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Enterobacter sakazakii and Salmonella in powdered infant formula

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CORRIGENDA

A minor error in the relative risk calculations for some of the preparation and handling scenarios where powdered infant formula (PIF) was reconstituted at 60°C has been identified. This means that the relative risk previously reported for certain scenarios where PIF was reconstituted at 60°C was an underestimation of the risk reduction at this reconstitution temperature. This change **does not affect the qualitative conclusions** of the expert meeting regarding the general nature of the impact of higher reconstitution temperatures in handling scenarios. The specific relative risk reductions for certain preparation and handling scenarios where PIF was reconstituted at 60°C in Tables 10, 11, 12, 15, 16 and 19 should be updated as shown below. (- indicates no change from what is currently presented in the report).

	Table 10	***Table 11***	***Table 12***
Preparation, storage and feeding scenarios	60°C	60 °C	60 °C
Refrigeration, re-warming, short feeding period	- 8.0	- 18.0	-31.1
Refrigeration, no re-warming, extended feeding period	- 13.7	- 24.1	-25.5
Refrigeration, no re-warming, short feeding period	- 13.7	- 30.5	-52.7
No refrigeration, re-warming, extended feeding period	-	-	-3.1
No refrigeration, re-warming, short feeding period	- 10.1	- 33	-98.1
No refrigeration, no re-warming, Extended feeding period	- 1.5	-	-4.2
No refrigeration, no re-warming, short feeding period	- 14.6	- 41.1	-98.1

	Table 15	***Table 16***
Preparation, storage and feeding scenarios	60°C	60°C
Refrigeration, re-warming, 0.33 hour feeding period	-6	-15
Refrigeration, re-warming, 1 hour feeding period	-	-3
No refrigeration, re-warming, 0.33 hour feeding period	-8	-28
No refrigeration, re-warming, 1 hour feeding period	-	-6

	Table 19
Scenarios	60°C
Cool room temperature (20°C)	
1: Mix in bottle, cool & hold for 1 hour, no re-warming, then feed within 20 minutes	-10
2: Mix in a bottle then cooled within 5 minutes to 37°C, and feeding for 20 minutes (37°C)	-14
3: 25 L container, no refrigeration, 1 hour holding, 30 minutes feeding	> - 100,000
4: 25L container, 1 hour prep time, refrigeration 6 hours, re-warmed, 30 minutes feeding	-44
5: 1L container, no refrigeration, 1 hour holding, 30 minutes feeding	-14
6: 1l container, 1 hour prep. time, refrigeration 6 hours, re-warm, 30 minutes feeding	-2.1
Warm room temperature (30°C)	
1: Mix in bottle, cool & hold for 1 hour, no re-warming, then feed within 20 minutes	-32
2: Mix in a bottle then cooled within 5 minutes to 37°C, and feeding for 20 minutes (37°C)	-41
3: 25 L container, no refrigeration, 1 hour holding, 30 minutes feeding	> - 100,000
4: 25L container, 1 hour prep time, refrigeration 6 hours, re-warmed, 30 minutes feeding	-2,044
5: 1L container, no refrigeration, 1 hour holding, 30 minutes feeding	-31
1 L container, 1 hour prep. time, refrigeration 6 hours, re-warm, 30 minutes feeding	-4.5

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The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) would like to express their appreciation to all those who contributed to the preparation of this report through the provision of their time and expertise, data and other relevant information. In particular, the work of Greg Paoli and Emma Hartnett in developing the risk assessment model is acknowledged.

Appreciation is also extended to all those who responded to the call for data that was issued by FAO and WHO and brought to our attention information that was not readily available in the mainstream literature and official documentation.

The preparatory work and expert meeting convened to prepare this report were coordinated by the Secretariat of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA). This included Sarah Cahill and Maria de Lourdes Costarrica in FAO, and Peter Karim BenEmbarek and Jørgen Schlundt in WHO. The Secretariat was supported by Kaye Wachsmuth, who also assisted in the finalization of the report. Publication of the report was coordinated by Sarah Cahill. Ruth Duffy edited the report. The work was supported and funded by the FAO Food Quality and Standards Service and the WHO Department of Food Safety, Zoonoses and Foodborne Diseases.

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DECLARATIONS OF INTEREST

Five of the 16 experts who participated in this meeting declared an interest in the topics under consideration:

Anna Bowen: Fifty per cent of her salary is covered by a commercial entity as part of a Cooperative Research and Development Agreement. While the company manufactures products for infants, it does not manufacture any infant formula products.

Jean-Louis Cordier: He is an employee of a manufacturer of powdered infant formula products.

Susan Dobson: She acted as a reviewer of a risk assessment on *Enterobacter sakazakii* in powdered infant formula for a commercial entity in Australia.

Stephen Forsythe: His research related to isolation media for *Enterobacter sakazakii* and the virulence of Enterobacteriaceae is funded by two commercial entities, one of which manufactures powdered infant formula.

