diet.

6.2.6 General considerations

The analysis presented above, can be used optimally where there is a clear definition of the situation that risk managers seek to address. In such situations, the analysis may help risk managers assess the value of establishing a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) for FUF. Since the meeting was not in a position to communicate or liaise directly with risk managers, the above analysis and presentation of the evidence attempts to address an anticipated range of possible risk management scenarios.

6.3 Answers to CCFH questions

The 39th session of CCFH identified seven specific questions to be addressed by the expert meeting in order to facilitate its deliberations as to whether it should establish a microbiological criterion for *E. sakazakii* (*Cronobacter* spp.) in FUF. The preceding sections have aimed to review the available data necessary to respond to these questions and present a scientific conclusion to an evaluation of that data, and the scientific evidence available has been highlighted above. The following aims to use that information to present a concise response to each of the specific questions posed by the CCFH.

Question 1: What is the number and incidence rate of confirmed *E. sakazakii* infection in infants up to 12 months, presented by month, as compared to the incidence rate in all other age groups, including young children (12–36 months), older children and adults?

Given the paucity of data, it is not currently possible to determine the number and incidence rate of confirmed *E. sakazakii* (*Cronobacter* spp.) infection in infants up to 12 months, by month, compared to the incidence rate in all other age groups. However, two data sets from laboratory-based surveillance systems were used to partly address this issue and provide some rough estimates of the rate of *E. sakazakii* (*Cronobacter* spp.) infections. Based on data from the United Kingdom, an estimated annual incidence rate for neonates in England and Wales was 17.60 per million population over the period 1992–2007. For infants aged 1–11 months and children 1–4 years and 5–9 years, the estimated incidence rates were 2.06, 0.7 and 0.22 per million population, respectively. This indicates a decreasing incidence rate of *E. sakazakii* (*Cronobacter* spp.) infection with age. The decline is by a factor of 8.5 between <1 month and 1–11 months, by a factor of 3 between 1–11 months and 12–48 months and between 1–4 years and 5–9 years. Considering the UK data as a whole for 1999–2007 and with neonates included in the infant category, the highest incidence rate occurred in those aged 75 years or more (3.75 per million population, followed by the group 65–74 years (2.65 per million population. For infants (<12 months) the rate was 2.45, and for young children (1–4 years) it was 0.65.

Based on data from the Philippines from 1998–2007, the incidence rates of *E. sakazakii* (*Cronobacter* spp.) isolation from any site for infants less than 12 months, and among children 12–23 months and 24–35 months, were 1.4, 0.05 and 0.25, respectively, per million population per year. Among older children, the incidence rates were 0.16 and 0.11 per million population per year among children 36–48 months and those 5–9 years respectively. Thus, the incidence of isolation of *E. sakazakii* (*Cronobacter* spp.) from any site decreased by a factor of approximately 10 between infants and children 1–4 years old, and did not substantially decrease further among children 5–9 years old. Focusing only on the incidence of invasive cases, a decrease by a factor of 4 was observed between infants <1 year and children 24–35 months old. No invasive cases were reported in the 12–23 month age group for this period.

The data available in the United Kingdom and the Philippines enables the provision of a snapshot of the possible incidence rate of *E. sakazakii* (*Cronobacter* spp.) infection across different age groups. However, it is also important to be cognisant of the limitations of this dataset, which is representative of only two countries and is based on laboratory surveillance, with little information on the cases themselves. While the data from both countries is not directly comparable due to the age breakdowns that were available, these datasets broadly indicated a similar pattern of decreasing incidence with age in infants and young children. Two further studies used to estimate the incidence of invasive *E. sakazakii* disease were also considered. One was undertaken by the US CDC, which queried FoodNet sites within the USA for invasive cases among infants in 2002. They identified 4 cases for an estimated incidence of 10 invasive cases among a million infants per year. Stoll and colleagues (2004) looked for cases among a network of 19 neonatal intensive care units in the USA, and estimated 9.4 invasive cases per 100 000 very-low-birth-weight infants. Further information is available in Section 3.3.

Question 2: Critically review all documented cases of confirmed *E. sakazakii* infections in infants between 6 and 12 months of age and consider specifically (i) the clinical history and outcomes, as well as (ii) the strength of the descriptive, epidemiological and/or microbiological evidence concerning the origin or source of these infections.

Collectively, there are roughly 120 reported documented cases among children less than 3 years of age (see Annex 1). Eight well documented cases are known to have occurred among children 6–35 months old. Six of these occurred among infants 6–11 months old, 5 of which were invasive (isolated from blood, CSF, brain tissue or urine). Three of the 5 case-patients had other active medical problems (one received feeds *via* continuous infusion through a gastrostomy tube; one had severe combined immunodeficiency syndrome; one had recently undergone surgical correction for vesico-ureteral reflux). Of the remaining 2 infants with invasive disease onset between 6–11 months of age, one had a history of premature birth (33 weeks estimated gestational age) and the other had no known medical problems. Outcomes are known for 3 of the 5 patients with invasive disease; all survived. All 5 of the case-patients with invasive disease consumed PIF, and in 3 of these case-patients the associated PIF was tested. In one case the PIF did not yield *E. sakazakii* (*Cronobacter* spp.), but both the prepared formula within the hospital and the blender used to prepare the formula yielded *E. sakazakii* (*Cronobacter* spp.). In the second case, only around 100 g of the associated PIF was available for testing; this did not yield *E. sakazakii* (*Cronobacter* spp.). In the third case, investigators were not certain they had obtained the correct lot of PIF; however, the lot they tested did not yield *E. sakazakii* (*Cronobacter* spp.). No other source of *E. sakazakii* (*Cronobacter* spp.) was identified for any of the 5 cases with invasive disease in this age group.

Two cases occurred among toddlers 12–35 months old. Both cases were invasive. Both case-patients had ongoing medical problems: one had a posterior fossa dermoid cyst, and the other had Kasabach-Merrit syndrome and had recently received chemotherapy. The outcome is known for one case-patient; this child survived. One of the case-patients consumed powdered formula marketed for infants 9–24 months old. The opened can of powdered formula associated with this case yielded *E. sakazakii* (*Cronobacter* spp.) with a PFGE pattern indistinguishable from the clinical isolate. Sealed cans of powdered formula and environmental samples from the case-patient's home and childcare setting did not yield *E. sakazakii* (*Cronobacter* spp.). The other case-patient is not known to have consumed powdered formula products, and no source of infection was identified for this patient.

The expert meeting recognized the potential for reporting bias, favouring the report of newborn infections over older infants, as newborns tend to have more severe illness and more

frequently occur in clusters (in the hospital environment), which makes detection and reporting easier than sporadic community cases, more common among older infants.

Further information is available in Section 3.2.

Question 3: Estimate the relative risk of *E. sakazakii* infections in infants 6–12 months of age, associated with the consumption of follow-up formula, as well as any other sources as identified in the previous question.

The previous FAO/WHO expert meeting concluded that among infants, those of 0–2 months are of greatest risk of *E. sakazakii* (*Cronobacter* spp.) infection. The small amount of data available makes it difficult to distinguish differences in risk in the 2–12 months age group. Furthermore, all the data available comes from a few developed countries and thus cannot be considered representative of the global picture.

Six cases among infants 6–12 months are reasonably well described. Feeding history is known for 5 of 6 cases and of these 5, all consumed PIF. In one case, hospital-prepared powdered infant formula and the blender used only to prepare the formula within the hospital yielded *E. sakazakii* (*Cronobacter* spp.). The organism was not isolated from the factory-sealed containers of PIF lots associated with the cases. However, these cases have occurred sporadically rather than as part of an outbreak. Thus, as epidemiological methods could not be used to implicate a source of infection, investigators were required to rely on microbiological testing of suspect vehicles. This makes identification of infection vehicle extremely difficult among sporadic cases. However, given the challenges in obtaining and testing PIF products linked to cases in older infants, the evidence suggests that powdered formula can be a vehicle of *E. sakazakii* (*Cronobacter* spp.) infections among older infants.

Nevertheless, the meeting noted that given that the number of documented cases, in a few developed countries, among infants of 6–12 months is much less than for younger infants, and that 60% of the invasive cases in infants of 6–12 months had underlying conditions, there would appear to be a decrease in risk as the infant gets older. This is also supported by the laboratory-based surveillance data from the UK and the Philippines, where in the 0–12 month age group most isolations were from infants in the first 2 months of life. However, caution is advised in considering any extrapolation of these data to countries with different socioeconomic conditions and stages of development, and where, for example, the immune status of the infant population might be quite different.

However, a decrease in risk is also biologically plausible given the changes that occur in the immune system of the infant from birth to 11 months. Neonates have a transitory immuno-deficiency affecting a broad number of immune functions and that increases the risk of infection. There are numerous contributory factors, as described in Section 3.4, that prevent adequate responses to pathogenic material. Neonates between 2 and 6 months of age become IgG deficient due to catabolism of maternal IgG. IgM expression is genetically constrained until 2 months of age.

Finally, it should be noted that the documented invasive cases mentioned above all occurred in the USA, where FUF is not marketed for infants. Thus, all cases had consumed PIF, although in some cases the PIF was explicitly marketed for older infants (e.g. from 9 months of age). Therefore there was no data available on cases linked specifically to FUF. In addition, no other sources were identified in these cases.

Question 4: What is the number and incidence rate of immunocompromised infants up to 12 months, presented by month, as compared to the number and incidence rate of immunocompromised in all other age groups, including young children (12–36 months), older children and adults, and does this vary regionally?

While there is general agreement that immunocompromised infants are more susceptible to infection, the meeting was unable to identify a way of clearly defining the immune status of the population of concern. Although some countries, such as the USA, estimate that approximately 4% of the total population is immunocompromised, the inability to clearly define immune status was reflected in the lack of information provided by countries responding to the Call for Data and in the literature. The meeting noted that a number of factors contribute to immune status, including nutritional status, zinc deficiency, HIV status, other clinical conditions, pharmaceutical treatment, low birth weight and premature birth. The prevalence of such factors vary widely, often regionally and according to socioeconomic conditions and the state of development of a country, and thus the meeting concluded that there will also be a wide variation in the incidence of immunocompromised infants. For example, malnutrition is a major cause of immunodeficiency leading to immunocompromised infants and children. Weight is one indicator of malnourishment and given that the percentage of underweight children under 5 years of age varies from 5 to 42%, a similar variation was considered to exist for the prevalence of infants and young children in an immunocompromised state. Thus, the meeting concluded that, in certain parts of the world, a substantial proportion of infants and young children might be immunocompromised.

