Enterobacter Sakazakii and Other Microorganisms in Powdered Infant Formula

CONTENTS

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DECLARATIONS OF INTEREST

Two out of the 16 experts which participated in this meeting declared an interest in the topics under consideration:

Dr Forsythe: His research is related to a commercially available tool for diagnosis of *Enterobacter sakazakii* in infant formula.

Dr Zwietering: His unit at the University of Wageningen performs scientific research which is financially supported by a company producing powdered infant formula.

FOREWORD

The 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 levels. Increasing food-borne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms 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 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 which 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 numbers of food-borne 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 from as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing all this data and information and to better understand 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 food-borne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment, particularly quantitative MRA, is recognized to be a resource-intensive task requiring a multidisciplinary approach. Yet food-borne illness is one of the most widespread public health problems, creating social and economic burdens as well as human suffering, making it 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 Food Quality and Standards Service, FAO, and the Food Safety Department, WHO, are the lead units responsible for this initiative. The two groups have worked together to develop the area of 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 have already from the present work clear indications 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

a_w	Water activity
BAM	Bacteriological Analytical Manual (USFDA)
BfR	Federal Institute for Risk Assessment (Germany)
BHI	Brain Heart Infusion
BPW	Buffered peptone water
BSI	Bloodstream infection
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
cfu	Colony forming unit
EE	*Enterobacteriaceae* enrichment
FAO	Food and Agriculture Organization of the United Nations
FSMP	Formula for special medical purposes
g	Gram(s)
GHP	Good hygienic practice
GMP	Good manufacturing practice
HACCP	Hazard Analysis Critical Control Point (System)
HEPA	High efficiency particle air
HIV	Human immunodeficiency virus
ISO	International Organization for Standardization
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LBW	Low birth weight
MPN	Most probable number
MRA	Microbiological risk assessment
NEC	Necrotizing enterocolitis
NGO	Non-governmental organization
PC	Contamination from the infant formula
PE	Contamination from the environment
PFGE	Pulsed field gel electrophoresis
PIF	Powdered infant formula
RAPD	Random amplified polymorphic DNA
RCP	Recommended Code of Practice
RIVM	National Institute of Public Health and the Environment (the Netherlands)
SIDS	Sudden infant death syndrome
TTC	Time to consumption
ULPA	Ultra low particle air
USFDA	United States Food and Drug Administration
VHPSS	Victorian Hospital Pathogen Surveillance Scheme
VLBW	Very low birth weight
VRBG	Violet red bile glucose
WHO	World Health Organization

EXECUTIVE SUMMARY

Consistent with the need to provide safe feeding for all infants,[1] the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly convened an expert meeting on *Enterobacter sakazakii* and other microorganisms in powdered infant formula (WHO, Geneva, 2-5 February 2004). The meeting was organized in response to a specific request to FAO/WHO for scientific advice from the Codex Committee on Food Hygiene to provide input for the revision of the Recommended International Code of Hygienic Practice for Foods for Infants and Children. It also aimed to provide pertinent information to the member countries of both organizations.

After reviewing the available scientific information, the expert meeting concluded that intrinsic contamination of powdered infant formula with *E. sakazakii* and *Salmonella* has been a cause of infection and illness in infants, including severe disease which can lead to serious developmental sequelae and death. No link has been established between illness and other microorganisms in powdered infant formula, although such a link was considered plausible for other Enterobacteriaceae.

E. sakazakii has caused disease in all age groups. From the age distribution of reported cases, it is deduced that infants (children <1 year) are at particular risk. Among infants, those at greatest risk for *E. sakazakii* infection are neonates (≤28 days), particularly pre-term infants, low-birth-weight infants or immunocompromised infants. Infants of HIV-positive mothers are also at risk, because they may specifically require infant formula and they may be more susceptible to infection.[2] This, and low birth weight, may be of particular concern for some developing countries where the proportion of such infants is higher than in developed countries.

It is important to note that powdered infant formula meeting current standards is not a sterile product and may occasionally contain pathogens. The meeting did not identify a feasible method, using current technology, to produce commercially sterile powders or completely eliminate the potential of contamination.

E. sakazakii is an opportunistic pathogen emerging as a public health concern. Little is known about its ecology, taxonomy, virulence and other characteristics. Recent data, however, point to differences in the microbial ecology of *Salmonella* and *E. sakazakii*.

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[1] As a global public health recommendation, infants should be exclusively breastfed for the first 6 months of life to achieve optimal growth, development and health. Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe complementary foods while breastfeeding continues until up to 2 years of age or beyond. Infants who are not breastfed require a suitable breastmilk substitute, for example an infant formula prepared in accordance with applicable Codex Alimentarius standards. Information provided in this connection to mothers and other family members who need to use it should include adequate instructions for appropriate preparation and the health hazards of inappropriate preparation and use (WHO, 2002).

[2] The UN guidance for these infants is that where replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding is recommended, and powdered infant formula may be an option. Some of these infants may be HIV-positive and thus immunocompromised.

Data from the infant food industry and national control authorities indicate that the detection of *Salmonella* in finished powdered infant formula is rare. The current Codex specification for *Salmonella* is the absence of organisms in 60 samples of 25 g each. *E. sakazakii* is more commonly found than *Salmonella* in the manufacturing environment, which is a potential source of post-heat-treatment contamination. Specific criteria for *E. sakazakii* are not included in the current Codex Code.

Even low levels of contamination of *E. sakazakii* in powdered infant formula were considered to be a risk factor, given the potential for multiplication during the preparation and holding time prior to consumption of reconstituted formula.

Based on a preliminary risk assessment, the inclusion of a lethal step at the point of preparation and a decrease in the holding and feeding times effectively reduced risk. A combination of intervention measures had the greatest impact.

Summary of recommendations

The expert meeting made recommendations to FAO, WHO, Codex, their member countries, NGOs and the scientific community. These are summarized below.

- In situations where infants are not breastfed, caregivers, particularly of infants at high risk, should be regularly alerted that powdered infant formula is not a sterile product and can be contaminated with pathogens that can cause serious illness; they should be provided with information that can reduce the risk.

- In situations where infants are not breastfed, caregivers of high-risk infants, should be encouraged to use, whenever possible and feasible, commercially sterile liquid formula or formula which has undergone an effective point-of-use decontamination procedure (e.g. use of boiling water to reconstitute or by heating reconstituted formula).[3]

- Guidelines should be developed for the preparation, use and handling of infant formula to minimize risk.

- The infant food industry should be encouraged to develop a greater range of commercially sterile alternative formula products for high-risk groups.

- The infant food industry should be encouraged to reduce the concentration and prevalence of *E. sakazakii* in both the manufacturing environment and powdered infant formula. To this end, the infant food industry should consider implementing an effective environmental monitoring programme and the use of Enterobacteriaceae rather than coliform testing as an indicator of hygienic control in factory production lines.

[3] Nutritional and other factors need to be considered, e.g. alteration of nutritional content, risk from burns due to handling boiling or hot water/formula, and potential for germination of bacterial spores. The formula should thereafter be cooled and handled appropriately.

- In revising its code of practice, Codex should better address the microbiological risks of powdered infant formula and, if deemed necessary, include the establishment of appropriate microbiological specifications for *E. sakazakii* in powdered infant formula.

- FAO/WHO should address the particular needs of some developing countries and establish effective measures to minimize risk in situations where breastmilk substitutes may be used in exceptionally difficult circumstances, e.g. feeding infants of HIV-positive mothers or low-birth-weight infants.

- The use of internationally validated detection and molecular typing methods for *E. sakazakii* and other relevant microorganisms should be promoted.

- Investigation and reporting of sources and vehicles, including powdered infant formula, of infection by *E. sakazakii* and other Enterobacteriaceae should be encouraged. This could include the establishment of a laboratory-based network.

- Research should be promoted to gain a better understanding of the ecology, taxonomy, virulence and other characteristics of *E. sakazakii* and of ways to reduce its levels in reconstituted powdered infant formula.

1. INTRODUCTION

Consistent with the need to provide safe feeding for all infants,[1] the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened a meeting on *Enterobacter sakazakii* and other microorganisms in powdered infant formula in WHO headquarters, Geneva, Switzerland from 2 to 5 February 2004. This meeting was part of the FAO/WHO activities on the provision of scientific advice to Codex and to their member countries and was convened in response to a specific request for scientific advice on this issue from the Codex Committee on Food Hygiene (CCFH). The meeting was chaired by Dr John Cowden, Scottish Centre for Infection and Environmental Health. Dr Martin Cole, Food Science Australia served as rapporteur. A total of 16 experts from 12 countries participated in the meeting in their independent capacities and not as representatives of their governments, employers or institutions. There were also two representatives from the infant formula industry for the general exchange of information only; they did not participate in the final drafting of conclusions or recommendations.

The meeting was supported by a number of background papers on the epidemiological and microbiological issues related to microorganisms in powdered infant formula, industry practices in the production of these products, the variety of products and their preparation for consumption (Appendix A). Prior to the meeting, a short electronic discussion group was organized to consider possible approaches for assessing the risk posed by pathogens in powdered infant formula; the outcome of this discussion acted as support material to this meeting. The electronic discussion was convened by the Director of the WHO Collaborating Centre for Risk Assessment of Pathogens in Food and Water in the Netherlands and included meeting participants with experience in that area. A number of other papers and relevant data submitted in response to an FAO/WHO public call for data were also considered during the deliberations of the meeting (Appendix B).

1.1 BACKGROUND AND OBJECTIVES

The issue of pathogens and in particular *E. sakazakii* in infant formula was brought to the attention of the 35th session of the Codex Committee on Food Hygiene (CCFH) by the United States of America. In raising this issue the United States had also prepared a risk profile of *E. sakazakii* in powdered infant formula for consideration by the committee. At the same time the 24th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) requested that the CCFH revise the Recommended International Code of Hygienic Practices for Foods for Infants and Children (CAC, 1979) in order to address concerns raised by pathogens that may be

[1] As a global public health recommendation, infants should be exclusively breastfed for the first 6 months of life to achieve optimal growth, development and health. Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe complementary foods while breastfeeding continues until up to 2 years of age or beyond. Infants who are not breastfed require a suitable breastmilk substitute, for example an infant formula prepared in accordance with applicable Codex Alimentarius standards. Information provided in this connection to mothers and other family members who need to use it should include adequate instructions for appropriate preparation and information concerning the health hazards of inappropriate preparation and use (WHO, 2002).

present in infant formula. As a result, the 35th session of the CCFH established a drafting group to initiate the revision of this code. The committee noted that as well as *E. sakazakii* there were a number of other pathogens of concern that may be present in powdered infant formula, such as *Clostridium botulinum*, *Staphylococcus aureus* and other Enterobacteriaceae that may need to be considered when revising the code. In reviewing the risk profile on *E. sakazakii* in powdered infant formula, the committee was of the opinion that it could be improved by incorporating information from other sources such as industry. However, it was also recognized that there are still many data gaps in relation to *E. sakazakii* in powdered infant formula. The committee thus requested FAO and WHO to convene at the earliest opportunity an expert meeting on pathogens of concern in powdered infant formula, including *Enterobacter sakazakii* and *Clostridium botulinum*.

In the interim, the risk profile prepared for the CCFH[2] has been updated to include additional information. It provides background information on the epidemiology of food safety concerns associated with powdered infant formula. In addition to *E. sakazakii*, it addresses other Enterobacteriaceae, including *Salmonella*, as well as staphylococci and clostridia. Consideration is also given to the manufacture and use of powdered infant formula. The unique issues associated with underweight infants and infants in neonatal intensive care units are outlined. Other issues that are raised include the use of powdered infant formula for babies born to HIV-positive mothers and those issues facing developing countries, such as the availability of potable water.

Canada is leading the drafting group working on the revision of the Codex Code of Hygienic Practices for Foods for Infants and Children (CAC, 1979). This Codex drafting group is examining the code to determine its adequacy, to establish the need for additional guidance and to identify specific issues related to powdered infant formula that should be included. This includes identifying the most relevant pathogenic and opportunistic microorganisms and providing guidance on these within the code.

The objective of the requested expert meeting was not clearly defined by the CCFH, although the need for additional information on *E. sakazakii* was made clear. However, as *E. sakazakii* and other microorganisms consumed in powdered infant formula affect a susceptible population group in neonates and infants and can cause severe and life-threatening conditions, there is a need to manage this risk. In keeping with the principles recommended for the effective interaction between risk managers and risk assessors (FAO/WHO, 2002), FAO and WHO liaised with the two aforementioned groups to ensure complementarity to the ongoing work and to assure that they provided the required scientific advice.

The objectives of the meeting were the following:

- Review the available scientific information on the human health consequences of ingesting microorganisms of concern in powdered infant formula and information on those microorganisms (including occurrence in formula); briefly review current production, distribution and preparation systems for powdered infant formula; prepare a report on the state of the science, including the public health impact, current control measures and significant gaps in the knowledge base.

[2] Currently available at ftp://ftp.fao.org/codex/ccfh36/fh04_12e.pdf.

- Determine approaches which could be used to evaluate the risk associated with microorganisms of concern in powdered infant formula and to reduce the risk.

- Using an agreed approach, analyse the efficacy of current practices with regard to public health protection and identify potential risk reduction options (evaluate and compare efficacy if and where possible).

This report summarizes the deliberations, findings and conclusions of the meeting.

2. EPIDEMIOLOGY AND PUBLIC HEALTH ASPECTS

2.1 ORGANISMS OF CONCERN

2.1.1 *Enterobacter sakazakii*

Enterobacter sakazakii is a gram-negative, non-spore-forming bacterium belonging to the Enterobacteriaceae family. On occasion, it has been associated with sporadic cases or small outbreaks of sepsis, meningitis, cerebritis and necrotizing enterocolitis. While *E. sakazakii* has caused disease in all age groups, the focus of this meeting was on cases that are reported in infants under 28 days old. Although incomplete, the published data on these infants indicate that approximately half of them had a birth weight of less than 2 000 g, and two-thirds were premature, being born at less than 37 weeks gestation. It is also likely that immunocompromised or medically debilitated infants are more susceptible to infections with *E. sakazakii*. The pattern of disease in term infants is less clear, with some having a major congenital abnormality (e.g. neural tube defects and Trisomy 21 [Down syndrome]), while others have no reported evidence of a compromised host defence, yet have been afflicted with *E. sakazakii* sepsis or meningitis (Lai, 2001). *E. sakazakii* bacteraemia has also been identified among older infants and infants at home (CDC, unpublished data). In addition, asymptomatic infants have been identified with *E. sakazakii* in their stools or urine (Biering et al., 1989; CDC, 2002; Block et al., 2002) and stool carriage has been demonstrated for up to 18 weeks (Block et al., 2002).

Mortality rates from *E. sakazakii* infection have been reported to be as high as 50 percent or more, but this figure has declined to under 20 percent in recent years. Significant morbidity in the form of neurological deficits can result from infection, especially among those with bacterial meningitis and cerebritis. While the disease is usually responsive to antibiotic therapy, a number of authors have reported increasing antibiotic resistance to drugs commonly used for initial treatment of suspected *Enterobacter* infection. Reports have also been made of β-lactamases and cephalosporinases from *E. sakazakii* (Pitout et al., 1997). Long-term neurologic sequelae are well recognized (Lai, 2001; Clark et al., 1990).