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

The activities of Drs Cordier, Forsythe and Zwietering were considered to represent potential conflicts of interest. Therefore, these experts did not participate in the final adoption of the conclusions and recommendations of the meeting.

FOREWORD

Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food at both national and international level. Increasing food-borne disease incidence over recent 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 patients and/or foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately new tools 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 number 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 as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing these data and information and gaining a better understanding of the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as identify the types of data necessary for estimating and optimizing mitigating interventions.

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

combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at international level.

The Food Quality and Standards Service (FAO) and the Department of Food Safety, Zoonoses and Foodborne Diseases (WHO) are the lead units responsible for this initiative. The two groups have worked together to develop MRA at international level for application at both national and international level. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few.

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

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and the work to date clearly indicates that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide member countries, the 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

ATCC	American Type Culture Collection
BAM	Bacteriological Analytical Manual (FDA)
BGA	Brilliant green agar
BPW	Buffered peptone water
°C	Degree Celsius
cal	Calorie
CCFH	Codex Committee on Food Hygiene
CCP	Critical control point
CDC	Centers for Disease Control and Prevention
CDT	Cytolethal distending toxin
cfu	Colony forming unit
CI	Confidence intervals
cm	Centimetre
DFI	Druggan-Forsythe-Iverson
DW	Deionized water
EC	European Communities
EE	Enterobacteriaceae enrichment (broth)
ESIA	*Enterobacter sakazakii* isolation agar
ESPM	*Enterobacter sakazakii* chromogenic plating medium
ESSM	*Enterobacter sakazakii* screening medium
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FSMP	Formula for special medical purposes
g	Gram
GHP	Good hygiene practice
GMP	Good manufacturing practice
HACCP	Hazard Analysis and Critical Control Point (System)
IAFP	International Association for Food Protection
IDF	International Dairy Federation
ISDI	International Special Dietary Foods Industry
ISO	International Organization for Standardization
IU	International units
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
kcal	Kilocalorie
kg	Kilogram
mg	Milligram
ml	Millilitre
MLC	Mean log concentration
mLST	Modified lauryl sulfate tryptose (broth)

MPN	Most probable number
NGO	Non-governmental organization
NICU	Neonatal intensive care unit
OR	Odds ratio
PCR	Polymerase chain reaction
PIF	Powdered infant formula
TS	Technical Standard
TSA	Tryptic soy agar
USDA	United States Department of Agriculture
VRBGA	Violet red bile glucose agar
VRBLA	Violet red bile lactose agar
WHA	World Health Assembly
WHO	World Health Organization
XLD	Xylose lysine deoxycholate agar
µg	Microgram
UHT	Ultra-heat treated

EXECUTIVE SUMMARY

The 37th Session of the Codex Committee on Food Hygiene (2005) requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to extend the scientific advice provided by the expert meeting on "*Enterobacter sakazakii* and other microorganisms in powdered infant formula" held in Geneva in 2004 (FAO/WHO, 2004). Accordingly, a technical meeting was convened on *E. sakazakii* and *Salmonella* in powdered infant formula (FAO, Rome, 16-20 January 2006) to consider any new scientific data and to evaluate and apply a quantitative risk assessment model for *E. sakazakii* in powdered infant formula (PIF). This technical meeting also aimed to provide input to Codex for the revision of the Recommended International Code of Hygienic Practice for Foods for Infants and Children.

A review of *E. sakazakii* infections worldwide expanded the findings of the 2004 meeting. While noting that *E. sakazakii* has caused invasive infection in all age groups, the meeting reiterated the findings of the 2004 FAO/WHO meeting that infants appear to be the group at particular risk. It also highlighted that neonates (≤28 days) and infants under 2 months of age are at greatest risk. An analysis of 45 cases indicated that there appear to be two distinct infant groups in terms of the syndrome developed as a result of an *E. sakazakii* infection – premature infants who develop bacteraemia outside of the neonatal period (with most cases occurring in infants under 2 months of age) and term infants who develop meningitis during the neonatal period. This difference in timing of infection may however be related to differences in timing of exposure to *E. sakazakii* rather than differences in susceptibility, but it was also noted that any infant could develop either syndrome at any age.

In considering the report of a recent outbreak investigation of nine cases and two deaths associated with the consumption of *E. sakazakii*-contaminated PIF, the meeting noted the value of coordinated disease surveillance and rapid response. The combined efforts of public health and regulatory officials and manufacturers were considered to be an important aspect of the management of the risks associated with *E. sakazakii* and *Salmonella* in PIF.

Based on the research findings reported since 2004, the meeting noted that *E. sakazakii* is now recognized as a genotypically and phenotypically diverse bacterial species. While specific methods for the isolation and identification of *E. sakazakii* have been developed, studies are ongoing to enhance this methodology and to address the virulence and the ecology of this pathogen.