Question 5: Taking into consideration the information generated in the above four questions, and given the application of risk management options as advocated in the Code, what is the relative risk reduction achieved by the application of microbiological criteria, as proposed in Annex 1 of the Code, to follow-up formula?

Data are scarce on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in products categorized as follow-up formulae for infants between 6 and 12 months. The absence of such data is likely to be due to the fact that currently there are no mandated microbiological criteria for *E. sakazakii* (*Cronobacter* spp.) for these products, and hence the absence of specific testing. Reports from two recent surveys on *E. sakazakii* (*Cronobacter* spp.) indicate only one positive sample in over 100 products tested. For FUF manufactured on lines shared with PIF, it is likely that the prevalence of *E. sakazakii* (*Cronobacter* spp.) contamination is the same in both products; however, for FUF manufactured on dedicated lines (where the hygiene requirements are less stringent) the prevalence is probably higher.

Given the same initial level of contamination, the implementation of a sampling plan will reduce the prevalence of contamination of the powder to the same extent in FUF as it will in PIF. Thus, in such a scenario, the relative reduction in risk of exposure to *E. sakazakii* (*Cronobacter* spp.) is the same. The relative reduction in risk of exposure to *E. sakazakii* (*Cronobacter* spp.) will of course be different if the contamination levels are different. The effectiveness of sampling plans (implemented and enforced) in reducing relative risk for product contaminated at different levels is discussed in detail in Section 4.3 of the 2006 Expert Meeting (FAO/WHO, 2006). The level of contamination in the product at the end of production is dependent on the stringency of the hygiene conditions during production and the microbiological quality of the dry-mix ingredients. As noted in Section 4.2, in the case of production lines where more lenient or no specific hygiene requirements for Enterobacteriaceae exist, compared to those on lines manufacturing PIF, mean levels of contamination of 10^{-3} cfu/g are likely. With such levels of contamination, the implementation of the *E. sakazakii*

(*Cronobacter* spp.) sampling plan that is recommended for PIF (30 samples of 10 g) would, based on the risk assessment model, result in up to a third of the product being rejected (depending on the within-lot and between-lot variation) and would also result in a relative reduction in the risk of exposure and illness due to consumption of the product (Table 11). However, as described in Chapter 4 of the report of the 2006 Expert Meeting (FAO/WHO, 2006), in such cases a sampling plan could be an incentive for the implementation of improved hygiene management in order to reduce contamination levels and therefore reduce the amount of product that is rejected as a result of implementing the plan.

While the available data seem to indicate a decrease in the risk of illness in infants as they get older, there is still inadequate information to develop a dose-response model for *E. sakazakii* (*Cronobacter* spp.). Thus, it is not currently possibly to quantify the potential differences in susceptibility that may exist between infants of different ages. While the meeting noted ongoing work in this area, it is still in an early stage of development. The meeting did note the importance of the immune status of the infant or young child with regard to susceptibility to *E. sakazakii* (*Cronobacter* spp.). Immune status can be a function of many factors, including age, nutritional wellbeing and other illnesses, among others. So while general statements are possible in terms of potential differences in the risk of illness in different age groups, this could not be incorporated into the risk assessment model.

Table 11. Impact of a sampling plan (n=30, s=10 g) on the probability of rejection of a lot of FUF and the relative reduction in risk of *E. sakazakii* (*Cronobacter* spp.) exposure/infection with different levels of contamination in FUF at the end of production.

Mean log (cfu/g)	Between-lot standard deviation	Within-lot standard deviation	Probability of rejection of lot	Relative risk reduction
10^{-2}	0.5	0.5	0.83	5.27
10^{-2}	0.8	0.5	0.76	24.3
$10^{-2.5}$	0.5	0.5	0.59	2.9
$10^{-2.5}$	0.8	0.5	0.57	11.34
10^{-3}	0.5	0.5	0.33	1.83
10^{-3}	0.8	0.5	0.37	5.76
$10^{-3.5}$	0.5	0.5	0.14	1.34
$10^{-3.5}$	0.8	0.5	0.20	3.25
10^{-4}	0.5	0.5	0.05	1.13
10^{-4}	0.8	0.5	0.09	2.07
$10^{-4.5}$	0.5	0.5	0.02	1.05
$10^{-4.5}$	0.8	0.5	0.04	1.5
10^{-5}	0.5	0.5	0.006	1.02
10^{-5}	0.8	0.5	0.014	1.23

Question 6: Identify and describe active and passive surveillance systems for *E. sakazakii* in countries.

No active surveillance systems for *E. sakazakii* (*Cronobacter* spp.) disease have been identified. A very small number of jurisdictions have mandatory passive surveillance systems. Most countries responding to the Call for Data reported having a foodborne disease surveillance

system and/or an outbreak reporting system that theoretically encompasses *E. sakazakii* (*Cronobacter* spp.) infection. However, it is noteworthy that the national foodborne disease reporting systems did not appear to identify cases. The majority of *E. sakazakii* (*Cronobacter* spp.) cases reported across all ages have been identified through voluntary passive reporting. More details on existing surveillance systems can be found in Section 3.1.

Question 7: What is the proportion of infants less than 6 months of age that consume follow-up formula and does this vary regionally?

The data on consumption was variable, but the meeting noted that marketing recommendations regarding the introductory age to FUF were not always followed. Based on the available consumption data, infants less than 6 months of age do consume FUF. A number of European countries and one African country reported "early use", i.e. consumption of the product by infants younger than that recommended. For example in the UK, FUF is marketed for infants 6 months of age or older; however, a survey (undertaken in 2005 by an independent research company MORI on behalf of the National Childbirth Trust Charity and UNICEF-UK) reported that, of the 272 out of 1000 surveyed caregivers who had used FUF, 17% had introduced it into their infants diet by 3 months (MORI, 2005). A further survey undertaken in the UK in 2005 (Infant Feeding Survey of 9416 caregivers) reported that 2% of caregivers had introduced FUF into their infants by 4 weeks and 4% by 8 weeks (Bolling et al., 2007). This is significant in light of the fact that neonates and infants less than 2 months are considered at greatest risk of *E. sakazakii* (*Cronobacter* spp.) infections (FAO/WHO, 2004, 2006). The reasons for starting feeding FUF earlier than recommended are discussed in Section 5.2 (consumer attitudes).

In relation to the 6 to 12 month age group, it is clear that a large proportion of infants in the 6–12 month age group are consuming FUF, e.g. 65% in Guatemala and 60% in Ireland. Very little information was submitted on the consumption of FUF by young children (1–3 years), although there are indications of decrease in FUF consumption with increasing age. In the USA, consumption of infant formula (USA regulations do not distinguish between PIF and FUF in the 6–12 month age group) decreases from 67.3% at 6 months to 36.4% at 12 months.

While the data submitted is limited, it suggests that there is likely to be regional variation in the consumption of FUF. Furthermore studies on infant formula consumption illustrate differences in consumption patterns, both between regions and within regions. Such variations could be considered to be equally applicable to FUF consumption.

7. Summary and conclusions

Enterobacter sakazakii was defined as a species in 1980 by Farmer et al., although it was commented in that paper that these organisms were thought to represent multiple species. Recently, molecular methods have been employed to clarify the taxonomic relationship of *E. sakazakii* strains. These studies showed that *E. sakazakii* actually comprise at least 6 species and forms a distinct group of Enterobacteriaceae. This group has now been classified in a new genus, *Cronobacter* gen. nov., within the Enterobacteriaceae. This change in nomenclature has just been validated with the publication of the paper describing the new genus in the June 2008 edition of the *International Journal of Systematic and Evolutionary Microbiology* (Iversen et al., 2008).

Cronobacter spp. is synonymous with *E. sakazakii.* The new genus is composed of six species. There is no data that currently shows that any one of these species is not a risk to neonatal health. Therefore, based on the recent studies, the meeting concurred that all six species in the genus *Cronobacter* should be considered to be pathogenic, as each one has been linked retrospectively to clinical cases of infection in either infants or adults. In addition, it was concluded that there are currently no regulatory implications of the new taxonomic changes.

Laboratory methods for the detection of *E. sakazakii* (*Cronobacter* spp.) have improved in recent years to allow the reliable detection of the organism. International standardization of improved methods by ISO and FDA-AOAC is currently underway. All currently validated laboratory methods will continue to facilitate the recognition of all species defined within the new taxonomy. These methods remain applicable for the *Cronobacter* spp.

The response to the Call for Data revealed a very mixed picture in terms of the extent of surveillance for *E. sakazakii* (*Cronobacter* spp.) infections and the resulting numbers and incidence of infections in neonates, other infants, children and adults. Globally, there appear to be very few surveillance data for *E. sakazakii* (*Cronobacter* spp.). Although a couple of passive surveillance systems exist, no active surveillance system for *E. sakazakii* (*Cronobacter* spp.) disease has been identified. Most countries reported having a foodborne disease surveillance system and/or an outbreak reporting system that would encompass *E. sakazakii* infection, if cases were linked to a food vehicle and/or occurred as part of a recognized outbreak of disease. However, it is noteworthy that instances were reported where cases were identified by outbreak or voluntary passive reporting but not by the national foodborne disease reporting system or even the mandatory reporting system for *E. sakazakii* (*Cronobacter* spp.).

Collectively, there are approximately 120 individually documented cases among infants and children less than 3 years of age. Six well documented cases are known to have occurred among children 6–11 months and two cases in the 12–35 month age group. Of the 5 invasive (urine, blood, CFS, brain tissue) cases in the 6–11 month age group, 3 had other active medical problems. While this appears to indicate few cases in the 6–35 month age group, this information must be considered in light of the lack of surveillance systems, under-reporting, and other limitations noted in the report in identification of cases.