While the reservoir for *E. sakazakii* is unknown in many cases, a growing number of reports have established powdered infant formula as the source and vehicle of infection (Biering et al., 1989; Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). In several investigations of outbreaks of *E. sakazakii* infection that occurred among neonates in neonatal intensive care units, investigators were able to show both statistical and microbiological association between infection and powdered infant formula consumption (Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). These investigations included cohort studies which implicated infant formula consumed by the infected infants. In addition, there was no evidence of infant-to-infant or environmental transmission; all cases had consumed the implicated formula (Simmons et al., 1989; Van Acker et al., 2001; CDC, 2002). The formula consumed by infected infants in each of these outbreaks yielded *E. sakazakii*; in two outbreaks, formula from previously unopened cans from the same

manufacturing batch also yielded *E. sakazakii*. A combination of typing methods (plasmid analysis, antibiograms, chromosomal restriction fragment analysis, ribotyping, multilocus enzyme electrophoresis) were used to evaluate the isolates from each outbreak as to their relatedness. Though the typing methods differed, the isolates among cases and those obtained from the implicated formula shared the same typing pattern in each of these investigations.

In addition, the stomach of newborns, especially of premature babies, is less acidic than that of adults: a possible important factor contributing to the survival of an infection with *E. sakazakii* in infants. The frequency of intrinsic *E. sakazakii* contamination in powdered infant formula is of concern, even though intrinsic concentration levels of *E. sakazakii* appear to be typically very low.

In a study of the prevalence of *E. sakazakii* contamination in 141 powdered infant formulas, 20 were found culture-positive, yet all met the microbiological specifications for coliform counts in powdered infant formula (<3 cfu/g) of the current Codex code (Van Acker et al., 2001; Muytjens, Roelofs-Willemse, and Jasper, 1988). Such formula has been linked to outbreaks (Van Acker et al., 2001). Furthermore, outbreaks have occurred in which the investigators have failed to identify lapses in formula preparation procedures (Van Acker et al., 2001; CDC, 2002). Thus, it seems that neither high levels of contamination nor lapses in preparation hygiene are necessary to cause infection from *E. sakazakii* in powdered infant formula. While it can be assumed that lapses in preparation hygiene or extended holding at non-refrigerated temperatures could lead to increases in the levels of contamination at the time of consumption, it is not possible to assess the contribution that these factors have on the cases of infection that have been associated with powdered infant formula that contained low levels of *E. sakazakii*. Thus it must be currently assumed that low levels of *E. sakazakii* in infant formula (<3 cfu/100 g) can lead to infections.

Formula preparation equipment contaminated by *E. sakazakii* has been demonstrated to have caused two outbreaks (Noriega et al., 1990; Block et al., 2002), but the original source of *E. sakazakii* was not determined in either case. Environmental swabbing of formula preparation areas in the course of outbreak investigations has not demonstrated *E. sakazakii* in the general environment. *E. sakazakii* has been identified in the environments of milk powder production facilities and other food production facilities, as well as in households (Kandhai, Reij, and Gorris, 2004). Not all infants with *E. sakazakii* infection have been exposed to powdered infant formula, and *E. sakazakii* infections can also occur in adults (Lai, 2001). Thus, although an environmental source of *E. sakazakii* infection other than infant formula has not been strictly identified, other sources undoubtedly exist. The relative contribution of powdered infant formula sources and other sources to the burden of *E. sakazakii* disease is unknown.

There is very little known about virulence factors and pathogenicity of *E. sakazakii*. The work done by Pagotto et al. (2003) was the first describing putative virulence factors for *E. sakazakii*. Enterotoxin-like compounds were produced by some strains. Using tissue cultures, some strains produced a cytotoxic effect. Two strains (out of 18 isolates) were capable of causing death in suckling mice by the peroral route. Therefore, there appear to be differences in virulence among *E. sakazakii* strains, and some strains may be non-pathogenic. Brain abscesses due to *E. sakazakii* and the related bacterium (*Citrobacter koseri*) are morphologically similar and may be due to similar virulence mechanisms (Kline, 1988).

2.1.2 Other relevant organisms of concern

Although liquid, ready-to-feed infant formula is commercially sterile, powdered infant formula is not. Enterobacteriaceae were present in 52 percent of 141 different formulas from 35 countries in one 1988 study (Muytjens, Roelofs-Willemse, and Jasper, 1988). Enterobacteriaceae are also common aetiologies for systemic infection in neonates and, to a lesser extent, older infants. *E. sakazakii* may be a sentinel organism, receiving attention due to its relative rarity. Other Enterobacteriaceae from powdered infant formula may also be responsible for systemic infections in infants, but there is little reported information to determine their role. One outbreak of *Citrobacter freundii* infections in a neonatal intensive care unit did identify formula as the vehicle of infection, though it was unclear if the formula was intrinsically (the source) or extrinsically contaminated.

Salmonella contamination of infant formula has been responsible for multiple outbreaks (Picket and Agate, 1967; Rowe et al., 1987; CDC, 1993; Usera et al., 1996; Threlfall et al., 1998; Olsen et al., 2001; Bornemann et al., 2002). Similar to *E. sakazakii*, low-level intrinsic contamination of powdered infant formula with *Salmonella* was epidemiologically and microbiologically associated with infections in infants in these outbreaks. Rates of salmonellosis are also highest in infants compared to any other age group (Olsen et al., 2001). The factors that give newborns a relatively high risk of infection include the relative gastric achlorhydria, the buffering capacity of the milk, the use of high iron infant formula and the need for frequent diaper changing (Miller and Pegues, 2000). Unlike *E. sakazakii* and other Enterobacteriaceae, however, *Salmonella* is rarely found in surveys of powdered infant formula. In the study surveying 141 different formulas by Muytjens, Roelofs-Willemse, and Jasper (1988), no samples yielded *Salmonella*.

Powdered infant formula has never been convincingly identified as a vehicle or source of infection for sporadic cases as opposed to outbreaks of infection with *Salmonella*, but this may well be due to the greater difficulty of identifying vehicles for sporadic infection. It would be illogical to conclude that sporadic infection due to powdered infant formula never occurred, but its frequency is unknown. Ongoing, large, sporadic case-control studies in the United States will be valuable in determining any potential association between significant numbers of sporadic salmonellosis cases and powdered infant formula.

2.2 SCOPE/CASE DESCRIPTION

The meeting considered illnesses in infants (i.e. children <1 year) linked to microorganisms (or their toxins) associated with powdered infant formula either epidemiologically or microbiologically.

2.2.1 Identification of products considered

The products under consideration were those in powdered form, specially manufactured and presented to be used by infants, either as a breastmilk substitute after preparation with water, or to modify prepared breastmilk substitutes or fortify human milk. Included products are, therefore, infant formula (as defined in Codex Stan 72-1981[1]), follow-up formula (as defined in Codex Stan

[1] Available at: ftp://ftp.fao.org/codex/standard/en/CXS_072e.pdf.

156-1987[2]), formula for special medical purposes intended for infants (as defined in Appendix V of Codex Alinorm 04/27/26[3]), formulas for special medical purposes for the partial feeding of infants (covered by Codex Stan 180-1991[4]) and human milk fortifiers.

Breastmilk substitutes are needed when infants do not have access to breastmilk for various reasons. Commercial infant formulas are usually used as breastmilk substitutes for normal healthy infants under 6 months, and are formulated industrially in accordance with the appropriate Codex standards. Formulas for special medical purposes intended for infants are breastmilk substitutes for sick infants (patients). Although other milks may be used after 6 months, follow-up formulas can substitute for breastmilk in the older infant who also eats complementary food. These three types of formula only need the addition of water to be ready for consumption and, therefore, are often also available in liquid, ready-to-feed form. However, formulas for special medical purposes for the partial feeding of infants require the addition of measured amounts of other foods, quite often also in powdered form, to satisfy the special nutritional needs of the individual infant patient.

Human milk fortifiers are powdered supplements which can be added to expressed human milk when the nutritional requirements of low-birth-weight infants (<2 500 g) and especially very low-birth-weight infants (<1 500 kg) are not satisfied by human milk alone. This can include thickening agents, such as starches or simple cereals which are specially manufactured for the purpose of increasing the consistency of the liquid food, and can be added to formula intended for infants with gastro-oesophageal reflux.

Throughout the following report, the term "powdered infant formula" includes all of the products mentioned above, but excludes cereals.

2.2.2 Case definition

The meeting considered illnesses in infants (i.e. children <1 year) due to microorganisms (or their toxins) associated with powdered infant formula consumption either epidemiologically or microbiologically. Figure 1 provides a graphic account of the issues included and excluded in the scope of the work. The area of intersection of all three circles, i.e. area 7, represents the scope of the meeting.

It should be noted that certain illnesses in infants caused by powdered infant formula may have been excluded, including those for which no microorganism has been reliably identified (area 5 in Figure 1) and those for which the microorganism has not been reliably associated with powdered infant formula (area 4 in Figure 1). In addition, bacteria detected in infant formula were not considered by the meeting if there was no evidence that these bacteria are associated with illness in infants (area 6 in Figure 1).

[2] Available at: ftp://ftp.fao.org/codex/standard/en/CXS_156e.pdf.
[3] Available at: ftp://ftp.fao.org/codex/alinorm04/al04_26e.pdf.
[4] Available at: ftp://ftp.fao.org/codex/standard/en/CXS_180e.pdf.

Young children (i.e. >1 year) consume powdered formula and may experience illness associated with the microbiological contamination of powdered formula. However, these illnesses were not considered by the meeting, as it was believed that the spectrum of illness could be adequately represented by infants under 1 year (and subpopulations of infants <1 year) and these same groups were considered to be the populations most at risk.

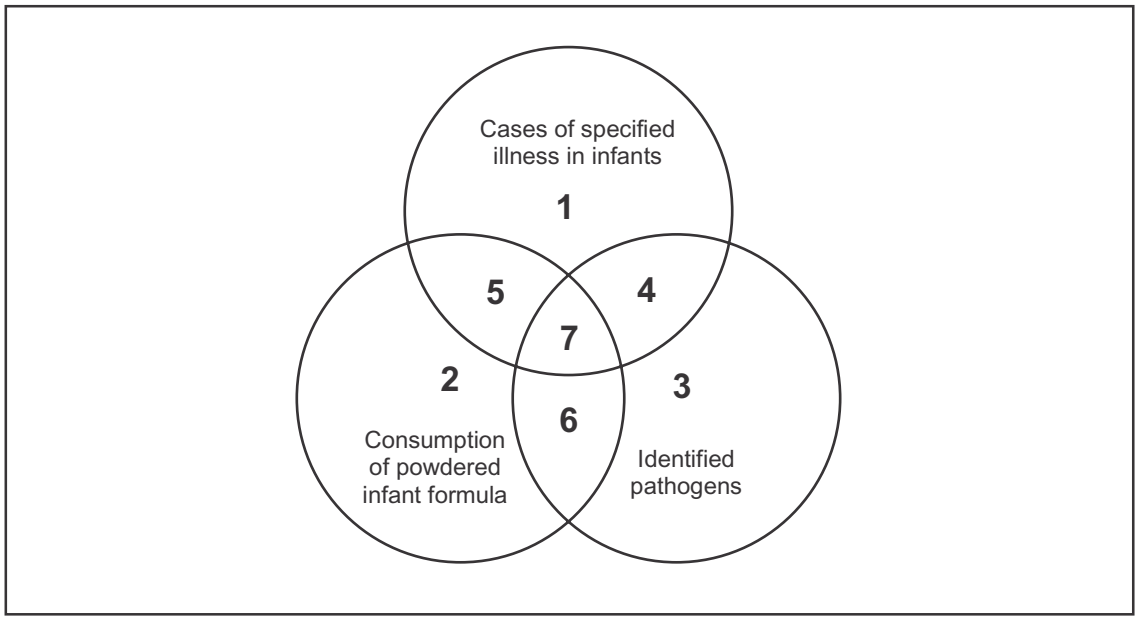

Figure 1. Graphic representation of the issues considered in defining the scope of the meeting.
1. Cases of illness in infants
2. Consumption of powdered infant formula
3. Identified pathogens
4. Illness in infants caused by a specific microorganism (powdered infant formula not related to illness)
5. Illness in infants associated with consumption of powdered infant formula (microorganism unknown)
6. Specific microorganisms in powdered infant formula but not causing illness
7. Specific microorganisms in powdered infant formula that resulted in cases of illness in infants = scope

3. HAZARD IDENTIFICATION

The microorganisms or microbial toxins of concern with powdered infant formula, and the strength of the evidence of a causal association between their presence in powdered infant formula and illness in infants, were categorized as follows:

3.1 CATEGORY "A" ORGANISMS – CLEAR EVIDENCE OF CAUSALITY

Enterobacter sakazakii and *Salmonella enterica* are in category "A" because both are well-established causes of illness in infants (e.g. systemic infection, necrotizing enterocolitis [NEC] and severe diarrhoea), and they have been found in powdered infant formula. Contaminated powdered infant formula has been convincingly shown, both epidemiologically and microbiologically, to be the vehicle and source of infection in infants.

The presence of *E. sakazakii* in powdered infant formula (and its association with illness in infants) is more likely than other Enterobacteriaceae or other *Enterobacter* species to be detected, because of the paucity of other vehicles or modes of transmission for *E. sakazakii* in this age group, and because it is facilitated by the use of molecular fingerprinting detection techniques. In other words, there may in fact be more instances of powdered infant formula-borne infection with Enterobacteriaceae than with *E. sakazakii*, but the former elude detection. Although there are clearly some differences in the microbial ecology of *S. enterica* and *E. sakazakii*, many of the risk-reduction strategies aimed at controlling *E. sakazakii* are also likely to control other *Enterobateriaceae*, especially other *Enterobacter* species.

3.2 CATEGORY "B" ORGANISMS – CAUSALITY PLAUSIBLE, BUT NOT YET DEMONSTRATED

Other Enterobacteriaceae are in category "B" because they are well-established causes of illness in infants (e.g. systemic infection, NEC and severe diarrhoea) and have been found in powdered infant formula, but contaminated powdered infant formula has not been convincingly shown, either epidemiologically or microbiologically, to be the vehicle and source of infection in infants. These organisms include, for example: *Pantoea agglomerans* and *Escherichia vulneris* (both formally known as *Enterobacter agglomerans*), *Hafnia alvei*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *C. freundii*, *Klebsiella oxytoca* and *Enterobacter cloacae*.

These organisms are increasing in importance as neonatal pathogens and, being Enterobacteriaceae (known to be present in low levels in powdered infant formula), are potential candidates as powdered infant formula-borne pathogens. For example, infant formula has been implicated as the vehicle of infection in an outbreak of *C. freundii* infection (Thurm and Gericke, 1994). In this event, however, it was not shown how the feed became contaminated.