The meeting reviewed a risk assessment model that had been developed to describe the factors leading to *E. sakazakii* infection in infants and to identify potential risk mitigation strategies. The risk assessment model enables this by facilitating the comparison of different levels of product contamination and of different preparation, handling and feeding scenarios. In addition, it provides the means to evaluate microbiological criteria and sampling plans in terms of the risk reductions achieved and the percentage of product lot rejected. The risk assessment model estimates the relative risk of illness from *E. sakazakii* posed to infants from intrinsically contaminated PIF, but it

does not consider contamination or recontamination from the environment or other sources post-manufacture.

After review and discussion of the quantitative risk assessment model on *E. sakazakii* in PIF, the technical meeting found it to be accurate and valid, based on the approach taken, the assumptions made and the interpretation of data. New data from industry and on consumer and hospital practices related to the use of PIF were incorporated into the model, which was then used to evaluate different risk-reduction scenarios.

Although the risk assessment does not model specific industry practices, it can evaluate the impact of different levels of contamination in the dry PIF product before use. As requested by Codex, the meeting used the risk assessment model to evaluate the relative risk reduction associated with different risk management interventions for *E. sakazakii*. Preparation, storage and handling practices for PIF were addressed as a series of scenarios. One scenario was selected as the baseline, and others were compared to determine their relative risk to the baseline. In general, the use of 70°C mixing water to reconstitute PIF significantly reduced risk. In other scenarios, reconstitution of PIF with 40° and 50°C mixing water (unless consumed immediately), holding bottles at room temperature and long feeding periods were associated with increased relative risk. The meeting acknowledged that not all PIF products were formulated to be mixed at 70°C and recognized that, if the use of hot water is recommended, specific labelling may be required.

The meeting concluded that some of the current preparation instructions on PIF product labels and those recommended by health authorities may lead to increased risk of *E. sakazakii* illnesses, and that these should be reviewed in the light of the risk assessment results. The meeting also considered that the assessment could be used by FAO/WHO to develop guidance, as requested by the World Health Assembly (WHA) in 2005, on “the preparation, use, handling, and storage of infant formula so as to minimize risks where infants cannot be or are not fed breast milk”.

The risk assessment model was also used to evaluate risk reductions associated with the implementation of microbiological criteria and related sampling plans. The model was found to be a valuable tool to assess and compare a series of criteria and sampling plans and their impact on risk reduction and on the amount of product rejected compared to a situation where no sampling plan was implemented. The meeting concluded that this tool could be applied for *E. sakazakii* and/or Enterobacteriaceae by risk managers within Codex and FAO and WHO member countries.

While the risk assessment model cannot be used to evaluate the impact of specific industry practices, such as wet- and dry-cleaning processes, the meeting did consider the available information on industry practices. The meeting noted that data available from the PIF industry indicate a reduction in both *E. sakazakii* and *Salmonella* in PIF through strict separation of the wet and dry phases of product manufacture, and through the use of dry-cleaning procedures (or at least minimizing the amount of water used for cleaning procedures whenever possible in specific segments of the manufacturing environment). However, it was noted that it may not be feasible to eliminate the use of water when producing some products, e.g. hypoallergenic powdered infant formula.

Salmonella has been considered a hazard in PIF and related milk-based products for several decades. A review of recent and historical outbreaks of salmonellosis linked to intrinsically contaminated PIF confirmed its public health importance and the need for risk management vigilance. Outbreaks were only identified by laboratory-based food-borne disease surveillance systems and usually by some unique quality of the epidemic strain, e.g. a rare serotype or the ability to ferment lactose. The meeting recognized that salmonellosis due to contaminated PIF was probably under-reported.

The meeting agreed with the 2004 Geneva meeting that current microbiological criteria for *Salmonella* in PIF are considered adequate. The meeting further concluded that a risk assessment for *Salmonella* in PIF was possible. However, it considered that the extensive information currently available on *Salmonella* and its behaviour provided a good basis for risk management decisions and that there was currently no obvious need for a quantitative risk assessment on *Salmonella* in PIF.

RECOMMENDATIONS

The meeting first re-endorsed the recommendations made by the 2004 FAO/WHO meeting on this issue. The additional recommendations made by the expert meeting are the following:

To member countries, FAO and WHO

- Develop prevention strategies for *E. sakazakii* infections caused by contaminated PIF that address the different stages of production and preparation and use of PIF, taking into consideration the risk to infants – both within and beyond the neonatal period and of any immune status.

- Develop educational messages on the safe handling, storage and use of powdered infant formula, including the health hazards of inappropriate preparation and use; target healthcare workers, parents and other caregivers, in both hospitals and the community, since *E. sakazakii* infections have occurred in hospital and home settings.

- Review and revise product labels, as appropriate, to enable caregivers to handle, store and use the product safely, and to make clear the health hazards of inappropriate preparation.

- Encourage member countries to establish surveillance and rapid response networks, and facilitate coordinated investigation by clinicians, laboratorians, and public health and regulatory officials, to enable the timely recognition and cessation of outbreaks of illness associated with *E. sakazakii* and the identification of contaminated PIF.