The data available do not enable a detailed breakdown of numbers of cases by month for infants. However, there were some laboratory surveillance data for England and Wales wherein data are available for infants under 1 month for 1992–2007, and for the Philippines, wherein data were available for infants under 1 month and at two-month intervals up to 12 months for

1998–2007. Based on the data from England and Wales, an estimated annual incidence rate for neonates was 17.60 per million population over the period 1992–2007. For infants aged 1–11 months, the estimated incidence rate was 2.06 per million population, and among children 1–4 years, 0.70 per million population. These data indicate a sharply decreasing rate of severe illness between infants <1 month and 1–11 months. While it was not possible to calculate incidence data at monthly intervals based on the information available from the Philippines, these data also indicated a decreasing incidence with age.

While there is general agreement that immunocompromised infants are more susceptible to infection, the meeting was unable to identify a way of clearly defining the immune status of the population of concern. Although some countries, such as the USA, estimate that approximately 4% of the total population is immunocompromised, the inability to clearly define immune status was reflected in the lack of information provided by countries responding to the Call for Data and in the literature. The meeting noted that a number of factors contribute to immune status, including nutritional status, HIV status, other clinical conditions, pharmaceutical treatment, low birth weight and premature birth. The prevalence of such factors varies widely among countries and thus the meeting concluded that there will also be a wide variation in the prevalence of immunocompromised infants. For example, underweight and stunting, two indicators of malnutrition, a major cause of immunodeficiency, vary in prevalence in children under 5 years of age, from 5–42% and 12–41%, respectively. It was therefore considered by the meeting that a similar variation exists for the incidence of infants and young children in an immuno-compromised state. Thus, the meeting concluded that in certain parts of the world a substantial proportion of infants and young children are immunocompromised.

Follow-up formulas are manufactured using processes that are almost identical to those used for other infant formulas as well as any other type of powdered dairy products for consumers >36 months of age, such as dairy-based beverages, fortified milk powders, and products used in medical nutrition.

The most significant differences between PIF and FUF lie in the fact that FUF may contain a wider variety of dry-mix ingredients and may be manufactured under different hygiene requirements in accordance with the legislation of the local jurisdiction. The wider variety of dry-mix ingredients is a consequence of the need for a more diversified diet for children aged 6 to 35 months, particularly for young children (>12 months). As these ingredients are often manufactured using completely different food processing technologies and consequently different hygiene control measures, their microbiological quality may not necessarily meet the most stringent requirements typically applied to infant formula. The stringency of the hygiene control measures required for a particular processing line will depend on the microbiological criteria for the range of products manufactured on that line. In the case of a dedicated line, the hygiene control measures will directly relate to the product being manufactured. However, in the case of shared lines, the products with the most stringent microbiological criteria will determine the overall hygiene control measures required, as well as the verification procedures for processing environment and the processing lines. The meeting therefore concluded that while PIF and FUF may be manufactured using nearly identical processes, other aspects of manufacturing, namely the dry-mix ingredients used and the stringency of hygiene control measures, can vary considerably. These aspects need to be taken into consideration when comparing the products and the extent to which microbiological criteria are needed.

Data on the prevalence of *E. sakazakii* (*Cronobacter* spp.) in products categorized as FUF for infants between 6 and 11 months are scarce. The absence of such data is most likely due to the fact that there is no mandated requirement for testing FUF for *E. sakazakii* (*Cronobacter*

spp.). Reports from two recent surveys on *E. sakazakii* (*Cronobacter* spp.) indicate only one positive sample in over 100 products tested. For FUF manufactured on shared lines with PIF, it is likely that the incidence of *E. sakazakii* (*Cronobacter* spp.) contamination is the same in both products; however, for FUF manufactured on dedicated lines (where the hygiene requirements may be less stringent), the prevalence is probably higher. The relative reduction in risk associated with the implementation of a sampling plan for FUF will be expected to be the same as for PIF with the same level of contamination.

Based on the available data, the meeting concluded that FUF is commonly consumed by infants less than 6 months of age in both developing and developed countries, despite existing regulations and label recommendations. Data from developed countries also showed that a substantial percentage of caregivers to infants do not use basic hygiene and the recommended procedures within their country for safely preparing and feeding infant formula. It is likely that infant caregivers in developing countries, where hygiene and cooling require greater effort, do not have safer practices than those in developed countries. This suggests that a substantial proportion of caregivers to infants worldwide fail to follow all of the preparation and feeding practices recommended to reduce the risks of microbiological hazards associated with a non-sterile product. Educational, socioeconomic and hygiene factors may be important to explain why caregivers fail to follow the recommended practices.

8. References

Acheson, D.W.K. & Lubin, L.F. 2008. Vulnerable populations and their suceptibility to foodborne disease. pp. 290–319, *in:* B.M. Lund and P.R. Hunter (editors). *The Microbiological Safety of Food in Healthcare Settings*. Blackwell Publishing Ltd, Oxford, UK.

Block, C., Peleg, O., Minster, N., Bar-Oz, B., Simhon, A., Arad, I. & Shapiro, M. 2002. Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of *Enterobacter sakazakii*. *European Journal of Clinical Microbiology and Infectious Diseases*, 21: 613–616.

Bolling, K., Grant, C., Hamlyn, B. & Thornton, A. 2007. Infant Feeding Survey 2005. The Information Centre for Health and Social Care, and the UK Health Departments. Available at: http://www.ic.nhs.uk/statistics-and-data-collections/health-and-lifestyles/infant-feeding/infant-feeding-survey-2005.

Bowen, A. & Braden, C. 2006. Invasive *Enterobacter sakazakii* disease among infants. *Emerging Infectious Diseases*, 12(8): 1185-1189.

CAC [Codex Alimentarius Commission]. 1987. Codex Standard for Follow-up Formula. Codex Standard No. 156-1987. Available at http://www.codexalimentarius.net/download/standards/293/CXS_156e.pdf

CAC. 2003a. Report of the 24th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, Berlin, Germany, 4–8 November 2002. Doc. Alinorm 03/26A. Available at: http://www.codexalimentarius.net/web/archives.jsp?year=03.

CAC. 2003b. Codex Alimentarius Commission, 25th Session, Rome, 30 June–05 July 2003. Report of the 35th Session of the Codex Committee on Food Hygiene, Orlando, Florida, United States of America, 27 January–1 February 2003. Doc. Alinorm 03/13A. Available at: http://www.codexalimentarius.net/web/archives.jsp?year=03.

CAC. 2004. Codex Committee on Food Hygiene, 36th Session, Washington DC, United States of America, 29 March–3 April 2004. Risk profile on *Enterobacter sakazakii* and other microorganisms in powdered infant formula. Prepared by the United States of America and Canada. Available at: ftp://ftp.fao.org/codex/ccfh36/fh04_12e.pdf.

CAC. 2004b. Food Hygiene Basic Texts. Available at: ftp://ftp.fao.org/codex/Publications/Booklets/Hygiene/FoodHygiene_2003e.pdf.

CAC. 2008a. Report of the 31st Session of the Codex Alimentarius Commission. Geneva, Switzerland, 30 June–4 July 2008. Doc. Alinorm 08/31/REP. Available at: ftp://ftp.fao.org/codex/Alinorm08/al31REP_adv.pdf.

CAC. 2008b. Code of Hygienic Practice for Powdered Formulae for Infants and Young Children. CAC/RCP 66-2008. Available at: http://www.codexalimentarius.net/download/standards/11026/cxp_066e.pdf.

Carletti, C. & Catttaneo, A. 2008. Home preparation of powdered infant formula: is it safe? *Acta Paediatrica*, 97: 1131–1132.

Caubilla-Barron, J., Hurrell, E., Townsend, S., Cheetham, P., Loc-Carillo, C., Fayet, O., Prere, M.-F. & Forsythe, S.J. 2007. Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *Journal of Clinical Microbiology*, 45(12): 3979–3985.

CDC [Centers for Disease Control and Prevention]. 2008. Global AIDS Program. BOTUSA. Available at http://www.cdc.gov/botusa/CDC/FDA Infant Feeding Practices Study II Web Tables. Forthcoming at: www.cdc.gov/ifps

Cordier, J.L. 2007. Production of powdered infant formulae and microbiological control measures. Chapter 6 *in*: J.M. Farber and S.J. Forsythe (editors). *Emerging issues in food safety*: Enterobacter sakazakii. ASM Press, Washington DC, USA.

Define Research and Insight. 2006. Powdered Infant Formula Qualitative Research. April 2006. Funded by U.K. Food Standards Agency. Available at: http://www.food.gov.uk/multimedia/pdfs/powderinfantform.pdf.

Drudy, D., O'Rourke, M., Murphy, M., Mullane, N., O'Mahony, R., Kelly, L., Fisher, M., Sanjaq, S., Shannon, P., Wall, P. & Fanning, S. 2006. RAPD analysis of *Enterobacter sakazakii* strains from environmental and clinical sources. *International Journal of Food Microbiology*, 110: 127–134.

Fanning, S. & Forsythe, S.J. 2007. Isolation and identification of *E. sakazakii. In:* J.M. Farber and S.J. Forsythe (editors). *Emerging issues in food safety:* Enterobacter sakazakii. ASM Press, Washington DC, USA.

FAO/WHO. 2004. *Enterobacter sakazakii* and other micro-organisms in powdered infant formula: Meeting report. *Microbiological Risk Assessment Series*, No. 6. 59 p. Rome, Italy. Also available at: ftp://ftp.fao.org/docrep/fao/007/y5502e/y5502e00.pdf.

FAO/WHO. 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting report. *Microbiological Risk Assessment Series,* No. 10. 95p. Rome, Italy. Also available at: ftp://ftp.fao.org/docrep/fao/007/y5502e/y5502e00.pdf.

FAO/WHO. 2007. Guidelines on the safe use of powdered infant formula. Available at: http://www.fao.org/ag/agn/agns/jemra_riskassessment_enterobacter_docs_en.asp

Farmer, J.J. III, Asbury, M.A., Hickman, F.W. & Brenner, D.J. 1980. The Enterobacteriaceae Study Group (USA). *Enterobacter sakazakii*:a new species of "Enterobacteriaceae" isolated from clinical specimens. *International Journal of Systematic Bacteriology*, 30: 569–584.