3.3 CATEGORY "C" ORGANISMS – CAUSALITY LESS PLAUSIBLE OR NOT YET DEMONSTRATED

Other microorganisms are in category "C", either because, despite causing illness in infants (e.g. systemic infection, NEC and severe diarrhoea), they have not been identified in powdered infant formula, or, although having been identified in powdered infant formula, they have not been implicated as causing such illness in infants. These organisms include *Bacillus cereus, Clostridium difficile, C. perfringens, C. botulinum, Staphylococcus aureus* and *Listeria monocytogenes*.

Bacillus cereus, a spore-forming gram-positive rod commonly found in the environment, is an acknowledged enteropathogen. Enterotoxigenic *B. cereus* has been isolated from reconstituted milk-based formula (Rowan and Anderson, 1998). Although one confirmed common source outbreak associated with infant formula has been reported in Chile (Cohen et al., 1984), no evidence of intrinsic contamination of the infant formula with *B. cereus* was provided. Thus, a causal association between powdered infant formula and *B. cereus* infection was not demonstrated.

Clostridium difficile is a frequent colonizer of newborns, usually without clinical manifestations. One study, sparked by the finding of stools positive for *C. difficile* in two infants dying of sudden infant death syndrome (SIDS), showed significantly greater colonization of newborns fed on formula than breastfed infants (Cooperstock et al., 1982). However, no direct link with powdered infant formula was established.

4. HAZARD CHARACTERIZATION

4.1 POPULATIONS AT RISK

While *E. sakazakii* has caused disease in all age groups, on the basis of the age distribution of reported cases, it was deduced that the group at particular risk is infants (i.e. children <1 year). Among infants, those who are immunocompromised and neonates (≤28 days) are considered to be at greatest risk, particularly neonates of low birth weight (<2 500 g according to WHO [1994]). Infants of HIV-positive mothers are also of concern, because they may specifically require infant formula[1] and they may be more susceptible to infection.

The United States FoodNet 2002 survey (C. Braden, personal communication, 2004) estimated that the rate of *E. sakazakii* infection among infants (based on isolation of the organism from sterile sites only) was 1 per 100 000, whereas the rate among low-birth-weight neonates was 8.7 per 100 000.

A review of cases in infants (including from outbreak investigations) reported in English-language literature from 1961 to 2003 found that 25 of 48 cases (i.e. 52%) of *E. sakazakii*-induced illness were amongst infants of low birth weight. While the increased risk cannot be firmly established from these data, it does strongly support the conclusion that low-birth-weight neonates are a high risk group for *E. sakazakii*-induced illness.

A common observation is that the age of patients with salmonellosis is distributed according to a bimodal distribution with peaks in children and the elderly. The reasons for a relative excess of cases in the very young include increased susceptibility upon first exposure or the increased likelihood of medical care being sought for the very young, and they may also be more likely to be tested than other age groups. Whatever their susceptibility to infection, once infected, infants (particularly medically immunocompromised infants) are more likely to suffer severe consequences or death from salmonellosis. Infants who are breastfed are less likely to become infected by *Salmonella*. In a case-control study to identify risk factors for sporadic salmonellosis, case patients were 44.5 times more likely to have a liquid diet containing no breastmilk and 13.2 times more likely to reside in a household where a member had diarrhoea (Rowe et al., 2004).

4.2 DOSE-RESPONSE

Due to the limited information available on *E. sakazakii*, particularly in terms of the number of organisms that ill patients were exposed to, it was not possible to develop a dose-response curve for this pathogen. For the purpose of undertaking a risk assessment, a fail-safe estimate of the infectivity per organism (r value of the exponential model) based on some estimate values can be

[1] The UN guidance for these infants is that where replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding is recommended, and powdered infant formula may be an option.

made, assuming that all infected servings contain only one organism. Since growth will occur, this will always be a fail-safe value and the real value will be an even lower number.

In the Netherlands, ten cases of *E. sakazakii* infections in infants were reported over 40 years (1). A total of 12 500 babies are born per million people in the country per year. Of these babies, 2% are assumed to be born with a birth weight below 2 000 g. This means 250 babies of 2 000 g or less are born per million people per year. It should be noted that these assumptions were made based on the specific information available in one particular country and that the number of low-birth-weight babies will vary from one country to another. Also, low-birth-weight babies are generally considered to be of 2 500 g or less.

Because powdered infant formula is a source of nutrition for many infants at risk, a very large number of servings are consumed. Thus, there is a small possibility that even one or a few organisms in reconstituted powdered infant formula could cause illness. It was assumed that 300 feedings are provided to a baby in 1 month, and that within the baby's life the period of risk is 1 month. At the population level, where it is estimated that 250 babies per million people per year weigh 2 000 g or less, 75 000 feeds (250 babies × 300 feeds) are consumed. In a 40-year period in the Netherlands, at the population level, 45 000 000 feeds were consumed by babies of 2 000 g, i.e. 75 000 (feeds per year per million people) × 40 (no. of years) × 15 (million population).

The probability (P) of infection is equal to the number of cases/number of exposures to one organism. For the approximation of the dose-response relation at low doses, P = rD, where r is the infectivity per organism and D is the dose or the amount of organism ingested. Since it is assumed that all infected servings just contain one organism, D = 1. Therefore P = r*1, which is equal to the number of cases/number of exposures to one organism. Thus r = number of cases/number of exposures to one organism. If the probability of one organism being present in the feed is 0.025 (prevalence), the number of contaminated feedings per 40 years to babies of 2 000 g or less in this country, i.e. the number of exposures to one organism, is estimated as 45 000 000 × 0.025 = 1 125 000 (2). As indicated earlier, the number of cases in this country in 40 years is 10 (1). Then r is calculated as follows:

$$(1)/(2) = 10/1\ 125\ 000 = 8.9 \times 10^{-6}$$

This value is based on a calculation using selected values such that the infectivity is overestimated. If the dose is less than 10 000, it can be assumed that "1 -exp(rD) = (-rD)" and the effect of dose is in the linear range.

Pagotto et al. (2003) found only 2 out of 18 strains to be lethally infectious to suckling mice by the oral route, with only 1 of 4 mice dead at a dose of 10^7 cfu/mouse. This results (for the infective strains) in an r value of 2.5×10^{-8} (0.25×10^{-7}), meaning that all doses below 10^7 will probably give linear behaviour.

Based on these two estimates, it can be concluded that the best guess is that the dose-response relation at low doses is linear, but the basis of these two numbers is clearly not strong enough to conclude a value of the dose-response parameter relevant for human neonates.

5. EXPOSURE ASSESSMENT

5.1 EXPOSURE TO INFANT FORMULA/BREASTFEEDING RATES

It is impossible to estimate on a global basis the percentage of all infants who receive one of the products under consideration. This is due, on the one hand to the variable rates of breastfeeding in different populations and, on the other hand to the availability of the products in different parts of the world.

Exclusive breastfeeding rates differ from one country to the next. In Scandinavian countries, for example, 95% of babies are breastfed shortly after birth with almost 75% of those still being breastfed at 6 months of age. In other European countries, initial breastfeeding rates are below 30%, decreasing to almost no exclusive breastfeeding at 6 months. Available data on rates and exclusivity of breastfeeding from Australia in 1995 (Donath and Amir, 2000) and from Germany in 1997/98 (Kersting and Dulon, 2002) allow an estimate of the percentage of infants at different ages exposed to infant formula (Table 1).

Infant formula may be a direct source, an indirect source (contributing to a reservoir of *E. sakazakii* in the environment), and/or a vehicle for *E. sakazakii*-induced illness; it may also be neither the source nor the vehicle for *E. sakazakii*-induced illness. The meeting considered, on the basis of the information available, that in between 50 and 80% of cases, powdered infant formula is both the vehicle and the source (direct or indirect) of *E. sakazakii*-induced illness. To estimate a range of the proportion of cases due to powdered infant formula versus some other source, the meeting considered data from the United States in 2003 (C. Braden, personal communication, 2004). For sporadic cases of *E. sakazakii* sepsis and meningitis, six out of seven cases had exposure to powdered infant formula; the exposure was unclear in the remaining case. Thus, for at least 85% of these cases, powdered infant formula was a potential source. A review of 48 *E. sakazakii* cases in English language literature since 1961 revealed that at least 25 cases (52%) were directly linked to powdered infant formula.

Table 1. Estimated percentage of (healthy term) infants exposed to powdered infant formula or follow-up formula in Australia and Germany.

Age	Australia, 1995 (Donath and Amir, 2000) n = 2 874	Germany, 1997/98 (Kersting and Dulon, 2002) n = 1 717
1 month	29%	-
2 months	-	42%
3 months	40%	-
4 months	-	51%
6 months	57%	61%

Reconstituted powdered infant formula is probably a common vehicle in transmitting *Salmonella* to infants, given its major role in the infant diet, but contamination of formula is more likely to occur from the preparer or preparation environment than from the manufacturing process. Infrequent occurrence of intrinsic contamination of powdered infant formula does occur and has resulted in outbreaks of illness, but this appears to be rare. Thus, the meeting considered that most cases of salmonellosis amongst infants were probably not caused by intrinsic contamination of powdered infant formula. Disease caused by contamination of powdered infant formula by rare serotypes is more likely to be detected. As stated above (section 2.1.2), it would be difficult to detect outbreaks or specific sources of salmonellosis due to common serotypes within the higher incidence of background illness.

5.2 DEVELOPING COUNTRIES

There is a dearth of information on contamination of powdered infant formula sold in developing countries, and there has also been no surveillance on the disease burden resulting from consumption of contaminated powdered infant formula in developing countries. However, even if there have been no studies on whether the product used in developing countries is contaminated, the potential risks of contamination cannot be ruled out given that reports from different developed countries have shown that some batches of powdered infant formula are contaminated. Many developing countries import powdered infant formula from processing plants in a few countries, for example Bangladesh. The incidence and levels of *E. sakazakii* are likely to be the same as in products evaluated in exporting countries of origin and reported in published surveys. The levels should remain stable during transport and distribution.

In many developing countries, the proportion of special subpopulations consisting of low-birth-weight infants and infants of HIV-infected mothers is higher than in developed countries; therefore, the use of powdered infant formula in these circumstances may be increasing. The basis of the higher demand for powdered infant formula is the recommendation for infants of HIV-positive mothers that – where replacement feeding is acceptable, feasible, affordable, sustainable and safe – all breastfeeding be avoided. (WHO, 2001). Human milk fortifiers are required to compensate the nutritional needs of very low-birth-weight infants. In circumstances when the mother cannot breastfeed or chooses not to breastfeed for any reason, special powdered infant formula may be required for feeding of low-birth-weight infants. Therefore, well-controlled studies need to be conducted to assess the extent of risk associated with contaminated powdered infant formula for infants in developing countries.

5.3 MICROBIAL ASPECTS OF MANUFACTURE AND USE OF POWDERED INFANT FORMULA

According to industry experts from the United States and Europe, powdered infant formula can be manufactured in different ways. A flow chart of the production and use of powdered infant formula highlights a number of points at which this product may be subject to microbial contamination (Figure 2).

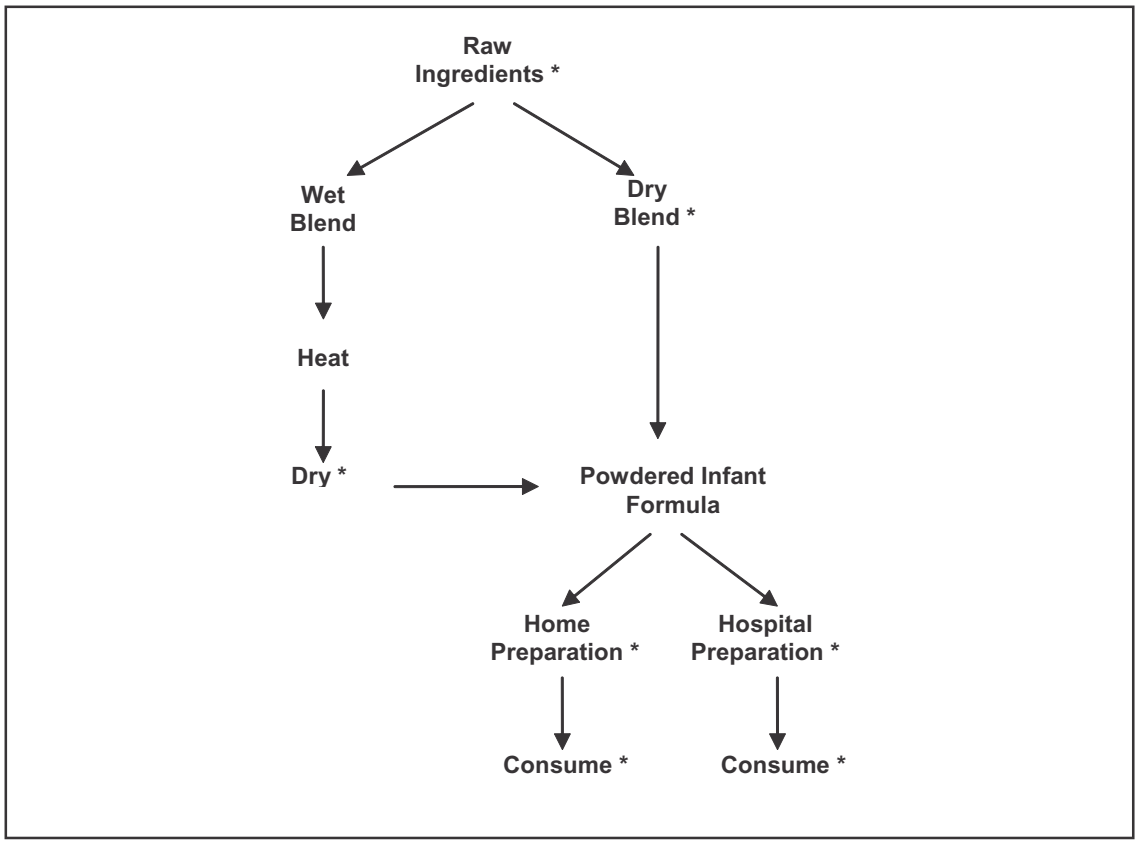

Figure 2. Flow chart for the production and use of powdered infant formula. The heat step during wet blending is assumed to effectively eliminate Enterobacteriaceae.
* = potential sites for environmental contamination.

5.3.1 Manufacture

Dry infant formula is manufactured according to three process types:

a. **Wet-mix process:** all ingredients are handled in a liquid phase and heat-treated (critical control point [CCP]), e.g. pasteurized or sterilized, and then dried.
b. **Dry-mix process:** individual ingredients are prepared, heat-treated as appropriate, dried and then dry-blended.
c. **Combined process:** part of the ingredients are processed according to (a), in order to produce a base powder to which the rest of the ingredients are added according to (b).

5.3.2 Control of ingredient quality

The main microbiological issues of current public health concern associated with powdered infant formula are related to the presence of *Salmonella* and other Enterobacteriaceae (coliforms) including *E. sakazakii*. The presence of these microorganisms may occur as a result of:

- contamination through ingredients not submitted to a heat treatment during the powdered infant formula manufacturing process (this applies for dry-mix and combined processes).

- contamination from the processing environment during the dry steps of the process, i.e. contamination post-thermal processing, presumably acquired from the processing environment during drying or packing (this applies for dry, wet and combined processes).