- Encourage countries to enhance laboratory-based surveillance, including reporting to Salm-Surv, the WHO salmonellosis worldwide surveillance network, since laboratory-based surveillance is the only way in which past outbreaks of salmonellosis associated with intrinsically contaminated PIF have been recognized.

- Encourage laboratories conducting surveillance for *Salmonella*, and manufacturers and regulators testing for *Salmonella* in PIF, to use isolation and diagnostic methods which can identify lactose-fermenting strains of this organism, since these have been the cause of some of the outbreaks of salmonellosis associated with PIF.

- Encourage scientists to determine the optimal isolation and identification methods for *E. sakazakii*, taking into account the new research data demonstrating genetic and phenotypic diversity in the species.

- Encourage research to determine ecological niches and virulence factors for *E. sakazakii* to better target risk mitigation strategies and control measures.

- Develop and review international guidelines – as requested by the 2005 WHA – educational messages and product labels regarding the preparation, storage and handling of PIF, considering the results of the *E. sakazakii* risk assessment model presented in this report.

- At this time, the meeting did **not** recommend that FAO/WHO conduct a quantitative risk assessment for *Salmonella* in PIF.

To Codex

- Make risk management recommendations based on the outputs of the JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment) risk assessment for *E. sakazakii* in PIF and the estimates of potential risk reductions of proposed control measures. In particular, give consideration to the elaboration of sampling plans and microbiological criteria for *E. sakazakii* and Enterobacteriaceae in PIF, and labelling recommendations for PIF, specifically in the revision of CAC/RCP 21-1979.

To member countries

- Apply the risk assessment in developing national risk management strategies for the reduction of risks associated with PIF, such as appropriate educational programmes.

- Encourage industry to effectively implement preventive measures and to strengthen those measures that further minimize entry of the microorganisms of concern into the manufacturing environment and avoid their multiplication therein.

To industry

- Effectively implement, to the extent possible and feasible, preventive measures, including the strengthening of those measures that further minimize entry of the microorganisms and avoid their multiplication, such as the exclusion of water from the processing environment to the extent possible and feasible. The most effective means of achieving the latter is considered to be the implementation of systematic dry-cleaning.

- Support research that allows further evaluation of the effectiveness of Enterobacteriaceae as an indicator organism pointing to conditions in the manufacturing environment or final product that have increased potential for harbouring *E. sakazakii* or *Salmonella*.

To FAO and WHO

- In future “calls for data”, provide more specific details with regard to the type and format of data needed in order to enable providers to target their efforts towards the provision of data which can be effectively used in the risk assessment process.

1. INTRODUCTION

In working to ensure the safety and availability of food for infants and young children – one of the more vulnerable groups in our society – the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) began addressing the issue of *Enterobacter sakazakii* and other microorganisms in powdered infant formula (PIF) in February 2004, through the implementation of a joint technical meeting (FAO/WHO, 2004). Follow-up activities included the provision of information and guidance on the risks associated with *E. sakazakii* and other microorganisms in PIF to Codex and member countries and the elaboration of a risk assessment tool to enable the evaluation of a range of intervention strategies. In 2005, the World Health Assembly (WHA) adopted a resolution requesting WHO, in collaboration with FAO, to develop international guidelines and educational messages regarding the preparation, storage and handling of PIF. In response to these developments, a second meeting was implemented from 16 to 20 January 2006. This focused specifically on *E. sakazakii* and *Salmonella* in PIF and addressed, in particular, a number of questions on these pathogens posed by the 37th Session of the Codex Committee on Food Hygiene (CCFH) (14-19 March 2005), the associated risk and means of evaluating control measures. This report documents the discussions and recommendations of that meeting.

The meeting was chaired by Mr Aamir Fazil, Public Health Agency of Canada. A total of 16 experts from 11 countries participated in the meeting in their independent capacities and not as representatives of their governments, employers or institutions. These included one expert from the infant formula industry and two academics whose research is being funded by the industry. While these experts participated in the general discussions and exchange of information, they did not participate in the final adoption of the conclusions and recommendations of the meeting.

1.1 BACKGROUND

As noted in the first FAO/WHO report on this issue (FAO/WHO, 2004), the 35th Session of the CCFH, following the review of a risk profile, began to address the concerns of member countries about the occurrence of *E. sakazakii* infections among infants due to consumption of contaminated PIF. The risk profile had concluded that *E. sakazakii* was emerging as a hazard in PIF causing infrequent but often severe infections in infants, particularly low-birthweight and/or immunocompromised neonates (≤28 days) in the hospital setting. Other Enterobacteriaceae, particularly *Salmonella*, were also documented as causing disease in infants through low level contamination of PIF. The 35th Session of CCFH undertook to revise the Recommended International Code of Hygienic Practice for Foods for Infants and Children (CAC/RCP 21-1979) and created a Codex Working Group led by the Canadian delegation to begin this work. To support development of the revised code (to be renamed the Code of Hygienic Practice for Powdered Formulae for Infants and Young Children), the CCFH also asked for expert advice from FAO/WHO.