FDA [US Food and Drug Administration]. 2007. Food Protection Plan: An Integrated strategy for Protection of the Nation's Food Supply.

Friedemann, M. 2007. *Enterobacter sakazakii* in foods and beverages (other than infant formula and milk powder). *International Journal of Food Microbiology*, 116: 1–10.

FSA/DH [Food Standards Agency/Department of Health]. 2007. Guidance for health professionals on safe preparation, storage and handling of powdered infant formula. Available at: http://www.dh.gov.uk/en/Healthcare/Maternity/Maternalandinfantnutrition/DH_4123674

Grummer-Strawn, L.M., Scanlon, K.S. & Fein, S.B. Infant feeding and feeding transitions during the first year of life. Forthcoming in *Pediatrics.*

Healy, B., Mullane, N., Collin, V., Mailler, S., Iversen, C., Chatellier, S., Storrs, M. & Fanning, S. 2008. Evaluation of an automated repetitive sequence-based PCR system for subtyping *Enterobacter sakazakii*. *Journal of Food Protectection,* 71: 1372–1378.

HPA [Health Protection Agency]. 2007. *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* spp. bacteraemia, England, Wales and Northern Ireland: 2003–2007. Available at: http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1201681252503.

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.

ISO [International Organization for Standardization]. 2006. ISO/TS 22964:2006 Milk and milk products – Detection of *Enterobacter sakazakii*.

Iversen, C. & Forsythe, S.J. 2004. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant milk and related products. *International Journal of Food Microbiology*, 21: 771–777.

Iversen, C. & Forsythe, S.J. 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*, 14: 443–454.

Iversen, C., Waddington, M., Farmer, J.J. III & Forsythe, S.J. 2006. The biochemical differentiation of *Enterobacter sakazakii* genotypes. *BMC Microbiology*, 6: Article No. 94.

Iversen, C., Lehner, A., Mullane, N., Bidas, E., Cleenwerck, I., Marugg, J., Fanning, S., Stephan, R. & Joosten, H. 2007. The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov., *Cronobacter sakazakii* subsp. *sakazakii* comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* comb. nov., *Cronobacter. turicensis* sp. nov., *Cronobacter. muytjensii* sp. nov., *Cronobacter. dublinensis* sp. nov. and *Cronobacter. genomospecies* I. *BMC Evolutionary Biology*, 7: Article No. 64.

Iversen, C., Mullane, N., McCardell, B., Tal, B.D., Lehner, A., Fanning, S., Stephan, R. & Joosten, H. 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter genomospecies* 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 58(6): 1442–1447.

Kandhai, M.C., Reij, M.W., Gorris, L.G.M., Guillaume-Gentil, O. & Van Schothorst. M. 2004. *Occurrence of Enterobacter sakazakii* in food production environments and households. *The Lancet,* 363: 39–40.

Koehler, K.M., Lasky, T., Fein, S.B., DeLong, S.M., Hawkins, M.A., Rabatsky-Her, T., Ray, S.M., Shiferaw, B., Swanson E. & Vugia D.J. 2006. Population-based incidence of infection with selected bacterial enteric pathogens in children younger than five years of age, 1996-1998. *The Pediatric Infectious Disease Journal*, 25: 129–134.

Khoshoo, V., Edell, D., Thompson, A. & Rubin, M. 2007. Are we overprescribing antireflux medications for infants with regurgitation? *Pediatrics*, 120(5): 946–949.

Labiner-Wolfe, J., Fein, S.B. & Shealy, K. Infant formula handing, education and safety. Forthcoming in *Pediatrics*.

MORI. 2005. Women's knowledge of formula milk and follow on milk advertising. Provided by CSPI in response to FAO/WHO Call for Data.

Mullane, N., Iversen, C., Healy, B., Walsh, C., Whyte, P., Wall, P.G., Quinn, T. & Fanning, S. 2007a. *Enterobacter sakazakii*: an emerging bacterial pathogen with implications for infant health. *Minerva Pediatrica*, 59: 137–148.

Mullane, N., Whyte, P., Wall, P.G., & Fanning, S. 2008. Development of a multi-locus variable number of tandem repeat for *Enterobacter sakazakii* in an infant formula processing facility. *Applied and Environmental Microbiology*, 116: 73–81.

Mullane, N., Whyte, P., Wall, P.G., Quinn, T. & Fanning, S. 2007b. Application of pulsed-field gel electrophoresis (PFGE) to characterize and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. *International Journal of Food Microbiology*, 116: 73–81.

Noriega, F., Kotloff, K., Martin, M. & Schwalbe, R. 1990. Nosocomial bacteremia caused by *Enterobacter sakazakii* and *Leuconostoc mesenteroides* resulting from extrinsic contamination of infant formula. *Pediatric Infectious Disease*, 9: 447–449.

Savino, F. & Castagno, E. 2008. Overprescription of antireflux medications for infants with regurgitation. *Pediatrics*, 121: 1070.

Stoll, B.J., Hansen, N., Fanaroff, A.A. & Lemons, J. 2004. *Enterobacter sakazakii* is a rare causeof neonatal septicemia or meningitis in VLBW infants. *The Journal of Pediatrics*, 144: 821–823.

Tekkok, I., Baeesa, S., Higgins, M. & Ventureyra, E. 1996. Abscedation of posterior fossa dermoid cysts. *Child's Nervous System*, 12: 318–322.

Townsend, S., Hurrell, E. and Forsythe, S.J. 2008. Virulence studies of *Enterobacter sakazakii* isolates associated with a neonatal intensive care unit outbreak. *BMC Microbiology*, 8: Art.No.64.

Townsend, S.M., Hurrell, E., Gonzalez-Gomez, I., Lowe, J., Frye, J.G., Forsythe, S.J. & Badger, J.L. 2007. *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. *Microbiology,* 153: 3538–3547.

Urmenyi, A.M. & Franklin, A.W. 1961 Neonatal death from a pigmented coliform infection. *Lancet*, 1: 313–315.

UNICEF. 2007. Children and AIDS. A stocktaking report. Actions and progress during the first year of Unite for Children, Unite against AIDS. Available at: http://www.unicef.org/publications/index_38048.html.

UNICEF. 2008. The State of the World's Children 2008. Available at: http://www.unicef.org/sowc08/docs/sowc08.pdf.

WHO. 2006. WHO HIV and Infant Feeding Technical Consultation held on behalf of the Inter-agency Task Team (IATT) on Prevention of HIV Infections in Pregnant Women, Mothers and their Infants: Consensus Statement. Available at: http://www.who.int/hiv/mediacentre/Infantfeedingconsensusstatement.pf.pdf.

WHO. 2008. Worldwide prevalence of anaemia 1993–2005; WHO Global database on anaemia. Edited by B. de Benoist, E. McLean, I. Egli and M. Cogswell. 48 p. Also available at: http://www.who.int/vmnis/publications/anaemia_prevalence/en/index.html.

Annex 1

Recorded cases of *E. sakazakii* (*Cronobacter* spp.) infections and colonisations in infants and young children

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
England	Urmenyi and White-Frankin, 1961	Male	3033 g (6lb 11oz)	38	11 d	Meningitis and sepsis	Died	Not determined	2(2)	Earliest reported cluster – same nursery and incubator. First 2 known cases, 1958
England	above	Female	2017 g (4lb 7oz)	32 (C-section)	5 d	Meningitis and sepsis	Died	Not determined	above	
Denmark	Jøker et al., 1965	Female	3250 g	Unknown	4 d	Meningitis	Recovered	Unknown	1	
USA (GA)	Monroe & Tift, 1979	Male	2600 g	Term	7 d	Bacteraemia	Recovered	Yes	1	First reported case of bacteraemia. Formula not analyzed nor any relationship suggested. No environmental testing
USA (IN)	Kleiman et al., 1981	Female	Unknown	Term	35 d	Meningitis - necrotizing cerebritis, absess formation	Recovered (severe sequelae)	Unknown	1	Previously healthy 5-week old.
USA (OK)	Adamson & Rogers, 1981	Male	Unknown	Normal pregnancy and delivery	35 d	Meningitis/Sepsis	Recovered	Unknown	1	Previously healthy 5-week old.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Netherlands	Muytjens et al., 1983; Smeets et al., 1998	Male	2830 g	36	5 d	Meningitis	Recovered	Yes	1	Hospital A, 9/77 *E. sakazakii* found in prepared formula and utensils but not in powdered formula. Plasmid profile of *E. sakazakii* in formula differed from the profile of *E. sakazakii* in all patients. Authors conclude that environmental strains did not cause the infection (assume this includes formula)
Netherlands	above	Female	2400 g	Term	3 d	Meningitis/Bacteraemia	Died	Yes	1(1)	Hospital A, 4/79 Co-morbidity- meningomyelocele.
Netherlands	above	Female	1670 g	32	3 d	Meningitis	Died	Yes	1(1)	Hospital A, 4/81
Netherlands	above	Male	1900 g	32	4 d	Meningitis	Died	Yes	1(1)	Hospital A, 4/81
Netherlands	above	Female	2690 g	Term	5 d	Meningitis/bacteraemia	Died	Yes	1(1)	Hospital A, 7/81
Netherlands	above	Male	2085 g	38	5 d	Meningitis/NEC	Died	Yes	1(1)	Hospital B and D, 2/78 Twin, incubator
Netherlands	above	Female	1370 g	Premature	5 d	Meningitis/NEC	Died	Yes	1(1)	Hospital C and D, 7/79
Netherlands	above	Female	850 g	30	9 d	Meningitis/Bacteraemia	Recovered (retarded)	Yes	1	Hospital E, 9/79
USA (MO)	Naqvi, Maxwell & Dunkle, 1985	Female	Unknown	Unknown	21 d	Meningitis/Cerebral abcesses	Recovered	Unknown	1	
Spain	UCD, 2008 Reina, J., et al. 1989	Unknown	Unknown	Neonate	Unknown	Conjunctivitis	Unknown	Unknown	1	The reference and information were Included in University College of Dublin summary submitted to Codex for consideration by the expert meeting, Washington, DC. July 2008. Original paper not available.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Greece	Arseni, et al., 1984	Unknown	Unknown	Premature	3 d	Bacteraemia	Died	Unknown	1	Source unknown. Co-infection with *Klebsiella pneumoniae*
Greece	Arseni, et al.,1987 See above	8 male 3 female	1000 g–2990 g	2 Premature 9 Unknown	2-58 d	Colonized	Varied	Unknown	11(4)	Sept. and Oct., 1984 11 neonates on a NICU colonized with *E. sakazakii*. Five of the 11 had clinical signs of sepsis and 4 died. *E. sakazakii* was not isolated from blood or CSF. nor from environmental sources. In most cases co-infections (colonization) with different/other micro-organisms such as *Pseudomonas* spp., *Klebsiella* spp., *Ser. Marcescens, E. cloacae* or *aerogenes*
USA (LA)	Willis & Robinson, 1988	Male	Unknown	Term	28 d	Meningitis/Bacteraemia (Hydrocephalus developed)	Recovered (sequelae)	Unknown	1	Two reported cases not related
USA (MA)	above	Male	2040 g	37	8 d	Meningitis/Bacteraemia	Recovered (sequelae)	Unknown	1	
Iceland	Biering et al., 1989 Clarke et al., 1990	Male	3144 g	36	5 d	Meningitis	Recovered (retardation, quadriplegia)	Yes and breast milk	3 (1)	First study to isolate *E. sakazakii* from unopened cans of formula (5) associated with illness in infants. Formula and patient isolates same by biotype, antibiotic and plasmid profile. No *E. sakazakii* isolated from preparation utensils or environment.
Iceland	above	Male	2508 g (Down)	Term	5 d	Meningitis	Died	Yes	above	
Iceland	above	Male	3308 g	38	5 d	Meningitis	Recovered	Yes	above	