It must be emphasized that dry-mix ingredients are not "raw"; they are processed by the suppliers to fulfil the same requirements as the finished powdered infant formula. The presence of Enterobacteriaceae is due to post-heat-treatment recontamination. The results of an unpublished industry survey of ingredients are summarized in Table 2 (J.L. Cordier, personal communication, 2004). In order to ensure that ingredients are microbiologically suitable, a number of factors need to be considered:

- The likelihood of occurrence in ingredients – some are considered to have a high risk of containing Enterobacteriaceae (e.g. starch) while others have a low risk (e.g. oils). The rating may depend on the local situation (Table 2).

- Selection of the supplier according to stringent criteria (e.g. appropriate control measures, good hygienic practices [GHPs], verification and release procedures in place).

- Testing of the ingredients to verify effectiveness of the above measures (not to ensure safety).

5.3.3 Processing

Powdered infant formula is produced from ingredients that may include milk, milk derivatives, soy protein isolates, carbohydrates, fats, minerals, vitamins and some food additives. These ingredients, in either liquid or powdered form, are typically mixed with water to form a liquid mix, which is then dried to a powder ($a_w \leq 0.3$) in large spray dryers. Prior to drying, the liquid mix is heated (pasteurized at 71.6°C for 15 seconds or 74.4°C for 25 seconds [for products containing starches or thickeners] or at higher temperatures [e.g. 105°-125°C for at least 5 seconds]), homogenized, in some cases

Table 2. Industry survey for the presence of Enterobacteriaceae and *E. sakazakii* in ingredients used in dry mixing operations for all types of powdered formula (up to 3 years).

Ingredients	n (10 g)	Coliform or Enterobacteriaceae positives	*E. sakazakii* positives
Vitamins	793	8	0
Skimmed milk powder	835	1	1
Dem. whey powder	23	3	0
Sucrose	1 691	28	0
Lactose	2 219	70	2
Banana powder/flakes	105	3	1
Orange powder/flakes	61	1	1
Lecithin	136	1	1
Starch	1 389	155	40

evaporated and sometimes stored in large, chilled holding tanks. Vitamins are added just prior to drying. During the drying process, the liquid mix is heated to approximately 82°C and is pumped under high pressure to spray nozzles or an atomizer mounted in a large drying chamber through which flows filtered, high-temperature air. Inlet air temperature ranges from 135° to 204°C, and the exhaust temperature ranges from 45° to 80°C. The liquid mix is dried nearly instantaneously in the hot air and the resultant powder falls to the bottom of the dryer for collection. Alternatively, it is collected from the exhaust stream in cyclone collectors or bag houses. The powder then passes from the drying chamber to a fluidized cooling bed where it is quickly cooled to below 38°C using cool, high efficiency particulate air (HEPA) > EU 10.[1] Next, the powder is sifted and pneumatically or mechanically transported to storage silos, tote bins or big bins, or directly to filling operations.

In some cases, manufacturers produce infant formula by first drying a wet mixture of the major ingredients (protein, fat and carbohydrate). This is typically called infant formula base powder. Then, in large mixers or blenders, the dry minor ingredients, such as vitamins, minerals and additional carbohydrates, are blended into the base powder to produce the final product formulation. This option allows for longer drying campaigns and reduces the frequency of changeovers between different product formulations. Another option is to blend all of the pre-dried ingredients together to make a finished infant formula powder. This process is more efficient from an energy standpoint and provides more flexibility in formulation modifications. In the dry-blending process, it is essential that the dry ingredients meet the same microbiological standards as the final product because they receive no additional heat treatment. Because incoming "raw" material testing alone does not guarantee conformance to the high quality standards required by the industry, manufacturers employing these processes maintain close relationships with their "raw" material suppliers and require strict adherence to good manufacturing practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) principles.

On completion of the drying or blending steps, the final product is conveyed from the storage silos or blenders to filling machinery where it is filled into cans or flexible containers. The containers are flushed with inert gas, sealed, coded, labelled and packed into shipping cartons. The finished product is typically held until it undergoes final testing, including nutrient content, uniformity and microbiological analysis.

5.3.3.1 Heat treatment

It has been suggested that the high thermal resistance of *E. sakazakii* strains in comparison to other members of the Enterobacteriaceae can possibly explain their high prevalence in powdered and prepared formula milk (Nazarowec-White and Farber, 1997a). However, recent studies suggest that the osmotolerance of the organism may be more important in this latter regard (Breeuwer et al., 2003). The ability to be osmotolerant may increase the risk of the organism becoming more dominant in the environment, thus increasing the risk of post-processing contamination of powdered infant formula. Previous work done by Nazarowec-White and Farber (1997b) and others

[1] The Eurovent 4/4 standard has classified HEPA (high efficiency particle air) and ULPA (ultra low particle air) filters in five different classes, EU 10 – EU 14, based on the efficiency determined by using the Sodium Flame test. EU 10 exhibits 95–99.9% efficiency, while EU 14 exhibits >99.999% efficiency.

(Nazarowec-White, McKellar, and Piyasena, 1999; Iversen, Lane, and Forsythe, 2004) showed that standard pasteurization practices are effective for the inactivation of *E. sakazakii*. Edelson-Mammel and Buchanan (2004) showed that a greater than 4-log reduction can be obtained by rehydrating dried infant formula with water pre-equilibrated to ≥ 70°C. This implies that preparing reconstituted formula using the latter approach (using 70°C for rehydration) is likely to result in a high probability that a serving would not contain this organism. Interestingly, there appeared to be two distinct phenotypes of *E. sakazakii*, and heat resistance varied as much as twentyfold (Edelson-Mammel and Buchanan, 2004). Figure 3 illustrates the difference in heat resistance and provides a comparison with other Enterobacteriaceae (Edelson-Mammel and Buchanan, 2004). A complete listing of D- and z- values can be seen in Table 3. In summary:

- There appears to be substantial diversity in thermal resistance among strains.
- Inactivation of the organism can occur very quickly at temperatures above 70°C.

This suggests that the use of relatively mild thermal treatments is a potential risk reduction strategy that can be directed towards reducing or eliminating *E. sakazakii* in reconstituted powdered infant formula.

5.3.4 Post-processing and packaging

The difficulty which has to be considered in the evaluation of potential treatments for inactivating microbial pathogens in powdered infant formula is the behaviour of vegetative cells in dry products, i.e. very frequently there is increased heat resistance. Based on currently available knowledge,

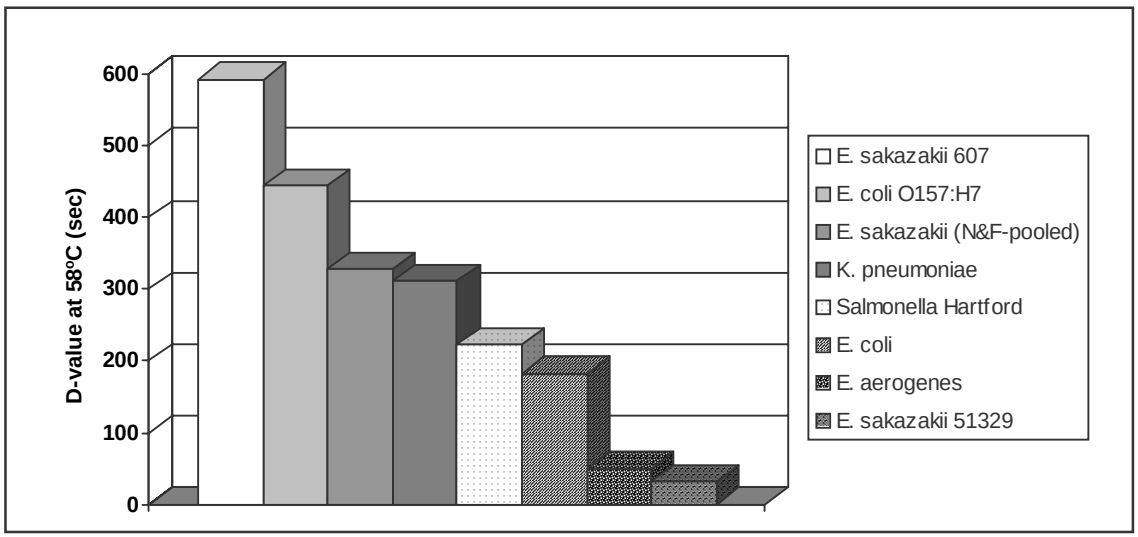

Figure 3. Heat resistance of different strains of *E. sakazakii* and other Enterobacteriaceae (Buchanan, 2003).
Note: E. sakazakii (N&F pooled) = pool of 10 strains as reported in Nazarowec-White and Farber (1997a).

sterilization of the final product in its dry form in a processing environment in cans or sachets seems only possible using irradiation. However, with the doses that are likely to be required to inactivate *E. sakazakii* in the dry state, the technology does not appear to be feasible due to organoleptic deterioration of the product.

A number of other technologies, such as ultra-high pressure and magnetic fields, may be potential candidates. These new technologies are at an early stage of development and currently none are suitable for dried foods. It is recommended that research be done in this field, bearing in mind the needs for quantitative validation of the killing effect.

5.3.5 Hazard Analysis Critical Control Point (HACCP) in the manufacture of powdered infant formula

In the United States and Europe, for many years now, infant formula manufacturers have recognized that GHP and HACCP play a primary role in the control of microbiological, chemical and physical hazards as well as allergens. Although there is currently no worldwide regulatory requirement for infant formula manufacturers to have HACCP plans, most (if not all) incorporate HACCP principles

Table 3. Decimal reduction time (D-value)[a] and z-value[b] for *E. sakazakii* in powdered infant formula.

D-value (min.)									z-value (°C)	Reference
52°C	53°C	54°C	56°C	58°C	60°C	62°C	65°C	70°C		
(Temperature at which D-values were determined)										
54.8 ±4.7		23.7 ±2.5	10.3 ±0.7	4.2 ±0.6	2.5 ±2				5.8	Nazarowec-White and Farber, 1997a[c]
	8.3, 20.2	6.4, 7.1	1.1, 2.4	0.27, 0.34, 0.4, 0.48					3.1, 3.6	Breeuwer et al., 2003[d]
				0.50						Breeuwer et al., 2003
			21.1 ±2.7	9.9 ±0.8	4.4 ±0.4		0.6 ±0.3	0.07	5.6	Edelson-Mammel and Buchanan, 2004[e]
		16.4 ±0.67	5.1 ±0.27	2.6 ±0.48	1.1 ±0.11	0.3 ±0.12			5.8 ±0.40	Iversen, Druggan, and Forsythe, 2004[f]
		11.7 ±5.80	3.9 ±0.06	3.8 ±1.95	1.8 ±0.82	0.2 ±0.11			5.7 ±0.12	Iversen , Druggan, and Forsythe, 2004[g]

[a] D-value is the time required for a 10-fold reduction in viable numbers of organisms at a given temperature.
[b] z-value is the temperature change required to change the D-value by a factor of 10.
[c] D-values of a pool of 10 strains (5 clinical isolates and 5 food isolates) of *E. sakazakii*.
[d] These D-values for 4 different strains of *E. sakazakii* were determined in phosphate buffer. The authors report that heat treatment in reconstituted powdered infant formula did not influence the D-value.
[e] D-value for *E. sakazakii* strain 607 described as the most heat resistant of a range of *E. sakazakii* strains that were examined in the study.
[f] Data for *E. sakazakii* type strain.
[g] Data for *E. sakazakii* capsulated strain.

into their control programmes as well as GHP. Quality of raw materials, air and liquid filters, sifter screens, magnets/metal detectors, pasteurization and storage temperatures are important control points and must be addressed specifically.

The heat treatments (CCP) applied are theoretically sufficient to ensure the destruction of eight or more log units of Enterobacteriaceae, including *Salmonella* and *E. sakazakii*, as well as other vegetative microorganisms, such as *L. monocytogenes* or *S. aureus*. Spore-formers, such as *B. cereus* and *C. botulinum*, are inactivated in part – to what extent depends on the processing conditions. Other heating steps are typically applied in a wet-mix process, but they are not considered CCPs; they include:

1. thermization or pasteurization of, for example, raw materials (e.g. incoming raw milk or raw whey);

2. preheating of the liquid formula before spray-drying; and

3. actual spray-drying.

Although these steps may have some killing effect (in particular, steps 1 and 2), they are performed for technological reasons and are not considered CCPs.

5.3.6 Monitoring

5.3.6.1 Methods for detection

Different genera and species of Enterobacteriaceae have been isolated from reconstituted powdered infant formula after enrichment (Muytjens, Roelofs-Willemse, and Jasper, 1988; Iversen and Forsythe, 2004), including *E. sakazakii*, *E. cloacae*, *C. koseri*, *C. freundii*, *Pantoea agglomerans* and *Escherichia vulneris* (the latter two formerly known as *E. agglomerans*). Specific detection methods are required to isolate and distinguish between closely related members of the Enterobacteriaceae.

- *Primary isolation:* Both *E. sakazakii* and *S. enterica* are isolated using pre-enrichment, enrichment and selective-differential agar. For *Salmonella*, multiple 25 g volumes (n = 60) are tested (CAC, 1979; ICMSF, 1986), whereas for *E. sakazakii* a most probable number approach is used with multiple 100 g, 10 g and 1 g volumes (Figure 4) (Muytjens, Roelofs-Willemse, and Jasper, 1988; Nazarowec-White and Farber 1997b; USFDA, 2002). For both organisms, presumptive isolates are confirmed using biochemical or gene-based tests (Anon., 1996; Kandhai, Reij, and Gorris, 2004). For *Salmonella*, methods have been validated by international organizations, but this is not the case for *E. sakazakii*. A characteristic of most *E. sakazakii* strains is the production of a yellow non-diffusible pigment below 37°C. However, this is not a unique trait and it is commonly found in the closely-related genus *Pantoea* which has also been isolated from reconstituted powdered infant formula (Muytjens, Roelofs-Willemse, and Jasper, 1988; Iversen and Forsythe, 2004). In addition, there are white *E. sakazakii* strains (Block et al., 2002). A second common trait used in presumptive identification is the production of α-