The FAO/WHO meeting convened from 2 to 5 February 2004 (FAO/WHO, 2004) confirmed and extended the conclusions of the risk profile considered by the CCFH, and produced an initial

risk assessment, which was considered preliminary in nature. A more complex risk assessment model was initiated during that session. The meeting made a number of recommendations to FAO, WHO, Codex, their member countries, NGOs and the scientific community, summarized as follows:

- In situations where infants are not breastfed, caregivers, particularly of infants at high risk, should be regularly alerted that PIF 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 appropriate, 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 heating of reconstituted formula).

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

- 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 should be enhanced or developed.

- The infant food industry should be encouraged to develop a greater range of commercially sterile 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 PIF. 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.

- In revising its code of practice, Codex should better address the microbiological risks of PIF, and establish appropriate microbiological specifications for *E. sakazakii* in PIF.

- 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-birthweight infants, or where there is lack of refrigerated storage.

- 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 PIF) 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 levels in reconstituted PIF.

The more complex risk assessment model was further developed by JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment) consultants and was presented to the Codex Working Group in November 2004. That group identified "handling and storage of powdered infant formula and the associated risks of *E. sakazakii* infection in infants" as a particular area of concern, given the low-level presence of pathogens in this product. It was acknowledged that the risk assessment model might also be applied to evaluate the relative risk associated with different levels of *E. sakazakii* in the powder at the end of manufacture as well as the risk reduction achieved through the implementation of different microbiological criteria, sampling and testing programmes. This JEMRA work (CCFH 05/37/9) and a draft revised code were presented at the 37th Session of CCFH, in Buenos Aires, Argentina, 14-19 March 2005.

After review and discussion of the draft revised code, the 37th Session of CCFH asked FAO and WHO for additional information and scientific advice and

"requested FAO/WHO to convene an Expert Meeting to look at the following issues:

i. Taking into consideration any existing and new information on *E. sakazakii* and existing and new data on *Salmonella*, [1] identify if possible the distribution of cases linked to the different types of powdered formula[2] as a function of age, and define specifically the age groups and other groups of infants and young children at greatest risk.

ii. Review the dose-response and growth models of *E. sakazakii*, using new data that is becoming available.

iii. Evaluate specific control measures for different manufacturing operations (depending on data provided by manufacturers of powdered formula), which could minimise product contamination by *E. sakazakii* and evaluate how microbiological criteria for Enterobacteriaceae can be used as an indication of process hygiene.

iv. a) In light of new data submitted by ISDI/industry request that the risk assessment be updated to take into consideration this new information and make the output available to the Working Group (in charge of redrafting the proposed draft Code – see paragraph 56) for the development of microbiological criteria; b) Use the risk assessment to evaluate the risk reduction associated with various control measures, microbiological criteria and sampling plans.

[1] The need for any risk assessment work on *Salmonella* will be reviewed following an initial literature review and consideration of available data.

[2] "Powdered formula" is used here to describe powdered infant formula, follow-up formula, formula for special medical purposes (FSMP) intended for infant, and human milk fortifiers, as described in Section 6.1 of the 2004 Meeting Report "*Enterobacter sakazakii* and other microorganisms in powdered infant formula" (FAO/WHO, 2004).

v. Request that the aspects of the risk assessment model addressing preparation, storage and handling of powdered formula be revisited to ensure that all currently used preparation procedures are evaluated."

In May 2005, the World Health Assembly (WHA) passed a resolution, WHA58.32, which acknowledged the outcome of the JEMRA and CCFH meetings. Among other things, the resolution urged member states to take certain protective and preventive actions – for example, training caregivers, making information available, working with manufacturers to continue to reduce hazards in PIF – in order to minimize the exposure of infants and young children to any hazards associated with PIF. The WHA resolution also requested: that Codex urgently complete its work related to PIF standard setting; that WHO and FAO develop guidelines for healthcare providers on the preparation, use, handling and storage of infant formula; and that WHO take the lead in supporting independently reviewed research in the area of pathogens in PIF.

Outbreaks and sporadic cases of illness and death in infants due to *E. sakazakii*- and *Salmonella*-contaminated PIF are probably under-reported. Nevertheless, reports of cases and outbreaks have continued during 2004 and 2005. In late 2004, consumption of *E. sakazakii*-contaminated PIF was linked to nine infections with two deaths in an outbreak among infants in France (InVS, 2006; Coignard *et al*., 2006) and to five infections with one death among infants in New Zealand (Ministry of Health, New Zealand, 2005). In 2005, *Salmonella* Agona linked to PIF caused an outbreak among infants in France (InVS, 2005).

1.2 OBJECTIVES

To respond in a timely way to the ongoing public health concerns regarding *E. sakazakii* and *Salmonella* in PIF, the meeting aimed to:

- Review the risk assessment model for accuracy and validity in terms of the approach taken, the assumptions made, the interpretation of data etc.

- Review any new epidemiological data that have become available, specifically regarding infants at greatest risk of *E. sakazakii* infection.