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
USA (TN)	Simmons et al., 1989; Clarke et al., 1990	Unknown	780 g	28	28 d	Sepsis	Recovered	Yes	4(0)	Cluster 7 Feb–14 Mar, 1988. All 4 fed same powdered protein hydrolysate formula in the hospital. No report of environmental testing or testing of unopened can. *E. sakazakii* isolated from open can of PIF and 4 cases had same plasmid and multilocus enzyme profile.
USA (TN)	above	Unknown	950 g	29.5	57 d	Sepsis	Recovered	Yes	above	
USA (TN)	above	Unknown	850 g	27.5	52 d	Sepsis	Recovered	Yes	above	
USA (TN)	above	Unknown	1270 g	34.5	13 d	Bloody diarrhoea	Recovered	Yes	above	
Portugal	Lecour et al.,1989	Unknown	Unknown	Unknown	Unknown	Meningitis	Died	Unknown	1(1)	Case series of 187 children with bacterial meningitis evaluated for treatment with cefotaxime. One infant died from *E. sakazakii* infection. No details provided.
USA (MD)	Noriega et al., 1990	Female	Unknown	Unknown	6 m	Bacteraemia *E. sakazakii* and *Leuconostoc mesenteroides* found in blood culture	Recovered	Yes	1	Described as extrinsic contamination. *E. sakazakii* found in the blender used to prepare PIF. Formula itself did not have any *E. sakazakii*. Clinical history remarkable for child undergoing bowel resection at day 1, on TPN for first 3 months of life. Fed via continuous gastric tube.
USA (OH)	Gallagher & Ball, 1991	Male	2520 g	35	2 d	Meningitis/Bacteraemia	Recovered	Unknown	1	
Germany	Ries, Harms & Scharf, 1994	Male	1420 g	31 premature	Unknown	Meningitis	Recovered	Unknown	1	Resulted in multiple cystic encephalomalacia
Canada (reported elsewhere as USA)	Tekkok, et al., 1996	Female	Unknown	Unknown	540d	Brain Abscess	Recovered	No	1	Tissue also yielded *Corynebacterium aquaticum* and *Enterobacter cloacae*

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Scotland	SCIEH Weekly Report, 11.3.97	Female	Unknown	Unknown	Neonate	Meningitis	Unknown	Unknown	1	First reported case since 1986 (computerization of records)
Brazil	Santos, M., 2000 (cases 1998)	Unknown	Unknown	Unknown	4 neonates 1 infant	Bacteraemia	Recovered	No	5	An outbreak in 4 hospital units in Rio de Janeiro, 26 Sept-16 Oct., 1998. All cases received IV fluids (parenteral nutrients). *E. sakazakii* was isolated from unused IV bags and from a sponge used to clean IV solution vials.
USA (NC)	Burdette & Santos, 2000	Female	3000 g	35	6 d	Meningitis	Recovered	Unknown	1	Became ill at home
USA	Lai, KK, 2001	Male	Unknown	Unknown	3y	Bacteraemia	Recovered	Unknown	1	Embryonic rhabdomyosarcoma Chemotherapy via central line
Belgium	Van Acker et al., 2001	Male (1)	850 g	27	55 d	Necrotizing enterocolitis	Recovered	Yes. Formula A	12(2) 6 E sak +	June-July 1998, NEC outbreak in hospital NICU *E. sakazakii* isolated from unopened Formula A 3 subtypes of *E. sakazakii* found in patents. 3/6 patient isolates matched formula isolates.
Belgium	above	Female (2)	1930 g	31	16 d	Necrotizing enterocolitis	Recovered	Yes. Formula P	above	No *E. sakazakii* found in Formula P
Belgium	above	Male (4)	965 g	27	33 d	Necrotizing enterocolitis	Died	Yes. Formula A	above	Strain of *E. sakazakii* different for patient sterile site and formula (both prepared and unopened powder)
Belgium	above	Male (7)	1100 g	28	9 d	Necrotizing enterocolitis	Recovered	Yes. Formula A	above	Patient culture was not fully tested
Belgium	above	Female (8)	590 g	27	39 d	Necrotizing enterocolitis	Recovered	Yes. Formula A	above	Same strain of *E. sakazakii* found in patient non-sterile site and formula (both prepared and unopened powder).
Belgium	above	Female (9)	1350 g	31	17 d	Necrotizing enterocolitis	Recovered	Yes. Formula A	above	Same strain of *E. sakazakii* found in patient non-sterile site and formula (both prepared and unopened powder).

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Belgium	above	Male (11)	1290 g	32	7 d	Necrotizing enterocolitis	Recovered	Yes. Formula A	above	Same strain of *E. sakazakii* found in patient non-sterile site and formula (both prepared and unopened powder).
Belgium	above	Male (3)	995 g	27	40 d	Necrotizing enterocolitis	Died	Yes, Formula A	above	Patient not cultured
Belgium	above	Female (5)	815 g	29	41 d	Necrotizing enterocolitis	Recovered	Yes, Formula A	above	Patient not cultured
Belgium	above	Female (6)	1200 g	28	22 d	Necrotizing enterocolitis	Recovered	Yes, Formula A	above	Patient culture negative for *E. sakazakii*
Belgium	above	Female (10)	1490 g	32	9 d	Necrotizing enterocolitis	Recovered	Yes, Formula A	above	Patient not cultured
Belgium	above	Male (12)	1550g	30	4 d	Necrotizing enterocolitis	Recovered	Yes, Formula A	above	Patient culture negative for *E. sakazakii*
USA (MN)	CDC, 2001	Unknown	Unknown	27	1 m	Urinary tract infection	Unknown	Yes	1	Unknown whether environmental testing was completed.
USA (MN)	CDC, 2001	Unknown	Unknown	34	1 m	Isolated from nasal secretions	Unknown	Unknown	1	Patient was fed 2 brands of infant formula, unclear whether PIF or liquid
USA (MN)	CDC, 2001	Unknown	Unknown	40	<1 m	Meningitis	Recovered	Yes	1	Unknown whether environmental testing was completed.
USA (GA)	CDC, 2002	Unknown	Unknown	Unknown	<1 y	“non-sterile” site	Unknown	Unknown	1	Unknown whether environmental testing was completed. Identified case during FoodNet assessment of invasive *E. sakazakii* disease incidence
USA (TN)	CDC, 2002	Unknown	Unknown	Unknown	<1 y	“non-sterile” site	Unknown	Unknown	5	Unknown whether environmental testing was completed. Identified cases during FoodNet assessment of invasive *E. sakazakii* disease incidence
USA (CO)	CDC, 2002	Male	760 g	25	<1 m	Isolated from tracheal aspirate	Unknown	No	1	Unknown whether environmental testing was completed.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Israel	Bar-Oz et al., 2001	Female	2155 g	36	4 d	Bacteraemia/ meningitis	Recovered (with VP shunts)	Yes	5	Two cases in neonates. Three faecal carriers, premature. *E. sakazakii* isolated from blender and prepared formula (one occasion) PFGE of *E. sakazakii* identical for 2 patients, 3 carriers, blender and prepared formula.
Israel	above	Female	620 g	27	9 d	Bacteraemia	Recovered	Yes	above	above
Israel	Block et al., 2002	Female	2720 g	Term	6 d	Meningitis	Unknown	Unknown	3	Searched hospital databases back to 1987. Found 6 cases. 2 are reported above (Bar-OZ), and one is 6-year-old bone transplant, not listed.
Israel	above	Female	Neonatal bacteraemia in a formula-fed full-term neonate	Term	Neonate	Bacteraemia	Unknown	Yes	above	above
Israel	above	Female	Unknown	36 C-section	Unknown	Conjuctivitis	Unknown	Unknown	above	above
Belgium	2002	Unknown	Neonate	Unknown	Unknown	Meningitis	Died	Unknown	1(1)	Data taken from http://www.babymilk.nestle.com/News/All+Countries/Belgium/Infant+formula+safety.htm
USA (WI)	CDC, 2002	Unknown	Unknown	37	<1m	Meningitis? CSF isolation	Recovered	Yes	1	Initial testing of open can of infant formula negative.
USA (MI)	CDC, 2002	Male	Unknown	37	<1m	Meningitis	Recovered. Neurological impairment	Yes	1	Openned and reconstituted formula positive for *E. sakazakii*. PFGE patterns did not match patient isolate.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
USA (MN)	CDC, 2002	Unknown	Unknown	36 premature	<1m	Meningitis/Bacteraemia	Died	Yes	1(1)	Unknown whether environmental testing was done
USA (TN)	Himelright et al., 2002	Male	1270 g	33.5	11 d	Meningitis	Died	Yes	10(1)	All 10 infants in NICU. All fed same PIF. Same *E. sakazakii* isolate found in both opened and unopened can of formula and in CSF of index patient (died). 2 suspected infections with *E. sakazakii* from tracheal aspirate with documented deterioration in clinical status. 7 colonizations
France	Caubilla-Barron, et al., 2007 (El Maadani, 1996 PhD Thesis)	Female (E)	1.47 kg	31	27 d	Asymptomatic	Recovered	Yes	18(4)	5 May–11 July 1994 NICU outbreak. Infant formula abuse likely (storage for 24 h and syringes used to deliver product were not refrigerated for 4–6 h before feeding). Different strains and clones isolated. 18 cases (4 deaths); however, isolate from one fatal case (R) was not confirmed as *E. sakazakii*
France	above	Female (I)	1.57 kg	31	12 d	Bacteraemia	Recovered	Yes	above	
France	above	Male (O)	2.09 kg	33	20 d	Asymptomatic	Recovered	Yes	above	
France	above	Female (B)	1.2 kg	29	16 d	Necrotizing enterocolitis	Recovered	Yes	above	
France	above	Male (J)	1.56 kg	32	15 d	Necrotizing enterocolitis	Died	Yes	above	
France	above	Male (D)	1.8 kg	32	17 d	Necrotizing enterocolitis	Recovered	Yes	above	
France	above	Male (K)	1.18 kg	30	87 d	Necrotizing enterocolitis	Recovered	Yes	above	
France	above	Female (B)	1.2 kg	28	13 d	Necrotizing enterocolitis	Recovered	Yes	above	
France	above	Male (H)	1.5 kg	31	19 d	Meningitis	Died	Yes	above	