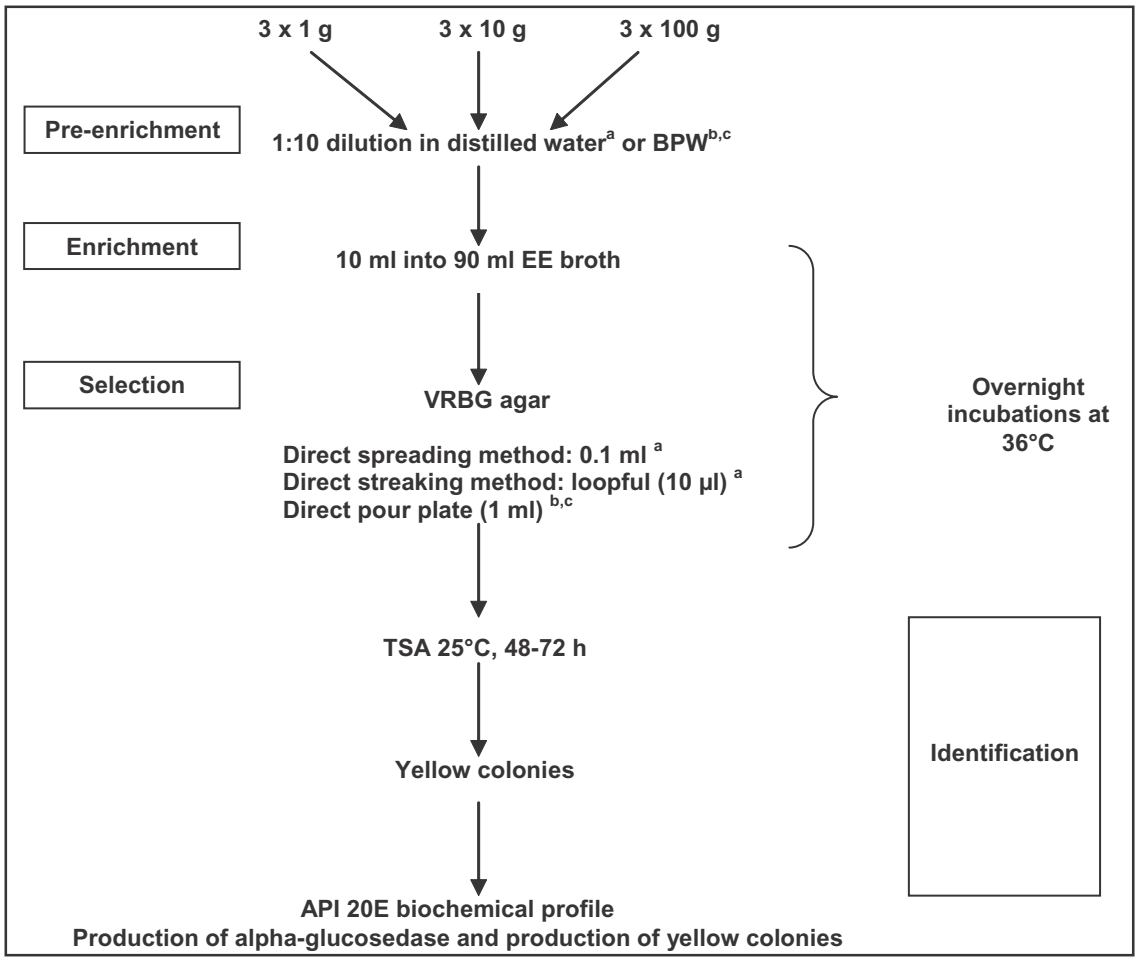

Figure 4. Quantitative *E. sakazakii* isolation procedure.
[a] USFDA (2002); [b] Muytjens, Roelofs-Willemse, and Jasper (1988); [c] Nazarowec-White and Farber (1997b) from Iversen and Forsythe (2003).
BPW, Buffered peptone water; EE Broth, *Enterobacteriaceae* enrichment broth;VRBG, Violet red bile glucose agar.

glucosidase (Muytjens, van der Rose-van de Repe, and van Druten, 1984; Iversen, Druggan, and Forsythe, 2004). Internationally-validated methods exist for the specified pathogens, *B. cereus*, *C. perfringens* and *S. aureus*, and the indicator organisms coliforms and Enterobacteriaceae (USFDA Bacteriological Analytical Manual; Health Canada Compendium of Methods, ISO, Geneva). No internationally validated methods exist for specific Enterobacteriaceae such as *E. sakazakii*, *E. cloacae* and *C. koseri*. Biochemical profiles are frequently used following primary isolation (Figure 4), but contradictions in identification may occur in different biochemical kits for the same strain (Iversen, Druggan, and Forsythe, 2004). Further research is required into the genetic diversity and distinguishing traits of *E. sakazakii* and related organisms.

- *Typing: Salmonella* isolates are subject to established typing methods, including serotyping, phage typing and antibiograms. Central alert centres exist (PulseNet and Salm-Net) to detect multinational *Salmonella* outbreaks due to a common food source (Swaminathan et al., 2001;

Rowe et al., 2004). Methods used to fingerprint *E. sakazakii* isolates include plasmid typing, ribotyping, pulsed field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) typing (Biering et al., 1989; Clark et al., 1990; Nazarowec-White and Farber, 1999). These methods enable the tracing of specific strains in powdered infant formula production and are very useful during epidemiological investigations, to observe if clinical and food isolates are indistinguishable (Smeets et al., 1998).

E. sakazakii isolates from powdered infant formula available in Canada and Canadian clinical isolates were characterized by phenotypic (biotype and antibiograms) and genotypic (ribotyping, RAPD and PFGE) methods (Nazarowec-White & Farber, 1999). There is currently at least one large food company that is using ribotyping of *E. sakazakii* to track the spread or trace the source of the organism in powdered milk plants. Molecular typing methods, such as ribotyping and PFGE, are very suitable tools for studying environmental contamination in plant processing environments, in trouble-shooting, as well as in tracing sources of contamination, and should, wherever feasible, be encouraged.

5.3.6.2 Monitoring and testing by industry

Sampling and testing regimes in processing facilities are integrated sampling plans which are implemented to demonstrate the efficiency of the control measures taken to eliminate or minimize the presence of *Salmonella* and other Enterobacteriaceae (including *E. sakazakii*) in finished products, as well as other specified pathogens such as *B. cereus* and *S. aureus*. Such sampling plans are not necessarily identical to the ones applied by official control laboratories – they may be as or more stringent, but with a different focus and different types of samples. Such integrated sampling plans are flexible and may be adapted to the findings. In particular, indications for deviations in the processing environment and line (indication for an increased risk of contamination) could lead to increased sampling (number and size of samples) and testing and investigation of the deviation.

Such sampling plans may vary among manufacturers and the parameters included (pathogens, indicators, visual inspections etc.) are adapted to the particular processing line. They integrate the following types of sample:

- dry-mix ingredients;
- finished products;
- food contact surfaces at critical processing steps; and
- environmental samples from the processing environment, in particular critical ones.

For a detailed consideration of the above, see ICMSF (2002).

5.3.7 Microbiological specifications

The current Codex microbiological specifications relating to mesophilic aerobic bacteria, coliforms and *Salmonella* for powdered infant formula (CAC/RCP 21-1979) are outlined in Table 4. These criteria were established many years ago and need to be reviewed in light of new developments and knowledge.

Table 4. Current Codex advisory microbiological specifications for dried and instant products.[a]

	Case	Class plan	n	c	Limit per g [b]	
					m	M
Mesophilic aerobic bacteria	6	3	5	2	10^3	10^4
Coliforms	6	3	5	1	<3 [c]	20
Salmonellae [d]	12	2	60	0	0	-

[a] Including products intended for consumption after the addition of liquid, dried infant formula, instant infant cereals etc.

[b] The microbial limits apply to the dry product (CAC/RCP 21-1979).

[c] <3 means no positive tube in the standard 3-tube MPN (most probable number) method (ICMSF, 1978).

[d] For Salmonellae, 25 g samples should be used.

The current microbiological specifications for *Salmonella* were considered by the meeting to be adequate and are near the limit of practical microbiological testing. However, there is currently no requirement to test for *E. sakazakii* and the meeting concluded that the current specifications should be reviewed based on the information presented to the meeting. A revision of the Codex specifications should include consideration of the following:

- microorganisms and reasons for concern;
- analytical methods to be used;
- sampling plan and size of analytical units;
- microbiological limits; and
- numbers of units to be in conformity.

Using current dry-mix technology, it does not seem to be possible to ensure that powdered infant formula is free from Enterobacteriaceae such as *E. sakazakii*. Even a more stringent microbiological specification may not be reliably effective at detecting very low numbers of organisms. Given the large quantity of the product consumed and the fact that even one contaminating bacteria is capable of growing to large numbers, a combination of risk reduction measures may be required for the effective management of the risk.

5.3.8 Reconstitution and use

5.3.8.1 Storage of open packs of formula

Edelson-Mammel and Buchanan (R. Buchanan, personal communication, 2004) studied the long-term survival of *E. sakazakii* in powdered infant formula by preparing a quantity of powdered formula to contain approximately 10^6 cfu/ml *E. sakazakii* when reconstituted according to the manufacturer's instructions. Over the course of approximately 1.5 years, the spiked dry infant formula was stored at room temperature in a closed screw-cap bottle. Periodically, samples of the formula were taken, hydrated, and the level of viable cells determined by plating in duplicate on tryptic soy agar plates. During the initial 5 months of storage, the level of viable *E. sakazakii* declined approximately 2.5 log cycles (6.0 log cfu/ml to 3.5 log cfu/ml) at a rate of approximately 0.5 log cycles per month. Over the course of the subsequent year, the level of viable *E. sakazakii*

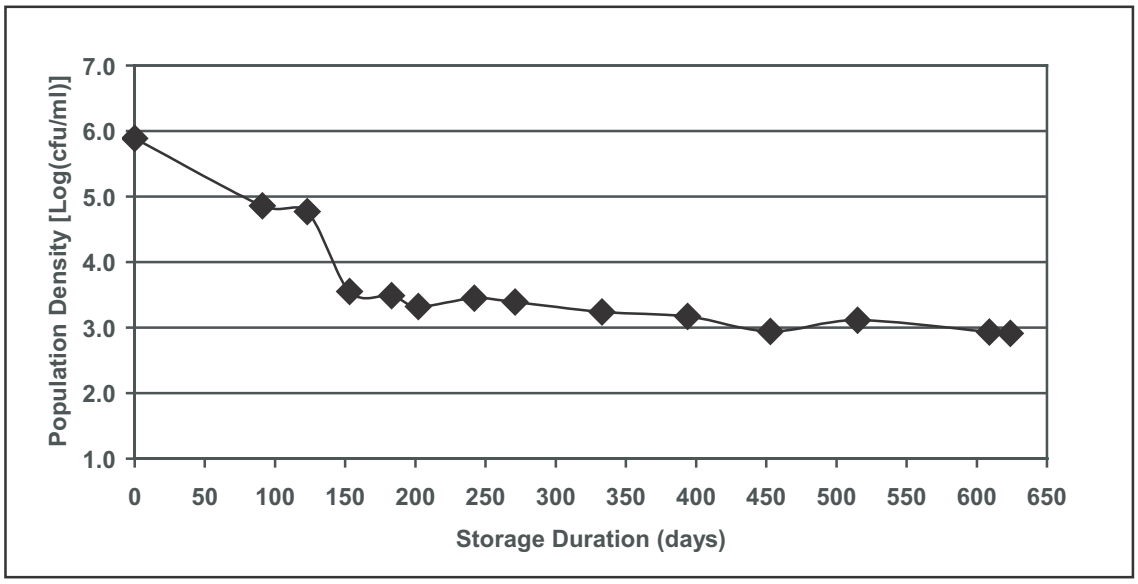

Figure 5. Long-term survival of *Enterobacter sakazakii* in powdered infant formula.

declined an additional 0.5 log cycles to approximately 3.0 log cfu/ml (Figure 5). These results clearly demonstrate that *E. sakazakii* can survive for extended periods in powdered infant formula.

Little is known about the fate of intrinsically contaminated powdered infant formula once opened and then stored at high ambient temperature and humidity which is characteristic of tropical countries. Current information indicates that the moisture content of powdered infant formula in such a setting would not increase to the extent that it may support growth of intrinsic contaminants.

5.3.9 Labelling and preparation

5.3.9.1 Labelling

Labelling of powdered infant formula is very comprehensive as a rule, with elements of information, advice and warning. The Codex standard for powdered infant formula requires: complete ingredient and nutrition labelling; advice on the feeding of infants ("breastfeeding is the best for your baby"); warning about inappropriate feeding of infants; and recommendations for the preparation, feeding and storage of the product as sold, opened and prepared for consumption. Depending on the law of the country, it may also contain information on particular properties of the product.

The recommendations for the preparation of infant formula, formula for special medical purposes intended for infants, and follow-up formula at home are detailed and often accompanied by illustrations. Presently, these recommendations include the following:

> *"Prepare each bottle freshly before feeding – boil water – put it into a clean bottle and cool it down to about 50°C – add measured amount of powder (number of scoops) –*

shake vigorously – cool to drinking temperature (skin test) – feed directly – discard residues in the bottle."

The labelling of formula for special medical purposes (FSMP) has to contain, in addition, more specific information about the product: what makes it special? what makes it suitable for the indication it is presented for? for what disease, disorder, medical condition is it intended? are there interactions with drugs? A warning statement should be included that the product is not intended to be eaten by healthy persons and that it is only to be used under medical supervision.

5.3.9.2 Preparation

In the home, manufacturers recommend that formula be prepared before each feeding using boiled water. It is recommended to boil the water and then cool it to 50°C before the addition of the measured amounts of the powdered product. Manufacturers recommend that label directions as described above should be followed carefully.

The reasons for the recommendation of cooling the water appear to be threefold. First, there does appear to be some nutrient loss associated with particular formulas, particularly loss of vitamin C. Second, clumping of formula upon rehydration with hot water can occur with certain formula powders. Finally, there are concerns that use of water at elevated temperatures could lead to increased incidence of burns either to the infant or to the formula preparer (the latter being especially pertinent to the inappropriate heating of bottles in microwave ovens). The United States Food and Drug Administration (FDA) provided data (Buchanan, 2003) on nutrient losses associated with rehydrating infant formulas with boiling water. No data were available to the workshop on the effect of the use of hot water on clumping or on burn issues.

After mixing the powder and water by shaking the bottle and cooling it to drinking temperature under water (cheek test), the formula is to be fed to the infant immediately. While rewarming of the bottle cannot be excluded with slow-feeding infants, it should be discouraged. For practical reasons, parents might be tempted to prepare all the bottles needed for one day in advance and keep them in the refrigerator. In this case, rapid cooling of the prepared formula and storage at low temperature are important factors with regard to the microbiological safety of the reconstituted formula.

In hospitals, practices will vary according to local arrangements and availability of trained personnel and facilities.[2] A centralized preparation of ready-to-feed formula and on-ward preparation are possible and both have advantages and disadvantages. For both, the availability of safe (sterile) water and aseptic conditions for the preparation are required. The transport of ready-to-feed preparations to the wards under sustained refrigeration and refrigeration on the ward up to the feeding time are important factors to control.

Infants who can coordinate sucking, swallowing and breathing will receive formula from the bottle which has been quickly warmed immediately prior to feeding. Feeding times can be prolonged

[2] See, for example: *Infant feedings; Guidelines for the preparation of formula and breast milk in health care facilities*. American Dietetics Association. 2004.

in sick and hypotonic infants and need to be controlled. Bottles should not be rewarmed. Formula remaining in the bottle should be discarded after a specified time limit.

In the case of immature or sick infants without coordinated sucking/swallowing, feeding by naso- or orogastreal tube or gastrotomy tube is practised. Formula can be applied continuously using a pump or by giving boluses which are adapted in volume to the tolerance of the infant (gastric volume and gastrointestinal motility). Continuous infusion into the gastrointestinal tract by pump requires control of the time of administration of one selected syringe volume as well as observation of the homogeneity of the formula in the syringe. Pre-administrative warming can be omitted. Handling of the infusion system should observe the same precautions as for parental feeding systems. Flushing of the tube after each feeding with sterile solutions may reduce microbial contamination and the formation of biofilms within the feeding delivery systems. Gastric residues of feeds and removed tube systems should be regularly checked for the presence of pathogenic bacteria.