- Review any new data that have become available in relation to *Salmonella* in PIF and if necessary revise the recommendations of the previous meeting with regard to this pathogen.

- Using the risk assessment model, estimate the potential risk reduction associated with the implementation of various microbiological criteria and their associated sampling plans for PIF production.

- Specifically address *E. sakazakii* in the product and Enterobacteriaceae as an indicator of process control.

- Using the risk assessment model, estimate the potential risk reduction of control measures implemented during reconstitution, storage and handling/feeding of PIF.

- Based on the risk assessment model and other relevant data, provide a response to the issues raised by the 37th session of the CCFH, as outlined above.

1.3 SCOPE

This meeting builds on the work done by the 2004 expert meeting (FAO/WHO, 2004), which recommended, among other things, that JEMRA expand and complete the more extensive risk assessment initiated at that meeting. Thus, the scope of this meeting was to address the risk associated with PIF intrinsically contaminated with *E. sakazakii* and *Salmonella*, and in doing so respond to the specific questions posed by CCFH. The meeting reviewed the risk assessment and applied it as a tool to provide estimates of relative risk reductions associated with different levels of contamination of PIF, different preparation and use scenarios, and a range of microbiological criteria and their associated sampling plans. In this manner the meeting addressed the requests of CCFH for scientific advice to be used in revising the current Recommended International Code of Hygienic Practice for Foods for Infants and Children.

Within the context of this work, PIF was considered to include products 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[3]), follow-up formula (as defined in Codex Stan 156-1987[4]), formula for special medical purposes intended for infants (as defined in Codex Alinorm 04/27/26, Appendix V[5]), formula for special medical purposes for the partial feeding of infants (covered by Codex Stan 180-1991[6]) and human milk fortifiers" as defined in the 2004 meeting (FAO/WHO, 2004). Throughout the report, the term "powdered infant formula" or PIF includes all the products mentioned above, but does not include cereals or other any other product added to breastmilk or used as breastmilk substitutes that are not specifically manufactured for that purpose.

This expert meeting reviewed the most recent available epidemiological and microbiological science related to *E. sakazakii* and *Salmonella* and their intrinsic contamination of PIF. The scientific review specifically addressed susceptible human populations, information from recent outbreaks of illness, new diagnostic assays, virulence and ecological factors of the organisms. The meeting also updated and/or expanded on previous information on industry and consumer practices relevant to *E. sakazakii*- and *Salmonella*-contaminated PIF.

1.4 MICROORGANISMS OTHER THAN *ENTEROBACTER SAKAZAKII* AND *SALMONELLA*

The 2004 meeting addressed the potential risk of a range of microorganisms in PIF and reported "clear evidence of causality" (category A) for *E. sakazakii* and *Salmonella*. Other microorganisms

[3] Available at: http://www.codexalimentarius.net/download/standards/288/CXS_072e.pdf.
[4] Available at: http://www.codexalimentarius.net/download/standards/293/CXS_156e.pdf.
[5] Available at: http://www.codexalimentarius.net/download/report/34/al04_26e.pdf.
[6] Available at: http://www.codexalimentarius.net/download/standards/293/CXS_156e.pdf.

were considered and assigned to one of the two other categories based on the strength of the evidence of a causal association between their presence in PIF and illness in infants.

This meeting agreed to retain the categorization scheme developed at the 2004 meeting. No outbreaks associated with the organisms in category B linked to their occurrence in PIF have been reported that would warrant considering the re-assignment of those organisms to category A. Hence the only organisms in category A, "clear evidence of causality", remain *Salmonella enterica* and *E. sakazakii*. It was noted that some organisms which have been isolated from PIF and are associated with neonatal infections had not been included in category B, "causality plausible, but not yet demonstrated". These include *Escherichia coli*, *Serratia* spp. and *Acinetobacter* spp. (Kaufman and Fairchild, 2004; Lee *et al*., 2004; Estuningsih *et al*., 2006). This information had not been published before the 2004 meeting and these organisms should now be included in category B. Similarly, with respect to category C, "causality less plausible or not yet demonstrated", the omission of coagulase-negative staphylococci which cause more neonatal infections in very low-birthweight neonates than *Staphylococcus aureus* (already in category C) was noted (cf. Kaufman and Fairchild, 2004; Lee *et al*., 2004).

In addition, in 2005 there were two papers reporting the case of a 5-month-old infant with botulism in 2001 and a possible link to PIF (Brett *et al*., 2005; Johnson *et al*., 2005). *Clostridium botulinum* was isolated from the baby's faeces, foodstuffs in the house, and both opened and unopened PIF of the same batch. Whilst *C. botulinum* type A was isolated from both the baby's faeces and the opened formula, the unopened formula contained *C. botulinum* type B that did not match the type isolated from the baby. It was considered that this single case did not warrant re-categorization of *C. botulinum*. No outbreaks due to the remaining organisms in category C have been reported and hence these organisms remain in this category. The updated categorization is presented in Table 1.