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
France	above	Female (F)	1 kg	28	28 d	Necrotizing enterocolitis	Died	Yes	above	
France	above	Female (A)	Unknown	Unknown	Unknown	No details Trachael isolate of *E. sakazakii*	Unknown	Unknown	above	
France	above	Unknown (R)	Unknown	Unknown	Unknown	Bacteraemia	Died	Unknown	above	
France	above	Male (C)	1.38 kg	29	18 d	Asymptomatic	Recovered	Yes	above	
France	above	Male (N)	Unknown	Unknown	Unknown	Digestive problems	Recovered	Yes	above	
France	above	Male (P)	Unknown	Unknown	Unknown	Digestive problems	Recovered	Yes	above	
France	above	Female (G)	Unknown	Unknown	Unknown	No details Sputum isolate of E sak	Unknown	Unknown	above	
France	above	Male (L)	1.45 kg	33	6 d	Necrotizing enterocolitis	Recovered	Yes	above	
France	above	Male (Q)	1.6 kg	41	13 d	Asymptomatic	Recovered	Yes	above	
Brazil	Barreira et al., 2003	Female	2.65 kg	Term	14 d	Meningitis	Fatal		1 (0)	Breast feeding - vertical transmission hypothesized
USA (CA)	Brown and Bowen, 2006	Male	Unknown	Term	240 d	Bacteraemia	Recovered	Yes	1 (0)	2003 case report Opened can of formula and home environment were tested and negative for *E. sakazakii*
Hungary	Hungary, Food Safety Office, 2003	Unknown	Unknown	Unknown	6 m	Gastric sore and nasal secretion	Unknown	Unknown	1	Submitted in response to FAO/WHO Call for Data
USA (MN)	CDC, unpublished data, 2003	Unknown	Unknown	33	10 m	Bacteraemia/ meningitis	Unknown	Yes	1	Unknown whether environmental testing was done

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
USA (KY)	CDC, 2003	Male	1155 g	30	<1 m	Meningitis/ bacteraemia	Recovered	No	2(1)	Twins tested positive. Open can of formula and water used to prepare meal were tested and were negative for *E. sakazakii*
USA (KY)	above	Male	1361 g	30	<1 m	Meningitis/ bacteraemia	Died	Yes	above	Above. This infant also had *E. sakazakii* grown from nasopharyngeal culture.
USA (UT)	CDC, 2003	Unknown	1681 g	31	1 m	Bacteraemia Omphalocele, and more	Unknown	Yes	1	Open and unopened cans of formula tested negative for *E. sakazakii*
USA (TX)	CDC, 2003	Female	540 g	23.5	1 m	Bacteraemia	Recovered	Yes	1	Unable to trace production lot number for PIF. No product tested
USA (NC)	CDC, 2004	Male	3068 g	Term	<1 m	Meningitis/ Bacteraemia	Unknown, seizures	Yes	1	Fed 3 types of PIF and breast milk. One open can tested negative. Kitchen environment (sink) of home yielded *E. sakazakii* with PFGE pattern indistinguishable from patient isolate.
USA	Stoll, et al., 2004	Male	1.091 kg	28	12 d	Bacteraemia	Recovered	No	1 (0)	Fed with a mixture of RTD Formula mixed with mother milk - no PIF
France	Coignard, et al., 2006	Female	1.995 kg	36	6 d	Meningitis	Died	Yes	9(2)	2004 case. *E .sakazakii* detected in Pregestimil (consumed by 8 infants); 4 infants infected, 5 colonized without signs of infection; 4 hospitals involved.
France	above	Male	1.98 kg	35	8 d	Meningitis	Died	Yes	above	
France	above	Male	1.42 kg	30	26 d	Eye infection	Recovered	Yes	above	
France	above	Female	3.250	37	26 d	Diarrhea	Recovered	Yes	above	
New Zealand	Jarvis and Martone, 2004	Female	Unknown	Premature	Unknown	Meningitis	Died	Yes and breast milk	5 (1)	4 other infants colonized with no symptoms. 2 types of infant formula *E. sakazakii* positive. Same strain *E. sakazakii* from case and 4 other infants and 1 type of PIF.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Hungary	Hungary Food Safety Office, 2004	Unknown	Unknown	Unknown	2 m	Unknown Nasal and aural secretion	Unknown	Unknown	1	Submitted in response to FAO/WHO Call for Data
USA (UT)	Bowen and Braden, 2006	Female	3011	36	10 m	Bacteraemia, see comments	Recovered	Yes	1	Case occurred in 2004 Severe combined immunodeficiency disorder PIF orally and by continuous drip at night. Open can tested negative for *E. sakazakii*
USA (MN)	CDC, upublished data, 2005	Female	3550	39	8 m	Urine isolate, see comments	Unknown	Unknown	1	Anomaly of chromosome 17 Fed infant formula, unclear if PIF
USA (MN)	CDC, 2005	Male	3401	38	<1 m	Meningitis	Recovered Hydrocephalus, seizures, developmental delay	Yes	1	Open can did not yield *E. sakazakii*. Clinical isolates had 2 different PFGE patterns.
Hungary	Hungary Food Safety 2005	Unknown	Unknown	Unknown	3 m	Unknown Urine isolate	Unknown	Unknown	1	Submitted in response to FAO/WHO Call for Data
USA (LA)	CDC, 2006	Female	3518 g	39	<1 m	Meningitis	Recovered Brain infarct	Yes	1	Open can did not yield *E. sakazakii*
USA (sc)	CDC, 2006	Female	2557 g	37	<1 m	Meningitis/ bacteraemia	Recovered	Yes	1	Open can did not yield *E. sakazakii*
USA (TN)	CDC, 2006	Male	2160 g	37	1 m	Meningitis	Recovered Multiple brain abscesses	Yes	1	Two open cans of formula and environmentl samples from patient's home did not yield *E. sakazakii*

Country	Reference (date of report)	Gender	Weight at birth	Ges-tation (weeks)	Age at illness onset	Illness/Symptoms	Out-come	Pow-dered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Hungary	Hungary Food Safety Office 2006	Un-known	Un-known	Unknown	3 m	Unknown Urine, sore secretion	Unknown	Unknown	1	Submitted in response to FAO/WHO Call for Data
USA (IA)	CDC, 2007	Male	Un-known	28	<1 m	Bacteraemia/clinical meningitis with brain cyst	Brain cysts and hydro-cephalus, last known to be on life support >1 m after onset	Yes	1	Infant devlivered by cesarean section. No open product available for testing. Brest milk and environmental samples tested negative for *E. sakazakii.*
USA (AZ)	CDC, 2007	Male	Un-known	35	<1 m	Bacteraemia/ meningitis	Died	No	2(1)	Twin had *E. sakazakii* in stool; different subtype Twins delivered by cesarean section, and patient in NICU during onset. Breast milk and environmental samples were negative for *E. sakazakii*
USA (AZ)	above	Male	Un-known	35	<1 m	Isolated from stool	Recov-ered	No		Twin of case above. PFGE pattern of stool isolate differed from twin's isolate.
USA (VA)	CDC, 2007	Male	1818	36 Premature	<1 m	Bacteraemia	Recov-ered	Yes	1	CDC tested one opened and one unopened can of formula but did not find E. sakazakii. FDA tested sealed product of same lot; no *E. sakazakii* was found. Swab of kitchen counter where formula was prepared and area where bottled water was stored yielded *E. sakazakii*; other samples from patient home were negative.
USA (IL)	CDC, 2007	Male	1815	32	<1 m	Meningitis/ bacteraemia	Recov-ered	Yes	1, possibly 2	Twin had NEC and clinical meningitis with "suspicious brain lesions". Six samples of breast-milk fortifier and mother's milk tested negative for *E. sakazakii*