5.3.10 Storage and handling of prepared formula

Farmer et al. (1980) examined 57 strains of *E. sakazakii* and reported growth of the organism at 25°, 36° and 45°C. Fifty of the tested strains grew at 47°C, but not at 4° or 50°C. Nazarowec-White and Farber (1997b) reported that minimum growth temperatures for *E. sakazakii* in Brain Heart Infusion (BHI) broth varied from 5.5° to 8°C; and strains actually began to die off slowly at 4°C. In addition, maximum growth temperatures for clinical and food isolates ranged from 41° to 45°C (see also Gavini, Lefebvre, and Leclerc, 1983). This has implications for enrichment broths which have a recommended incubation temperature of 45°C. Iversen, Lane, and Forsythe (2004) and Zwietering (personal communication, 2004) have measured the growth rate of *E. sakazakii* in powdered infant formula (Figure 6). Generation times for *E. sakazakii* in reconstituted infant formula varied at 10°C from 4.15 to 5.52 hours and at 22°C from 37 to 44 minutes. Lag times at 10° and 23°C ranged from 19 to 47 hours and 2 to 3 hours, respectively (Nazarowec-White and Farber, 1997b). Iversen, Lane, and Forsythe (2004) examined clinical and food strains and found that the generation times for *E. sakazakii* in reconstituted infant formula were 13.7 hours, 1.7 hours and 19-21 minutes at 6°, 21° and 37°C, respectively. The relationship between temperature and specific growth rate across the various studies is summarized in Figure 6. Therefore, it is evident that improper storage of contaminated reconstituted powdered infant formula can support rapid growth of *E. sakazakii*.

It is important to stress that the addition – in hospitals or at home – of ingredients such as starch or sugar to powdered infant formula may present a risk of contamination of the product. Such added ingredients need to comply with the same requirements as the powdered infant formula. However, the specific risk associated with the addition of such ingredients was not considered in this meeting.

5.3.11 Education

Many consumers, including those directly involved in caring for infants, are not aware that powdered infant formula is not a sterile product and may be contaminated with pathogens that can cause

serious illness, and they lack information on how handling, storage and preparation practices can influence the risk. Effective risk communication efforts for both the public and health professionals are needed. Information and education about basic hygiene practices in connection with food handling, storage and preparation at home also need to be emphasized.

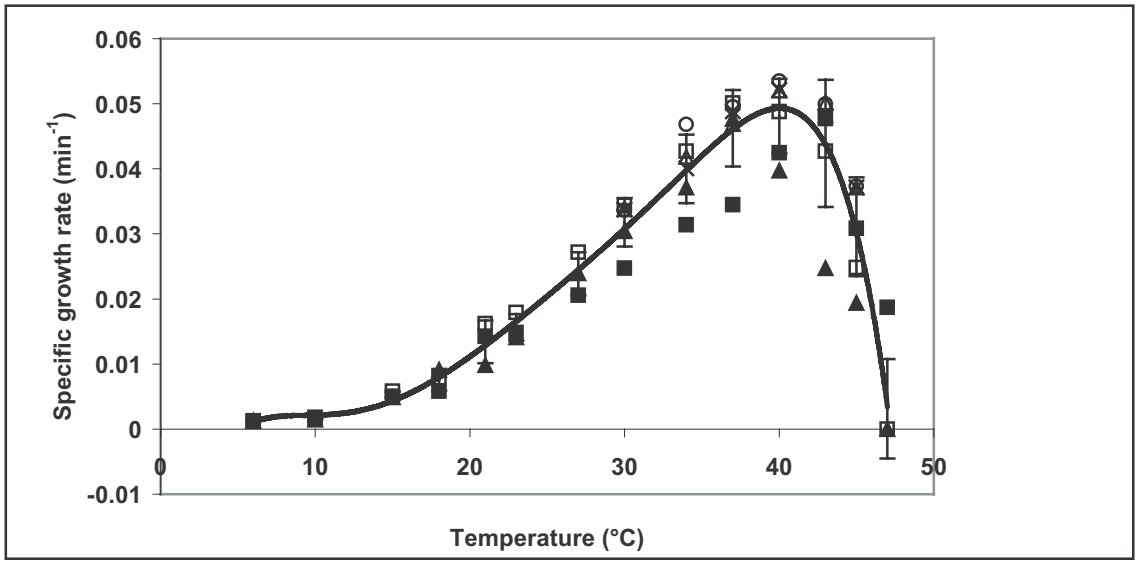

Figure 6. Growth rate of *E. sakazakii* (n=27) in reconstituted powdered infant formula according to temperature (Iversen, Lane and Forsythe, 2004; Zwietering personal communication, 2004).

6. RISK CHARACTERIZATION

6.1 APPROACHES AND OUTPUTS

As a means of providing a conceptual framework for the evaluation of the factors affecting the impact of *E. sakazakii* and other pathogens associated with powdered infant formula on public health, an initial risk assessment was undertaken. Due to the limited time available in the meeting, the risk assessment was, of necessity, preliminary in nature and primarily targeted to identifying key parameters that may need to be considered in evaluating and addressing risk reduction strategies. The objectives of the risk assessment were to address four questions:

- What are the factors that contribute to the microbial food safety risks associated with powdered infant formula and what is their relative importance?
- What are potential interventions that could mitigate these risks, and what is the relative efficacy of those interventions?
- What key scientific knowledge and/or data are needed to reduce the uncertainty associated with the estimates of risks and the estimates of the relative effectiveness of identified risk control options?
- What are potential consequences associated with the identified risk control options if implemented?

The risk assessment focused on two of the identified microbiological hazards, *E. sakazakii* and *S. enterica*. As noted in chapter 3, other members of the Enterobacteriaceae are occasionally associated with episodes of septicaemia or meningitis in infants. These appear to have pathogenesis similar to *E. sakazakii* and hence similar risk factors were assumed. To that end, an attempt was made to provide an estimate of the risk associated with these microorganisms by development of a simple "multiplier" to be used in conjunction with the risk estimate for *E. sakazakii*. Other microorganisms were not considered in the risk assessment, partly because of the divergent ecologies, aetiologies and technologies that would be needed to achieve enhanced risk control. This decision was also in keeping with the scope of the products considered within the risk assessment.

The range of products that could potentially be included within the designation, powdered infant formula – or that might be added to powdered infant formula – is extensive. For the purposes of risk assessment, the products considered were those that meet the definition of products sold in powdered form for subsequent rehydration and feeding in a liquid form as a complete or partial substitute for human milk. The range of products considered included materials specifically manufactured as supplements to human milk or infant formulas for the purposes of producing specific, desirable nutritional or physical characteristics. Examples of included products are: powdered infant formula, follow-up formula, FSMP intended for infants and human milk fortifiers. Products not included in the risk assessment were those that were not manufactured for the specific purpose of being added to human milk or infant formula. For example, this did not include the practice by certain consumers of adding honey to infant formula for the purposes of increasing consumption

rates. Thus, diseases, such as infections associated with the presence of low levels of *C. botulinum* spores in honey added to infant formula leading to intestinal colonization (i.e. infant botulism), were not considered in the risk assessment.

The risk assessment was intended to be global in its consideration of both developed and developing countries, however available surveillance data were largely limited to a small number of countries. In developing the risk assessment, both the risks associated with the inherent contamination of powdered infant formulas and that acquired during preparation and feeding were evaluated for the three consumption settings considered: neonatal hospital wards, neonatal intensive care units and the home. Factors associated with the microbiological quality of the water used for rehydrating the products were not considered, though it was fully appreciated that the presence of pathogenic microorganisms in water can greatly influence the microbiological safety of infant formula, particularly in developing countries. However, the risk control strategies for controlling this hazard can be significantly different from those considered in the current risk assessment.

Cases of salmonellosis and *E. sakazakii* infections associated with powdered infant formula have been documented in children older than 1 year; however, given that the rate of infection is substantially higher in infants, it was decided to concentrate the risk assessment on children of 1 year or younger. This population was in turn subdivided into groups and the effect of susceptibility was estimated in comparison with infants of 28 days or less (baseline). This decision was undertaken to take into account the apparent increased susceptibility associated with the youngest age group. Differences in apparent incidence rates were used as a means of estimating the relative susceptibility of the two age groups.

The flow chart in Figure 2 provides the simplified scheme for production, distribution and use that was used to assess the risks associated with both pathogens. Potential points where environmental contamination of the powdered infant formula or reconstituted formula prior to consumption could occur are indicated. In this flow chart, it is assumed that the pasteurization step(s) employed during the wet blending are of sufficient magnitude to eliminate both pathogens and that any contamination of that product is due to subsequent recontamination. As discussed in chapter 5 with dry-blending processes, most ingredients will have undergone a pasteurization treatment at some point in their manufacture. Modification to the general model describing the general flow chart were made in developing various “what-if” scenarios to consider the impact of different potential risk-reduction interventions.

6.2 EVALUATION OF POTENTIAL RISK REDUCTION OPTIONS FOR FORMULA-FED INFANTS

A simplified risk assessment for the purposes of articulating key concepts and providing simple “what-if” scenarios was developed. This risk assessment focused on the scientific information available in relation to disease in medical neonatal facilities. The basic approach of the risk assessment was to estimate the risk for a baseline scenario. Modifications to current practices or potential mitigation strategies were then compared relative to this baseline. A second, more complex risk assessment was conducted to more fully explore a wider range of factors that affect the microbiological risks associated with *S. enterica* and *E. sakazakii* in powdered infant formula.

While estimates derived from the more complex risk assessment generally agreed with the simpler version, there was insufficient time within the context of this meeting to verify the model's quality and present its findings. As a result, the more complex model was not applied to generate risk assessment conclusions.

In addition to demonstrating the interactions between the various factors that affect the risks, the risk assessment provided important insights into additional data that are needed to make informed decisions related to risk control. Details of how the more simple risk assessment was undertaken are provided in Appendix C.

The key findings of the risk assessments are listed below:

1. Key factors affecting the microbiological risks associated with powdered infant formula factors include:

    - the level of contamination in the powdered infant formula;
    - the level of hygiene in the preparation and delivery of the reconstituted formula;
    - inclusion of a bactericidal treatment at the time of preparation; and
    - the duration of the feeding period and the temperature.

2. Two factors that were predicted to produce the greatest reductions in the risks associated with *S. enterica* and *E. sakazakii* were:

    - the duration of the time to consumption; and
    - the inclusion of a bactericidal treatment at the point of rehydration.

3. The degree of risk reduction that can be achieved by reducing the levels of *E. sakazakii* and *S. enterica* in powdered infant formula is dependent in part on the extent of contamination that is attributable to the presence of the pathogens in the preparation environment.

4. Control measures can be combined to achieve a greater degree of risk reduction than that achieved through the use of any single control measure.

5. There is considerable uncertainty in all risk estimates due to a general lack of scientific data specifically related to powdered infant formula. This is particularly important in relation to information on the sources and contributing factors (e.g. duration of feeding periods) associated with both outbreaks and sporadic cases of disease.

7. RISK REDUCTION STRATEGIES FOR FORMULA-FED INFANTS

Possible control measures and their relative effectiveness in reducing the risk from *E. sakazakii* as estimated by the preliminary risk assessment are given below.

7.1 REDUCING THE CONCENTRATION/PREVALENCE OF INTRINSIC CONTAMINATION OF POWDERED INFANT FORMULA BY *E. SAKAZAKII*

The risk assessment estimated that a significant reduction in the frequency of contamination of powdered infant formula could reduce the relative risk between four- and fivefold (Appendix C, Table A1). Possible approaches for achieving this are described below:

- Employing a supplier assurance scheme and monitoring for raw materials, especially for ingredients not undergoing additional heat treatment prior to mixing.

- Reducing the level of Enterobacteriaceae in the production environment – the main source of contamination appears to be the manufacturing environment, therefore, such a reduction should also reduce the concentration and prevalence of contamination in the finished product. Key aspects could include an effective separation of wet and dry processing operations and an effective management programme of plant hygiene including an environmental monitoring programme within a HACCP plan.

- Monitoring and testing the concentration and prevalence of Enterobacteriaceae in finished products by industry.

- Tightening the current microbiological specifications for powdered infant formula.

These strategies were not specifically evaluated in the risk assessment. It should be noted that the level of relative risk reduction could be varied depending on the initial level of concentration/ prevalence of intrinsic contamination of powdered infant formula and the status of the risk mitigation strategies implemented in each manufacturing establishment.

7.2 REDUCING THE LEVEL OF CONTAMINATION THROUGH HEATING RECONSTITUTED POWDERED INFANT FORMULA PRIOR TO USE

The risk assessment estimated that the use of temperatures in excess of 70°C for reconstitution to achieve a 4-log reduction of *E. sakazakii* (Edelson-Mammel and Buchanan, 2004) could result in a 10 000-fold risk reduction. However, this intervention would need to be applied consistently to be effective. For example, if only 80% of servings received this treatment, the estimated risk reduction would be only fivefold (Appendix C, Table A5). Possible means for achieving this reduction are as follows:

- Where feasible, use of commercially available sterilized liquid products as a replacement for powdered formula, especially for high risk infants.

- Employment of an effective point-of-use pasteurization step following formula reconstitution (e.g. a number of hospitals use a commercial steamer in their formula preparation area).

- Use of hot water (70°-90°C) during the reconstitution of powder. A number of powders clump when water is used at very high temperatures. Other risks that need to be taken into consideration include scalding and the potential for activation of bacterial spores (FSANZ, 2003).

7.3 MINIMIZING THE CHANCE OF CONTAMINATION OF RECONSTITUTED FORMULA DURING PREPARATION

The risk assessment estimated that a significant decrease in the rate of environmental contamination would probably decrease the relative risk 1.2-fold (Appendix C, Table A3). In order to achieve this, one must ensure the use of good hygienic practice in the preparation area, through either guidelines (in the hospital) or labelling and education (in the home). This should include the prevention of cross-contamination from the environment and equipment (e.g. blenders) used during preparation.

7.4 MINIMIZING THE GROWTH OF *E. SAKAZAKII* FOLLOWING RECONSTITUTION PRIOR TO CONSUMPTION

The risk assessment estimated that extended holding times can greatly increase the relative risk of *E. sakazakii* if present. Due to the exponential nature of bacterial growth, the risk will also increase exponentially once the organism comes out of the lag period. For example, after 6 hours at 25°C, the relative risk increases thirtyfold and after 10 hours at 25°C, the relative risk increases 30 000-fold compared to the baseline (Appendix C, Table A2). Risk reduction can be achieved by:

- ensuring rapid cooling and storage below 10°C if not for immediate use; and
- minimizing the length of time between reconstitution and consumption.

Using current dry-mix technology, it does not seem possible to produce commercially sterile powders or to completely eliminate the potential of contamination. Furthermore, even low levels of *E. sakazakii* present in powdered infant formula have the potential to multiply during the preparation and holding prior to consumption. Therefore, a combination of intervention measures is recommended to effectively reduce the risk. On the basis of the preliminary risk assessment, it is clear that the inclusion of a bactericidal step at the point of preparation and a decrease in feeding time were the most effective control measures for reducing risk. A number of combined control measures and their likely effects on reducing risk are presented in Appendix C, Table A6.