Table 1. Categorization of the microorganisms or microbial toxins of concern in powdered infant formula based on the strength of evidence of a causal association between their presence in PIF and illness in infants.[a]

Category	Organisms included
Category A organisms – clear evidence of causality	*Enterobacter sakazakii*, *Salmonella enterica*
Category B organisms – causality plausible, but not yet demonstrated	*Pantoea agglomerans* and *Escherichia vulneris* (both formerly known as *Enterobacter agglomerans*), *Hafnia alvei*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Citrobacter freundii*, *Klebsiella oxytoca, Enterobacter cloacae*, *Escherichia coli*, *Serratia* spp. and *Acinetobacter* spp.
Category C organisms – causality less plausible, or not yet demonstrated	*Bacillus cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Listeria monocytogenes, Staphylococcus aureus* and coagulase-negative staphylococci

[a] This categorization was developed by the FAO/WHO expert meeting on *Enterobacter sakazakii* and other microorganisms in powdered infant formula, held 2-5 February 2004 (FAO/WHO, 2004).

2. EPIDEMIOLOGICAL AND MICROBIOLOGICAL ASPECTS OF *ENTEROBACTER SAKAZAKII*

2.1 EPIDEMIOLOGY AND PUBLIC HEALTH

Although *E. sakazakii* has caused illness in all age groups, certain groups are likely to experience higher disease rates. As reported in the first meeting (FAO/WHO, 2004) a United States FoodNet survey in 2002 estimated the annual rate of invasive *E. sakazakii* infection (based on isolation of the organism from sterile sites only) to be 1 per 100 000 infants (i.e. children <12 months of age), whereas the rate among low-birthweight neonates was 8.7 per 100 000 in the nine FoodNet surveillance sites (C. Braden, personal communication, 2004). Similarly, the annual incidence of invasive *E. sakazakii* infection was estimated at 9.4 per 100 000 infants of very low birthweight (<1 500 g) in a study conducted in 19 neonatal intensive care units (Stoll *et al*., 2004). Although the incidence worldwide of illness is not known for older paediatric or adult populations, published reports of bloodstream or central nervous system infections are rare: there are three reported cases among children aged >12 months (Arseni *et al*., 1987; Lai, 2001), and eight in adults, six of which among persons aged >70 years (Lai, 2001). The estimated incidence of *E. sakazakii* bloodstream infection among mixed ages in England, Wales and Northern Ireland ranged from 0.09 to 0.12 per 100 000 population between 2001 and 2003, based on 2001 census data (HPA, 2004). Therefore, children >12 months and adults are assumed to be at lower risk than infants for invasive infection with *E. sakazakii*.

Among infants, those born prematurely or with low birthweight (<2 500 g) are reported to be at highest risk for severe infection (Lai, 2001; FAO/WHO, 2004; Gurtler, Kornacki and Beuchat, 2005). However, in a review of 45 cases of bloodstream and central nervous system infections abstracted from the English language literature (n=32) and CDC (Centers for Disease Control and Prevention) case consultations (n=13), this group of infants appears, on average, to develop isolated *E. sakazakii* bacteraemia more often than meningitis (Table 2). A list of the cases on which this analysis is based is provided in Appendix E. While in some cases PIF was established as the source of *E. sakazakii*, in many cases PIF was neither epidemiologically nor microbiologically implicated as the source of infection. However, in such cases no other source of infection has been epidemiologically or microbiologically implicated.

The analysis indicated that premature infants tend to develop infection at a later median age – 35 days of life – than infants who develop meningitis. According to recent CDC case reports, bacteraemia cases have occurred as late as age 10 months in an immunosuppressed infant, and 8 months in a previously healthy infant (A. Bowen, personal communication, 2006). In contrast, meningitis appears to occur more frequently among infants born following term gestation and at a birthweight approaching the normal range. However, these infants also generally develop symptoms before the age of 1 week. Similar proportions of infants with bacteraemia (27%) and meningitis (33%) develop symptoms outside of the hospital setting. Infants are particularly susceptible to

Table 2. Characteristics of infants who develop different syndromes due to *E. sakazakii* infection.

Characteristics of infants	Bacteraemia N = 12		Meningitis N = 33	
	Median	Range	Median	Range
Birthweight (*g*)	850	[540-2 600]	2 454	[850-3 401]
Estimated gestational age (*weeks*)	27.8	[23.5-40]	37	[30-40]
Age at onset (*days*)	35	[7-300]	6	[2-35]

Table 3. Outcomes of *E. sakazakii* infection in infants according to syndrome.

Outcome	Bacteraemia		Meningitis	
	n/N	%	n/N	%
Seizures	1/12	8	11/33	33
Brain abscess or cyst	0	0	7/33	21
Hydrocephalus[a]	0	0	8/19	42
Developmental delay[a]	0	0	10/19	53
Mortality	1/12	8	14/32	44

[a] Outcome among survivors.

meningitis during the first few weeks of life and may be more likely to develop meningitis rather than isolated bacteraemia if exposed to *E. sakazakii* during this time. Consequently, although there appear to be two distinct infant risk groups – namely, premature infants developing isolated bacteraemia after 1 month of age and term infants developing meningitis during the neonatal period – the difference in timing of infection may be related to differences in timing of exposure to *E. sakazakii* rather than differences in susceptibility.