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
USA (IA)	CDC, 2007	Female	3714	41	1 m	Bacteraemia	Unknown	Yes	1	No open formula available, and production lot numbers were not known.
USA (GA)	CDC, 2007	Male	Unknown	29	1 m	Bacteraemia	Recovered	No	1	Infant had gastroschisis. Fed ready-to-eat formula and breast milk; neither yielded *E. sakazakii*
USA (GA)	CDC, 2007	Female	526 g	23	3 m	NEC Bacteraemia	Unknown	No	1	Infant had bowel perforation in weeks prior to bloodstream infection. Fed ready-to-eat formula. PFGE pattern of clinical isolate different from other GA case in 2007.
Canada	Health Canada Food Safety	Unknown	2.9 kg	Term	17 d	Meningitis	Recovered	Yes	1	Submitted in response to FAO/WHO Call for Data
India	Ray, et al. 2007	Female	1.4 kg	34	5 d	Meningitis	Died	Yes	1(1)	Case from 1992; Source assumed to be IF as infant on tube feeding - however no analyses performed
India	above	Female	Unknown	Unknown	60 d	Bacteraemia	Recovered	No	1	Case from 2006. Breast feeding - nosocomial infection assumed.
Spain	Aguirre Conde et al., 2007	Male	1.715 kg	31	5 d	Bacteraemia	Recovered	No	1	Infant fed with a mixture of mother milk and RTF formula
USA (MI)	CDC, unpublished data, 2007	Female	3438 g	40	13 m	Bacteraemia	Unknown	Yes	1	Kasabach-Merrit syndrome; recent chemotherapy Opened can yielded *E. sakazakii* isolate with same PFGE pattern as patient isolate. FDA tested 2 lots associated with patient and did not find *E. sakazakii*. Environmental samples from patient home and daycare provider did not yield *E. sakazakii*
USA (IA)	CDC unpublished data, 2008	Female	1875 g	37	<1 m	Meningitis? CSF isolation	Unknown	Yes	1	Infant delivered by cesarean section. Cultures of open cans and sealed formula and home did not yield *E. sakazakii*.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (weeks)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
USA (MD)	CDC unpublished data, 2008	Male	3289 g	37	<1 m	Meningitis? CSF isolation	Unknown Brain cysts	Yes	1	Infant delivered by cesarean section. Cultures of open and sealed cans of PIF did not yield *E. sakazakii.* However, investigators were not certain they had obtained the correct production lot.
USA (NE)	CDC, 2008	Male	Unknown	38	2 m	Isolated from tracheal aspirate	Unknown	Yes	1	Infant had cardiac defect. Open can of PIF did not yield *E. sakazakii.*
Japan	Japan Food Safety, 2008	Male	1269 g	27	22 d	Menigitis Brain abscesses	Recovered	Yes see comments	1	Formula given first few days, then breast milk Released from hospital at 85 d Submitted in response to FAO/WHO Call for Data

The following data is based on surveillance data from laboratories and, while cases are not described, it does indicate the age of patients and the site of isolation.

Country	Reference (date of report)	Gender	Weight at birth	Gestation (wk)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
England and Wales	UK Laboratory Summary 1992–2007	Unknown	Unknown	Unknown	<1 m	Isolated from blood and CSF			14	Submitted in response to FAO/WHO Call for Data 2008
England and Wales	UK Laboratory Summary 1997–2007	Unknown	Unknown	Unknown	1–11 m	Isolated from blood and CSF			18	Submitted in response to FAO/WHO Call for Data 2008
England and Wales	UK Laboratory Summary 1997–2007	Unknown	Unknown	Unknown	1–4 y	Isolated from blood and CSF			27	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	1 d	Isolated from blood			3	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	2–14 d	Isolated from blood (4) and urine (2)			6	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	1 m	Isolated from blood			3	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	2–6 m	Isolated from blood (2) and urine (1)			3	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	<1 y (exact age not given)	Isolated from blood (4) and umbilical cord (2)			6	Submitted in response to FAO/WHO Call for Data 2008

Country	Reference (date of report)	Gender	Weight at birth	Gestation (wk)	Age at illness onset	Illness/Symptoms	Outcome	Powdered formula	No. of cases (deaths)	Source of *E. sakazakii* & Comments
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	2 y	Isolated from blood (1) and urine (3)			4	Submitted in response to FAO/WHO Call for Data 2008
Philippines	Philippines Food Safety Authority 1998	Unknown	Unknown	Unknown	3 y	Isolated from blood (2) and urine (1)			3	Submitted in response to FAO/WHO Call for Data 2008

References for Annex 1

Adamson, D.H and Rogers, J.R. 1981. *Enterobacter sakazakii* meningitis with sepsis, *Clinical Microbiology Newsletter* 3. 19–20.

Aguirre Conde, A., Pérez Legorburu, A., Echániz Urcelay, I., Hernando Zárate, Z. & Arrate Zugazabeitia, J.K. 2007. Sepsis neonatal por *Enterobacter sakazakii* (Neonatal sepsis due to *Enterobacter sakazakii*). *Anales di Pediatria (Barcelona),* 66: 196–197.

Arseni, A., Malamou-Lada, H., Kostalos, C., Sta-I-Kou, A. & Koustia, H. 1984. A fatal case of sepsis in a premature newborn baby associated with *Enterobacter sakazakii* bacteremia. *Acta Microbiologica Hellenica*, 29: 402–407.

Arseni, A., Malamou-Ladas, E., Koutsia, C., Xanthou, M. & Trikka, E. 1987. Outbreak of colonization of neonates with *Enterobacter sakazakii*. *Journal of Hospital Infection*, 9(2): 143–150.

Bar-Oz, B., Preminger, A., Peleg, O., Block, C. & Arad, I. 2001. *Enterobacter sakazakii* infection in the newborn. *Acta Pædiatrica,* 90: 356–358.

Barriera, E.R., Costa de Souza, D., de Freitas Gois, P. & Fernandes, J.C. 2003. Miningite por *Enterobacter sakazakii* em recem-nascido: relato de caso. *Pediatria (Sao Paolo)*, 25(1/2): 65–70.

Biering, G., Karlsson, S., Clark, N.C., Jonsdottir, K.E., Ludvigsson, P. & Steingrimsson, O. 1989. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *Journal of Clinical Microbiology*, 27(9): 2054–2056.

Block, C., Peleg, O., Minster, N., Bar-Oz, B., Simhon, A., Arad, I. & Shapiro, M. 2002. *European Journal of Clinical Microbiology and Infectious Diseases*, 21:613–616.

Bowen, A. & Braden, C. 2006. Invasive *Enterobacter sakazakii* disease among infants. *Emerging Infectious Diseases*, 12(8): 1185–1189.

Burdette, J.H. & Santos, C. 2000. *Enterobacter sakazakii* brain abscess in the neonate: the importance of neuroradiologic imaging. *Pediatric Radiology*, 30: 33–34.

Caubilla-Barron, J., Hurrell, E., Townsend, S., Cheetham, P., Loc-Carillo, C., Fayet, O., Prere, M.-F. & Forsythe, S.J. 2007. Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *Journal of Clinical Microbiology*, 45(12): 3979–3985.

CDC [Centers for Disease Control and Prevention]. 2001. Information provided in response to the FAO/WHO Call for Data.

CDC. 2002. *Enterobacter sakazakii* infections associated with the use of powderedn infant formula – Tennessee, 2001. *MMWR–Morbidity and Mortality Weekly Report*, 51: 297–300. Reprinted in *Journal of the American Medical Association,* (2002) 287(17).

CDC, 2003. Information provided in response to the FAO/WHO Call for Data.

CDC, 2004. Information provided in response to the FAO/WHO Call for Data.

CDC, 2005. Information provided in response to the FAO/WHO Call for Data.

CDC, 2006. Information provided in response to the FAO/WHO Call for Data.

CDC, 2007. Information provided in response to the FAO/WHO Call for Data.

CDC, 2008. Information provided in response to the FAO/WHO Call for Data.

Chotpitayasunondh, T. 1994. Bacterial meningitis in children: etiology and clinical features, an 11-year review of 618 cases. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 25(1): 107–115.

Clark, N.C., Hill, B.C., O'Hara, C.M., Steingrimsson, O. & Cooksey, R.C. 1990. Epidemiologic typing of *Enterobacter sakazakii* in two neonatal nosocomial outbreaks. *Diagnostic Microbiology and Infectious Disease,* 13: 467–472.

Coignard, B., Vaillant, V., Vincent, J.-P., Lefleche, A., Mariani-Kurkdjian, P., Bernet, C., L'Heriteau, F., Senechal, H., Grimont, P., Bingen, E. &. Desenclos, J.-C. 2006. Infections sévéres é *Enterobacter sakazakii* chez des nouveau-nés ayant consommé une préparation en poudre pour nourrissons, France, octobre-décembre 2004. *Bulletin Epidemiologique Hebdomadaire,* 2–3/2006: 10–13. See: http://www.invs.sante.fr/beh/2006/02_03/beh_02_03_2006.pdf.

Gallagher, P.G. & Ball, W.S. 1991. Cerebral infarctions due to CNS infection with *Enterobacter sakazakii*. *Pediatric Radiology,* 21: 135–136.

Health Canada Food Safety. No date. Personal communication from Dr J. Farber, Health Canada.

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.

Food Safety Office, Hungary. 2003. Information provided in response to the FAO/WHO Call for Data.

Food Safety Office, Hungary. 2004. Information provided in response to the FAO/WHO Call for Data.

Food Safety Office, Hungary. 2005. Information provided in response to the FAO/WHO Call for Data.

Food Safety Office, Hungary. 2006. Information provided in response to the FAO/WHO Call for Data.

Japan Food Safety. 2008. Information received from the National Institute of Public Health in Japan in response to the FAO/WHO Call for Data.

Jarvis, W.R. & Martone, W.J. 1992. Predominant pathogens in hospital infections. *Journal of Antimicrobial Chemotherapy,* 29 (Suppl. A): 19–24.

Jøker, R.N., Norholm, T., & Siboni, K.E. 1965. A case of neonatal meningitis caused by a yellow Enterobacter. *Danish Medical Bulletin*, 12: 128–130.

Kleiman, M.B., Allen, S.D., Neal, P. & Reynolds, J. 1981. Meningocephalitis and compartmentalization of cerebral ventricles caused by *Enterobacter sakazakii*. *Journal of Clinical Microbiology*, 14(3): 352–354.

Lai, K.K. 2001. *Enterobacter sakazakii* infections among neonates, infants, children, and adults: case reports and a review of the literature. *Medicine (Baltimore),* 80: 113–122.

Lecour, H., Seara, A., Cordeiro, J. & Miranda, M. 1989. Treatment of childhood bacterial meningitis. *Infection*, 17: 343–346.

SCIEH [Scottish Centre for Infection and Environmental Health]. 1997. SCIEH Weekly Report 11.3.97.