The basic risk control principles demonstrated within the preliminary risk assessment for *E. sakazakii* would also hold true for *S. enterica*, although the specific risk reductions achieved would vary to some degree based on the mode and sources of *Salmonella* contamination and its growth and survival characteristics. Example risk reduction scenarios for *S. enterica* are also given in Appendix C.

8. KEY FINDINGS AND RECOMMENDATIONS

8.1 KEY FINDINGS

Intrinsic contamination of powdered formula with *E. sakazakii* or *Salmonella* can cause infection and illness in infants, including severe disease, and can lead to serious developmental sequelae and death. Other means for *E. sakazakii* illness in infants are plausible. A case where sterile formula was contaminated by starch has been documented, but other modes have not been clearly demonstrated. Means of transmission other than powdered infant formula have been demonstrated for infant salmonellosis.

The potential role of other Enterobacteriaceae has not been established but cannot be ruled out, given the limitations of current surveillance systems. While other pathogens (e.g. toxigenic bacteria) have been identified in powdered infant formula, their presence has not been implicated as causing illness in infants.

E. sakazakii has caused disease in all age groups. From the age distribution of reported cases, it is deduced that infants (children <1 year) are at particular risk. The infants at greatest risk from *E. sakazakii* infection are neonates (≤28 days), particularly pre-term infants, low-birth-weight infants or immunocompromised infants. Infants of HIV-positive mothers are also at risk, because they may specifically require infant formula and they may be more susceptible to infection.[1,2] The latter consideration, as well as low birth weight, may be of particular concern for some developing countries, where the proportion of such infants is higher than in developed countries.

There is a small but finite possibility that one or a small number of organisms in a serving could cause illness. This risk increases rapidly if the level of *E. sakazakii* is allowed to increase. Low numbers of *E. sakazakii* in powdered infant formula were also considered to be a significant risk factor, given the potential of even low numbers to multiply during preparation and holding prior to consumption of reconstituted formula.

There is very little known about virulence factors and pathogenicity of *E. sakazakii*. Phenotypic and genetic studies indicate diversity within the species. There are differences in the microbial

[1] The UN guidance for these infants is that where replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding is recommended, and powdered infant formula may be an option. Some of these infants may be HIV-positive and thus immunocompromised.

[2] As a global public health recommendation, infants should be exclusively breastfed for the first 6 months of life to achieve optimal growth, development and health. Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe complementary foods while breastfeeding continues until up to 2 years of age or beyond. Infants who are not breastfed require a suitable breastmilk substitute, such as an infant formula prepared in accordance with applicable Codex Alimentarius standards. Information provided in this connection to mothers and other family members who need to use it should include adequate instructions for appropriate preparation and the health hazards of inappropriate preparation and use (WHO, 2002).

ecology of *Salmonella* and other Enterobacteriaceae. Enterobacteriaceae, such as *E. sakazakii*, are more commonly found in the manufacturing environment, which serves as the primary source of post-pasteurization contamination. Data from industry and control authorities indicate that the detection of *Salmonella* in finished powdered infant formula is rare and microbial specifications in the current Codex code are probably adequate, in terms of existing technologies. Specific criteria for Enterobacteriaceae or *E. sakazakii* are not included in the current Codex code.

Using current mix technology, it does not seem possible to produce commercially sterile powders or to completely eliminate the potential of contamination. Based on a preliminary risk assessment, the inclusion of a bactericidal step at the point of preparation and a decrease in holding and/or feeding time of the reconstituted formula were most effective in reducing risk. A combination of intervention measures had the greatest impact.

8.2 RECOMMENDATIONS

8.2.1 To member countries, NGOs, FAO and WHO

- Encourage health professionals to investigate and report sources and vehicles (including powdered infant formula) of infection by *E. sakazakii* and other Enterobacteriaceae. Outbreaks from these organisms are events that should be investigated thoroughly to answer questions about the ecology of these organisms including the dose response.

- In situations where the mother cannot breastfeed or chooses not to breastfeed for any reason, alert caregivers of infants – both in the home and in healthcare facilities (particularly those at high risk) – to the fact that powdered infant formula is not a sterile product and that even a product meeting existing Codex standards can be contaminated with pathogens that can cause serious illness.

- Develop guidelines for the preparation, use and handling of infant formulas to minimize the risks.

- In situations where the mother cannot breastfeed or chooses not to breastfeed for any reason, encourage caregivers of infants, particularly those at high risk, to use, whenever possible and appropriate, commercially sterile formula (e.g. liquid) or formula which has undergone an effective point-of-use decontamination procedure (e.g. heating reconstituted formula).[3]

- Encourage industry to develop a greater range of alternative formula products that are commercially sterile for high risk groups.

- Encourage industry to reduce the concentration and prevalence of *E. sakazakii* in the manufacturing environment and powdered infant formula (in a context of risk reduction options).

[3] Nutritional and other factors need to be considered, e.g. alteration of nutritional content, risk from burns due to handling boiling or hot water or formula, and potential for increased risk from germination of bacterial spores. The formula should thereafter be cooled and handled properly.

- Encourage industry to use an effective environmental monitoring programme as an important component to an effective environmental management programme.

- Promote the use of Enterobacteriaceae rather than coliform testing as an indicator of hygienic control in factories.

8.2.2 To Codex (e.g. CCFH)

- Revise the code of practice and related text including the microbiological specifications to better address the microbiological risks of powdered infant formula.

- Establish appropriate microbiological specifications for *E. sakazakii* in powdered infant formula.

8.2.3 To member countries, FAO, WHO, Codex and NGOs

- Enhance risk communication, training, labelling and educational activities and approaches to ensure awareness of the issue and appropriate point of use procedures for preparation, storage and use of infant formula.

- Address the particular needs of developing countries in determining disease burden and establishing effective intervention measures for infants who cannot be breastfed for any reason. This includes determining the effects of external environmental factors on burden of disease, such as inadequate storage facilities (lack of refrigerators), lack of clean potable water, lack of fuel to heat water and unhygienic conditions in milk preparation milieu in home and hospitals. Address the capability of adults to implement control measures in the process of preparation and administration of formula and the capability of laboratory technicians to identify *E. sakazakii.*

8.2.4 To FAO, WHO and the scientific community

- Promote the use of internationally validated detection and molecular typing methods for *E. sakazakii* and related organisms.

- Establish a laboratory-based network to alert authorities of *E. sakazakii* outbreaks based on standardized reference methods with supporting central laboratory resources and training facilities.

- Promote research on ways to reduce the levels of *E. sakazakii* in reconstituted powdered infant formula, e.g. strict time-temperature control on rehydration, decreasing the time of feeding, addition of inhibitors, use of biopreservatives and acidification and combining treatments.

- Promote research to gain a better understanding of the ecology, taxonomy, characteristics and virulence of *E. sakazakii*. This will be important in underpinning information for the interpretation of epidemiology data and undertaking further risk assessments. The more complex risk assessment initiated in this meeting should be completed and expanded by JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment).

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Appendix A

LIST OF BACKGROUND PAPERS

Enterobacter sakazakii case reports and outbreaks involving infants as reported in the peer-reviewed English literature	Karl Klontz[1]
Formula associated infection *Enterobacter sakazakii* and *Salmonella* – Recent CDC experience	Chris Braden
Industry practices and standards: The management of *Salmonella* and *Enterobacteriaceae*	Jean-Louis Cordier
Thermal resistance and other characteristics of *Enterobacter sakazakii*	Robert Buchanan
Proposed draft revision of the recommended international code of hygienic practice for foods for infants and children	Jeffrey Farber
Canadian situation and approaches to addressing the issues related to powdered infant formula	Jeffrey Farber
Powdered infant formula industry practices and standards in the United States of America	Daniel March
Marketing and preparation of powdered infant formula	Hildegard Przyrembel
Practical considerations for risk based calculations for powdered infant formula	Marcel Zwietering
Risk assessment of *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: a discussion document	Prepared by Arie Havelaar[2] Presented by Greg Paoli
Enterobacter sakazakii in Israel: Summary	Colin Block

[1] Video presentation.
[2] Convened an electronic discussion group that addressed risk assessment approaches but unavailable to participate in the meeting.

Overview of powdered infant formula production, consumption, microbiological data and associated public health problems in the Philippines	Celia Carlos
Bacterial contamination of infant food formula in Bangladesh and the region	G. Balakrish Nair
Neonatal infections due to *Enterobacter sakazakii* in the Netherlands, 1975–2002	Harry Muytjens
Microbiological safety of infant formula and related dairy ingredients: summary of information from Australia	Martin Cole
Overview of powdered infant formula production, consumption, criteria, microbiological data and associated public health problems in Japan	Shizunobu Igimi

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Appendix B

DATA RECEIVED IN RESPONSE TO THE FAO/WHO CALL FOR DATA

Source	Information/Data received
Australia – Food Standards Australia New Zealand	*Bacillus cereus* in infant formula: Microbiological risk assessment report
Public Health Laboratory, University of Melbourne	Reports of *Enterobacter sakazakii* to the Victorian Hospital Pathogen Surveillance Scheme (VHPSS), 1990 to 2003
Canada – Office of the Codex Contact Point for Canada	*Enterobacter sakazakii* and powdered infant formula – information from industry
Germany – Federal Institute for Risk Assessment	Discussion by panel of experts on *Enterobacter sakazakii* in powdered milk based infant formula – meeting minutes
International Formula Council	*Enterobacter sakazakii* and powdered infant formula – documentation on behalf of major US infant formula manufacturers
International Special Dietary Foods Industry	Response to the specific issues raised in the FAO/WHO call for data
Japan – Ministry of Health, Labour, and Welfare	Advise to consumers and hospitals on the preparation of powdered infant formula

Source	Information/Data received
Netherlands (the) – Wageningen University	Occurrence of *Enterobacter sakazakii* in food production environments and households – *Lancet* 2004, 363: 39-40 Letter to the editor of *Trends in Food Science and Technology* on the risk of *Enterobacter sakazakii* in powdered milk formula
Philippines (the)	Data on *Enterobacter sakazakii* infections 2001, 2002
Poland – Wroclaw Medical University	Opinion on *Enterobacter sakazakii* contamination of powdered infant formula
United Kingdom – Nottingham Trent University (Stephen Forsythe)	Published papers: Risk profile of *Enterobacter sakazakii*. *Trends in Food Science and Technology*, **14**: 443-454. The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. *Letters in Applied Microbiology*, **38**: 378-382. Papers in press: • Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products • A selected differential medium for *Enterobacter sakazakii* Letters to the editor (*Trends in Food Science and Technology*, 15 February) "On the risk of *Enterobacter sakazakii* in infant milk formula"
United States of America – Food and Drug Administration	Food Advisory Committee, Contaminants and Natural Toxicants Subcommittee: Meeting on *Enterobacter sakazakii* contamination in powdered infant formula; 18-19 March 2003 – briefing material, including white papers, summary minutes, draft report

Appendix C

RISK ASSESSMENT

The development of "what-if" scenarios using a simple risk assessment model that compares the relative risk reduction potential of control measures for *Enterobacter sakazakii* and *Salmonella enterica* in powdered infant formula.

A simplified risk assessment was developed to consider the relative impact of different potential control measures on the microbiological safety of powdered infant formula. The purpose of the risk assessment was to demonstrate a number of concepts that need to be taken into account when quantitatively considering the relative efficacy of different risk reduction strategies. The scope of the risk assessment was restricted to the hospital environment, but the general principles considered should also be pertinent to the home environment. Figure A1 depicts a flow chart illustrating the scope of the powdered formula use encompassed in the risk assessment. The risk assessment was restricted to consideration of two of the identified microbiological hazards, *Enterobacter sakazakii* and *Salmonella enterica*, with a particular focus on the former. The risk estimates described in the risk assessments are based on the expected increase and decrease in risk as measured against a baseline level. That baseline was based on the following assumptions:

- The relative contribution of inherent contamination vs environmental sources to the presence of *E. sakazakii* in reconstituted powdered infant formula is 4:1 (i.e. 80% inherent contamination).
  - The probability of powdered infant formula being contaminated is 0.025.
  - The probability of infant formula being contaminated as result of environmental exposure during rehydration and preparation is 0.00625.

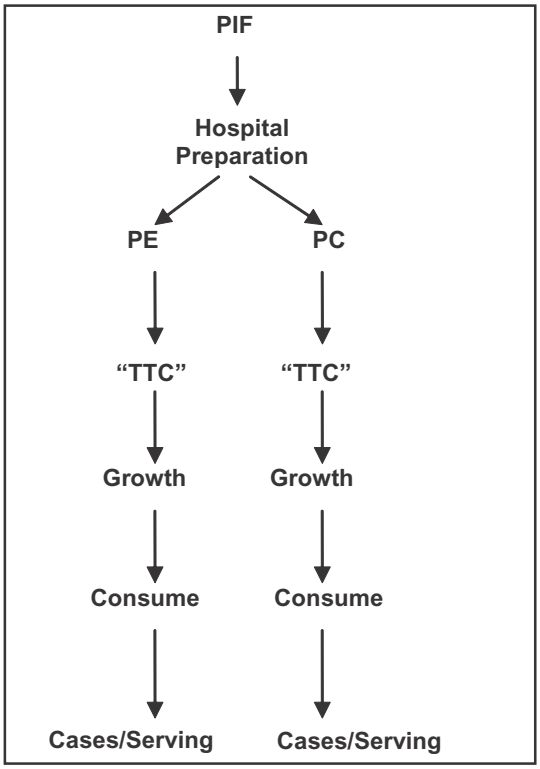

Figure A1. A flow chart depicting the scope of the powdered formula use encompassed in the risk assessment.
PIF, Powdered Infant Formula; PE, Contamination from the preparation environment; PC, Contamination from the infant formula; TTC, Time to consumption

- The “time to consumption” from preparation to complete consumption is 4 hours.
- The temperature of the reconstituted infant formula during feeding is 25°C.

The basic calculations employed in the risk assessment were to assume that *E. sakazakii* had an approximate 2-hour lag phase prior to initiating growth and that the powdered infant formula was rehydrated just prior to use (i.e. refrigeration and storage of the reconstituted product after preparation but before use). By then either manipulating the input data underlying the risk assessment or including additional models of potential treatments, a variety of “what-if” scenarios were developed, described below.

ENTEROBACTER SAKAZAKII

Scenario 1

The purpose of this scenario was to determine what could be achieved as a result of decreasing the frequency/extent of contamination of powdered infant formula with *E. sakazakii*. This decrease could be the result of any of a series of control measures performed at the manufacturing level prior to the sealing of the final product package. Examples of potential interventions could include enhancing plant sanitation, the successful implementation of microbiological criteria, or the adoption of some intervention technology. Five different contamination rate levels in intact packages were assumed, 0.025 (baseline), 0.0100, 0.0010, 0.0001 and 0.0000 (assumed level if able to treat the product after it was packaged). The calculated risk reductions are depicted in Table A1. The limiting factor regardin the degree of risk reduction that can be achieved by this approach is determined by the percent of the total servings of infant formula that become contaminated due to environmental contamination instead of contamination initially present in the powdered infant formula. Thus, the greatest reduction achieved would be limited by the assumed 20% that were environmentally contaminated.