Monroe, P.W. & Tift, W.L. 1979. Bacteremia associated with *Enterobacter sakazakii* (Yellow-pigmented *Enterobacter cloacae*). *Journal of Clinical Microbiology*, 10(6): 850–851.

Muytjens, H.L., Zanen, H.C., Sonderkamp, H.J., Kollee, L.A., Wachsmuth, I.K. & Farmer, J.J. III. 1983. Analysis of eight cases of neonatal meningitis and sepsis due to *Enterobacter sakazakii*. *Journal of Clinical Microbiology,* 18(1): 115–120.

Naqvi, S.H., Maxwell, M.A. & Dunkle, L.M. 1985. Cefotaxime therapy of neonatal Gram-negative bacillary meningitis. *Pediatric Infectious Disease*, 4(5): 499–502.

Noriega, F.R., Martin, M.A. & Scwalbe, R.S. 1990. Noscomial bacteremia caused by *Enterobacter sakazakii* and *Leuconostoc mesenteroides* resulting from extrinsic contamination of infant formula. *The Pediatric Infectious Disease Journal,* 9(6): 447–449.

Ray, P., Das, A., Gautam, V., Jain, N., Narang, A. & Sharma, M. 2007. *Enterobacter sakazakii* in infants: novel phenomenon in India. *Indian Journal of Medical Microbiology*, 25(4): 408–410.

Reina, J., Parras, F., Gil, J., Salva, F. & Alomar, P. 1989. Infecciones humanas por *Enterobacter sakazakii*. Consideraciones microbiologicas. *Enferm. Infecc. Microbiol. Clin.*, 7(3): 147–150.

Ries, M., Harms, D. & Scharf, J. 1994. Multiple cerebral infarctions in a premature baby with meningitis due to *Enterobacter sakazakii* leading to multicystic encephalomacia. *Klinische Padiatrie*, 206(3): 184–186.

Santos, M., Pessoa da Silva, C.L., Sampaio, J., Marangoni, D.V., Pinto, M. & Moreira, B.M. 2000. infection and control of *Enterobacter sakazakii* sepsis outbreak in four hospitals in Rio di Janiero, Brazil. *Infection Control and Hospital Epidemiology*, 21(2): 140.

Simmons, B.P., Gelfand, M.S., Haas, M., Metts, L. & Ferguson, J. 1989. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infection Control and Hospital Epidemiology*, 10: 398–401.

Smeets, L.C., Voss, A., Muytjens, H.L., Meis, J.F.G.M. & Melchers, W.J.G. 1998. Genetische karakterisatie van *Enterobacter sakazakii*-isolaten van Nederlandse patiënten met neonatale meningitis. *Ned. Tijdschr. Med. Microbiol.*, 6: 113–115.

Stoll, B.J., Hansen, N., Fanaroff, A.A. & Lemons, J. 2004. *Enterobacter sakazakii* is a rare cause of neonatal septicemia or meningitis in VLBW infants. *The Journal of Pediatrics*, 144: 821–823.

Tekkok, I., Baeesa, S., Higgins, M. & Ventureyra, E. 1996. Abscedation of posterior fossa dermoid cysts. *Child's Nervous System*, 12: 318–322.

Urmenyi, A.M.C. & White-Franklin, A. 1961. Neonatal death from pigmented coliform infection. *The Lancet*, 11 Feb. 1961: 313-315.

van Acker, J., de Smet, F., Muyldermans, G., Bougatef, A., Naessens, A. & Lauwers, S. 2001. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *Journal of Clinical Microbiology,* 39(1): 293–297.

Willis, J. & Robinson, J.E. 1988. *Enterobacter sakazakii* meningitis in neonates. *Pediatric Infectious Disease Journal*, 7(3): 196–199.

Annex 2

Data received in response to FAO/WHO Call for Data

Source	Information/Data received
Argentina – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
Austria (submitted by Maryse Arendt, Institute for Improvements around Birth of Initiativ Liewensufank, Luxembourg)	Säuglingsernährung heute 2006: Struktur- und Beratungsqualität an den Geburtenkliniken in Österreich; Ernährung von Säuglingen im ersten Lebensjahr (Infant Nutrition Today 2006: The quality of infrastructure and counselling services at birth clinics in Austria: Infant Nutrition in the first year of life)
Brazil – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
Nestlé – Dr JeanLouis Cordier	Specific answers to the questions posed in the call for data
Centre for Science in the Public Interest, USA	Infant and Young Child Feeding, Counselling: An Integrated Course
	The National Infant Feeding Survey 2005, Bolling, 2007
	NCT / UNICEF follow-on milk advertising survey topline results
	MORI survey: Women's knowledge of formula milk and follow on milk advertising.
	Guidance Notes on the Infant Formula and Follow-on Formula Regulations 2007: Revision 1 May 2008
	MINITeL Baby Food, Drinks and Milk, Market Intelligence, November 2007
	The National Childbirth Trust: IPSOS MORI omnibus data on formula milk brands–2007
	Specific answers to the questions posed in the call for data
Cuba – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
European Commission – Office of the Codex Contact Point	Scientific opinion of BIOHAZ Panel on the request from the Commission for review of the opinion on microbiological risks in infant formula and follow-on formula with regard to Enterobacteriaceae as indicators
	Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the microbiological risks in infant formula and follow-on formula.
European Food Safety Authority	Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs
Estonia - Health Protection Inspectorate	Specific answers to the questions posed in the call for data
France – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
	List of published *Enterobacter sakazakii* cases
Ghana - EatSafe Ghana (NGO)	Specific answers to the questions posed in the call for data
Guatemala – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
Hong Kong (China) – Food and Environmental Hygiene Department	Specific answers to the questions posed in the call for data
Hungary – Hungarian Food Safety Office	Disease Surveillance Information
International Formula Council (IFC)	Specific answers to the questions posed in the call for data
	List of published *Enterobacter sakazakii* cases
Ireland – Office of the Codex Contact Point, Food Safety Authority of Ireland	Specific answers to the questions posed in the call for data
Irish infant nutrition industry	IDACE –Best Practices in Powdered Formula Ingredient Manufacture
University College Dublin	Incidence of *Cronobacter* (*Enterobacter sakazakii*) in follow-up formula and infant drinks.

Source	Information/Data received
International special Dietary Foods Industry (ISDI)	Specific answers to the questions posed in the call for data Table on incidence and number of *E. sakazakii* infections in all age groups
Japan – National Institute of Public Health	Specific answers to the questions posed in the call for data
Jordan – Dr Reyad Shaker,	Research Papers:
Jordan University of Science and Technology	Effect of *Bifidobacterium breve* on the growth of *Enterobacter sakazakii* in rehydrated infant milk formula.
	Inactivation of *Enterobacter sakazakii* in Infant Milk Formula by Gamma Irradiation: Determination of D10-Value.
	Effects of Extended Dry Storage of Powdered Infant Milk Formula on Susceptibility of *Enterobacter sakazakii* to Hot Water and Ionizing Irradiation
	Isolation of *Enterobacter sakazakii* and other *Enterobacter* spp. from food and food production environments
	Detergent and Sanitizer Stresses Decrease the Thermal Resistance of *Enterobacter sakazakii* in Infant Milk Formula
Luxembourg – Direction de la Santé and Initiativ Liewensufank	Specific answers to the questions posed in the call for data
Malta – Department for Environmental Health	Specific answers to the questions posed in the call for data
	FOOD SAFETY ACT (CAP. 449), Infant Formulae and Follow-on Formulae, 2007
New Zealand – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
Dr Donald Campbell	Information sources and practices - preparation of Powdered infant formula in New Zealand - qualitative research (ERS)
Nicaragua – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
	Sistema Integrado de Vigilancia de Intervenciones Nutricionales (SIVIN) Informe de Progreso Nicaragua, 2003-2005 (Integrated surveillance system on nutrition Interventions: Progress Report Nicaragua 2003–2005)
Norway – The Norwegian Food Safety Authority	Specific answers to the questions posed in the call for data
Philippines – Office of the Codex Contact Point	Specific answers to the questions posed in the call for data
Antimicrobial Resistance Surveillance Programme and the National Statistics Office	Follow-up data on the surveillance programme and details of *E. sakazakii* isolates
	Follow-up data on national population statistics
Republic of Korea – Korea Food and Drug Administration	Specific answers to the questions posed in the call for data
Switzerland – Federal Office of Public Health	Specific answers to the questions posed in the call for data
Tunisia – INFOSAN Focal Point	Specific answers to the questions posed in the call for data
United Kingdom – Food Standards Agency	Specific answers to the questions posed in the call for data
Nottingham Trent University	Inter-laboratory Survey of *Cronobacter* in infant formulas and foods
United States of America – Centers for Disease Control and Prevention	Specific answers to the questions posed in the call for data
	Table of data on Infants and toddlers (<36 months old) with *Enterobacter sakazakii* isolated from blood, cerebrospinal fluid, urine or brain tissue and with illness onset between January 1998 and March 2008

FAO/WHO MICROBIOLOGICAL RISK ASSESSMENT SERIES

1 Risk assessments of *Salmonella* in eggs and broiler chickens: Interpretative Summary, 2002
2 Risk assessments of *Salmonella* in eggs and broiler chickens, 2002
3 Hazard characterization for pathogens in food and water: Guidelines, 2003
4 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Interpretative Summary, 2004
5 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Technical Report, 2004
6 *Enterobacter sakazakii* and microorganisms in powdered infant formula: Meeting Report, 2004
7 Exposure assessment of microbiological hazards in food: Guidelines, 2008
8 Risk assessment of *Vibrio vulnificus* in raw oysters: Interpretative Summary and Technical Report, 2005
9 Risk assessment of choleragenic *Vibrio cholerae* 01 and 0139 in warm-water shrimp in international trade: Interpretative Summary and Technical Report, 2005
10 *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report, 2006
11 Risk assessment of *Campylobacter* spp. in broiler chickens: Interpretative Summary, 2008
12 Risk assessment of *Campylobacter* spp. in broiler chickens: Technical Report, 2008
13 Viruses in food: Scientific Advice to Support Risk Management Activities: Meeting Report, 2008
14 Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report, 2008
15 *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula: Meeting Report, 2008