Table A1. Effect of reducing the frequency/extent of contamination of powdered infant formula on the relative risk of *Enterobacter sakazakii* contamination.

Frequency of contamination of powdered infant formula (PC)	Relative risk
0.0250	1.00 (baseline)
0.0100	-1.92-fold
0.0010	-4.29-fold
0.0001	-4.90-fold
0.0000	-4.99-fold

Scenario 2

The purpose of this scenario was to determine the impact that holding the product at room temperature until it is consumed has on the relative risk compared to an assumed norm of a 4-hour "time to consumption" (TTC). The results for TTC values of 0, 2, 4, 6, 8 and 10 hours were compared (Table A2). An additional scenario was examined, with 1% of the feeds at 10-hour TTC, and the remaining 99% of feeds at 4-hour TTC. It is apparent that the relative risk at the longer TTC values increases exponentially, reflecting the exponential growth of the pathogen. It is also important to note that the relative risk at 0 and 2 hours is the same because the assumed lag of 2 hours would have resulted in the microorganism not having grown in these samples. It is apparent that extended holding times can greatly increase the relative risk if *E. sakazakii* is present.

Table A2. Effect of "time to consumption" duration between rehydration and the completion of feeding on the relative risk of associated with *Enterobacter sakazakii*.

Time to consumption (h)	Relative risk
0	-30-fold
2	-30-fold
4	1.00 (baseline)
6	+30-fold
8	+1 000-fold
10	+30 000-fold
10*	+300-fold

Note: *If only 1% of the servings were held for 10 hours.

Scenario 3

In this scenario, the effect of decreasing the rate of contamination due to environmental sources due to enhanced hygiene in the hospital environment is considered. The baseline frequency of contamination was assumed to be 0.00625, and the reduced levels considered included 0.00100, 0.00010 and 0.000000. This scenario (Table A3) is very similar to scenario 1, in that the risk reduction achieved will be limited to the percentage of the servings that are contaminated by *E. sakazakii* present in the formula when the product is unsealed.

Table A3. The effect of decreasing the rate of environmental contamination on the relative risk of *E. sakazakii* in rehydrated infant formula.

Frequency of contamination of rehydrated infant formula due to environmental contamination (PE)	Relative risk
0.00625	1.00 (baseline)
0.00100	-1.20-fold
0.00010	-1.24-fold
0.00000	-1.24-fold

Scenario 4

The purpose of this scenario was to explore the impact of having different susceptibilities among different age populations. In this scenario, a 28-day neonate (newborn up to 4 weeks) was assumed to represent a baseline susceptibility and it was assumed that very low-birth-weight (VLBW, i.e. under 1 500 g) neonates were 10 times more susceptible, 6-month olds were tenfold less susceptible, and 12-month olds were 100 times less susceptible. (These numbers are totally hypothetical at this point and information of relative susceptibilities is a research data requirement.)

Due to the essentially linear nature of the dose-response relationship at these levels of contamination, the increases and decreases in risk are proportional to the increases and decreases in susceptibility (Table A4). This is a highly simplified consideration of the scenario, since there was no attempt to consider the differential in consumption rates at the various ages. However, one additional factor that was considered was the interaction with environmental temperature. An elevated environmental temperature (32ºC) was considered for the VLBW infants. Likewise, the same elevated temperature was considered for the neonates to determine the impact of being in a tropical climate with a higher ambient temperature. It is apparent that the higher environmental temperature increases the relative risk due to the increased growth of *E. sakazakii*.

Table A4. The hypothetical example of the impact that varying degrees of susceptibility could have on the relative risk associated with the presence of *Enterobacter sakazakii* in rehydrated infant formula. Also considered was the impact of an elevated environmental temperature (25°C vs 32°C).

Age group	Assumed relative susceptibility	Ambient temperature (°C)	Relative risk
VLBW	+10-fold	25	+10-fold
VLBW	+10-fold	32	+66-fold
Neonate	1.00 (baseline)	25	1.00 (baseline)
Neonate	1.00 (baseline)	32	+6.6-fold
6 months	-10-fold	25	-10-fold
12 months	-100-fold	25	-100-fold

Scenario 5

The effect of subjecting formula to a post-preparation treatment capable of decreasing the level of *E. sakazakii* by 4-log cycles (99.99%) was evaluated for its impact on relative risk. This type of treatment (e.g. rehydration of powdered infant formula with hot water, the subsequent heating of filled bottles) would decrease contamination due to both initial contamination of the powdered infant formula during manufacture and the environmental recontamination of the formula during preparation. The baseline in this instance would be formula prepared with room temperature water and not subjected to any post-preparation treatment vs formula that was treated (Table A5). As a means of demonstrating the impact of only performing such treatments some of the time, a third situation where only 80% of the servings of formula were subjected to post-preparation was also considered (Table A5). It is apparent that such post-preparation treatments can significantly reduce the relative risk; however, the total risk reduction would be dependent on using such treatments on a consistent basis.

Table A5. Reduction of relative risk associated with *Enterobacter sakazakii* in rehydrated infant formula if subjected to a post-preparation treatment that effectively reduced the levels of the pathogen by 4-log cycles (99.99%).

Treatment	Relative risk
Not treated	1.00 (baseline)
4-log treatment	-10 000-fold
80% of servings receive a 4-log treatment	-5-fold

Scenario 6

This scenario explores the impact of combining control measures on the relative risk. Three subscenarios were considered:

- reducing the frequency of inherent contamination (PC) from 0.025 to 0.001 and reducing the frequency of environmental contamination (PE) from 0.00625 to 0.00100;
- reducing the PC from 0.025 to 0.001 and shortening the TTC to 2 hours; and
- reducing the PC from 0.025 to 0.001, the PE from 0.006 25 to 0.00100, and shortening the TTC to 2 hours.

It is apparent that combining a series of treatments can effectively increase the degree of risk reduction achieved (Table A6). These results should be compared with the results achieved with individual treatments in Tables A1-A3.

Table A6. Effect of combining risk reduction activities on the relative risk of *Enterobacter sakazakii* associated with powdered infant formula.

Control measures	Relative risk
No control measures	1.00 (baseline)
Reduce PC to 0.001 and PE to 0.001	-16-fold
Reduce PC to 0.001 and TTC to 2 hours	-132-fold
Reduce PC to 0.001, PE to 0.001 and TTC to 2 hours	-480-fold

The basic risk control principles demonstrated in the above scenarios for *E. sakazakii* would hold true for *S. enterica*, but the specific risk reductions achieved would vary to some degree based on the mode and sources of *Salmonella* contamination and its growth and survival characteristics. As a means of demonstrating this,

two of the above scenarios were rerun for *S. enterica* so that the relative risk reductions expected with the microorganisms could be compared. In these scenarios the assumed TTC and environmental temperatures were kept the same, but the growth rate for *S. enterica* is somewhat slower than *E. sakazakii* and since the frequency of contamination with salmonellae, both initially in powdered infant formula and environmentally, appears to be substantially lower, the assumed PE and PC were both set at 0.0025.

SALMONELLA ENTERICA

Scenario 1

This is a repetition of *E. sakazakii* scenario 2 where the impact of TTC was examined. While the same pattern of relative risk reductions are observed for the two microorganisms, the somewhat slower growth rate of *S. enterica* results in the relative risk reductions being smaller (Table A7).

Table A7. Comparison of the impact of "time to consumption" durations on the relative risks associated with *Enterobacter sakazakii* and *Salmonella enterica* in powdered infant formula.

Time to consumption (h)	Relative risk – *E. sakazakii*	Relative risk - *S. enterica*
0	-31-fold	-12-fold
2	-31-fold	-12-fold
4	1.00 (baseline)	1.00 (baseline)
6	+31-fold	+12-fold
8	+1 000-fold	+150-fold
10	+30 000-fold	+1 100-fold

Scenario 2

This is a repetition of *E. sakazakii* scenario 6 that examined the effect of combining risk reduction strategies. In this scenario, the changes in PC, PE and TTC for *E. sakazakii* were kept the same as in *E. sakazakii* scenario 6. The changed conditions for *S. enterica* were a decrease in PC from 0.0025 to 0.0001, a decrease in PE from 0.0025 to 0.0010, and a decrease in TTC to 2 hours. Again, the pattern of relative risk reductions were similar for the two microorganisms, but the specific level of control achieved was dependent on the frequency and mode of contamination and the characteristics of the two microorganisms (Table A8).

Table A8. Effect of combining risk reduction activities on the relative risks of *Enterobacter sakazakii* and *Salmonella enterica* associated with powdered infant formula.

Control measures	Relative risk – *S. enterica*	Relative risk – *E. sakazakii*
No control measures	1.00 (baseline)	1.00 (baseline)
Reduce PC and PE	-4.5-fold	-16-fold
Reduce PC and TTC	-23-fold	-132-fold
Reduce PC, PE and TTC	-56-fold	-480-fold

BACKGROUND TO ASSUMPTIONS

Estimation of the ratio of *Enterobacter sakazakii* to other Enterobacteriaceae in neonatal and infant bloodstream infection (BSI) and meningitis:

Best guess	=	0.002
Upper bound	=	0.004
Lower bound	=	0.001

The proportion of *E. sakazakii* to all *Enterobacter* spp. was derived from data provided to the consultation by Dr Martin Cole (Australia) (Appendix B).

All age groups:	Reports of Enterobacter infections from blood/CSF specimens from 1990-2003
E. sakazakii	21
All other *Enterobacter* spp.	1 676
Estimated proportion	~1%

The proportion of *Enterobacter* spp. among all Enterobacteriaceae was derived from the publications listed below. It was estimated to be about 20%. The ratio of *E. sakazakii* to all Enterobacteriaceae is then 1% of 20% or 0.002 (best estimate). The upper and lower estimates are based on expert opinion.

The values below are rough estimates based on data extracted from a number of scientifically heterogeneous publications from several countries:

Country		n*	% *Enterobacter*/ Enterobacteriaceae
Philippines[a]	All infants up to 3 months	17 BSI 8 meningitis	18% in BSI 50% in meningitis
USA[b]	1st late onset sepsis BSI in VLBW infants	196	17%
USA[c]	Fulminant late onset neonatal sepsis	13	31%
Cameroon[d]	Neonatal BSI and meningitis	53	21%
Taiwan[e]	Neonatal meningitis	31	16%
Jordan[f]	Neonatal meningitis	53	19%
Mexico[g]	Neonatal meningitis	22	9%

* Best estimate of total Enterobacteriaceae from reference.

[a] Gatchalian, S.R., Quiambao, B.P., Morelos, A.M., Abraham, L., Gepanayao, C.P., Sombrero, L.T., Paladin, J.F., Soriano, V.C., Obach, M., & Sunico, E.S. 1999. Bacterial and viral etiology of serious infections in very young Filipino infants. *Pediatric Infectious Disease Journal*, **18**(10): S50-55.

[b] Karlowicz, M.G., Buescher, E.S., & Surka, A.E. 2000. Fulminant late-onset sepsis in a neonatal intensive care unit, 1988-1997, and the impact of avoiding empiric vancomycin therapy. *Pediatrics 2000*, **106**: 1387-1390.

[c] Kago, I., Wouafo Ndayo, M., Tchokoteu, P.F., Koki Ndombo, P., Ekoe, T., Doumbe, P., Tietche, F., & N'Koulou, H. 1991. [Neonatal septicaemia and meningitis caused by gram-negative bacilli in Yaounde: clinical

bacteriological and prognostic aspects.] *Bulletin de la Societe des Sciences Medicales du Grand-duche de Luxembourg*, **84**(5 pt 5): 573-581.

[d] Stoll, B.J., Hansen, N., Fanaroff, A.A., Wright, L.L., Carlo, W.A., Ehrenkranz, R.A., Lemons, J.A., Donovan, E.F., Stark, A.R., Tyson, J.E., Oh, W., Bauer, C.R., Korones, S.B., Shankaran, S., Laptook, A.R., Stevenson, D.K., Papile, L.A., & Poole, W.K. 2002. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD neonatal research network. *Pediatrics*, **110**: 285-291.

[e] Chang Chien, H.Y., Chiu, N.C., Li, W.C., Huang, F.Y. 2000. Characteristics of neonatal bacterial meningitis in a teaching hospital in Taiwan from 1984-1997. *Journal of Microbiology, Immunology and Infection*, **33**: 100-104.

[f] Daoud, A.S., Al-Sheyyab, M., Abu-Ekteish, F., Obeidat, A., Ali, A.A., & El-Shanti, H. 1996. Neonatal meningitis in northern Jordan. *Journal of Tropical Pediatrics*, **42**: 267-270.

[g] Sanchez-Saucedo, L.U., Gonzalez-Yunez, R.A., Avila-Figueroa, C., & Santos, J.I. 1990. Neonatal meningitis: observations on its etiology, mortality and sequelae (Spanish). *Boletin medico del Hospital Infantil de Mexico*, **47**(11): 772-776.

Estimation of the ratio of *E. sakazakii* susceptibility between high risk groups and other infants

Based on the United States of America 2002 FoodNet laboratory survey for *E. sakazakii* isolates and United States of America census population data:

- Four sterile site infections. Total FoodNet population of infants (<1 year old) of 402 407. Assuming all identified isolates were from infants, the rate is 1/100 000 population. The estimated population of LBW/VLBW infants in FoodNet was 46 202. Assuming all identified isolates were from LBW/VLBW infants, the rate is 8.7/100 000.

- Using LBW/VLBW population as the model susceptible subpopulation, the ratio of susceptibility is 8.7 to 1, or approximately 10 (best estimate). The estimated range is based on whether one infant (1/4 of the cases) in the FoodNet survey was misclassified using the above assumptions. Thus, the range would be 7.5 to 12.5.

It was not attempted to estimate the relative susceptibilities among different susceptible subpopulations: e.g. immunocompromised non-neonate infants including HIV-infected, LBW neonates and immunocompromised neonates.

Estimates of population sizes

Estimates of population sizes based on data from the 2000 United States census:[a]

Age	Estimated population
28 days or younger	336 130
29 days to less than 1 year	3 697 589
<1	4 033 719
1-4	15 575 428
5-9	19 900 837
10-19	41 512 600
20-29	39 185 524
30-39	42 871 294
40-49	44 303 788
50-59	33 772 415
60-69	21 192 111
≥70	26 020 990
Total	**288 368 706**

[a]Available from the U.S. Census Bureau (www.census.gov).

Estimates of case numbers, population and rates in United States FoodNet sites of *Salmonella* infections by age group, 2002

	≤28 days	29 days - <1 year	<1 year	1-4 years	5-9 years	10-19 years
Rate	52.0	147.3	139.4	59.29	18.52	10.92
Number	23	717	740	1 210	480	590
Population	44 237.2	486 611.8	530 849	2 040 976	2 592 406	5 403 341

	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years	≥70 years	**Total**
Rate	12.66	11.94	10.25	9.81	10.23	11.56	**15.99**
Number	643	708	618	447	270	365	**6 071**
Population	5 078 736	5 931 766	6 030 705	4 555 746	2 638 867	3 158 296	**37 961 688**

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