Outcomes differ depending on the type of infection (Table 3). Infants with bacteraemia tend to fare better; mortality among these cases is 10%. Conversely, those with meningitis frequently develop complications including seizures and brain abscesses, infarcts or cystic changes. Of the infants with meningitis in this series, 44% died and the majority of survivors experienced long-term neurological consequences.

In summary, although *E. sakazakii* has caused invasive infection in all age groups, the meeting reiterated the findings of the 2004 FAO/WHO meeting that infants appear to be the group at particular risk, with neonates and infants under 2 months at greatest risk. *E. sakazakii* meningitis tends to develop in infants during the neonatal period, which mirrors age-based meningitis rates due to other pathogens, and which may be due to immunologic or neurovascular processes in this age group (Schuchat *et al*., 1997). *E. sakazakii* bacteraemia tends to develop in premature infants outside of the neonatal period with most cases occurring in infants under 2 months of age. However, infants with immunocompromising conditions have developed bloodstream infections as late as age 10 months and previously healthy infants have also developed invasive disease outside the neonatal period. Infections have occurred in both hospital and outpatient settings and it was noted that as older infants generally live at home in the community, infections in such infants may be more likely to be under-reported.

2.1.1 Outbreaks: Update since 2003

In 2004, a small outbreak occurred in New Zealand and was linked to PIF used in a nursery. Subsequently, a premature infant died after contracting *E. sakazakii* meningitis. A follow-up investigation in the neonatal intensive care unit (NICU) found that four other babies had been colonized with the organism. The PIF which was implicated was microbiologically confirmed as the source. As a result of this outbreak, there was an inquiry into actions of sector agencies in relation to contamination of infant formula with *E. sakazakii*, and *E. sakazakii* invasive disease was made notifiable in New Zealand in 2005 (Ministry of Health, New Zealand, 2005).

Another outbreak due to *E. sakazakii* occurred in France in 2004 (InVS, 2006; Coignard *et al*., 2006). A total of nine cases were reported, with two deaths. Syndromes included fatal meningitis (2), conjunctivitis (1), haemorrhagic colitis (1) and colonization (5). All infants were premature and under 2 000 g (low birthweight), with the exception of the infant with colitis who weighed 3 250 g and was born at 37 weeks of gestation. When public health officials recognized two cases of *E. sakazakii* meningitis separated by 1 week, an investigation was initiated immediately. In total, five hospitals were involved in the investigation. A review of feeding practices found that all infants had been fed a hypoallergenic PIF, Pregestimil®. Four implicated lots produced over a 6-month period were contaminated at levels ranging from 1 to 10 cfu per 100 g. In addition, the two lots which had the highest incidence of contamination (22.4 and 32.1% positive) were implicated in eight of the nine cases. A review of hospital practices with regard to the preparation and storage of the PIF was undertaken. It was found that one hospital was not following recommended procedures for the preparation, handling and storage of the feeding bottles, and four hospitals were storing the reconstituted formula for storage periods of greater than 24 hours, and in domestic-type refrigerators where there was no temperature control or traceability. The two most severe cases had been fed PIF starting from birth. Although some of the implicated batch of product had also been sent to Algeria, no cases have been reported from that country. Whether this is due to surveillance limitations or actual absence of cases is not known. The investigation led to a voluntary recall and withdrawal of all implicated lots, various actions by the manufacturer, and recommendations from the French Food Safety Agency for the preparation, handling and storage of feeding bottles (AFSSA, 2005).

In addition to the outbreaks described above, worldwide there have been a number of recalls of PIF contaminated with *E. sakazakii* since 2004. Recalls due to contaminated PIF have occurred in Argentina and Brazil, and in Luxembourg a cereal-based infant formula was recalled due to contamination with high levels of Enterobacteriaceae and suspected contamination with *E. sakazakii* (Sécurité alimentaire – Grand-Duché de Luxembourg, 2005). In addition, there were a number of recalls linked to the French outbreak which occurred in countries around the world, including Brazil, Hong Kong, Ireland, the Gambia, Gabon and the United Kingdom.

It is important to point out that not only PIF, but other food sources, have been described as the potential source of *E. sakazakii* infections in infants. However, these food sources have been neither epidemiologically nor microbiologically confirmed as the source of infection. For example, in one case, a premature baby with *E. sakazakii* infection had only been fed breastmilk and ready-to-use sterile infant formula (Stoll *et al*., 2004). In another case, starch had been added to a sterile

ready-to-feed formula (FAO/WHO, 2004). A case of *E. sakazakii* meningitis where contaminated breastmilk was suspected was recently described in Brazil (Barreira *et al*., 2003). The young girl, who was 14 days old at the time of diagnosis, was only being fed breastmilk. The strain of *E. sakazakii* was resistant to both ampicillin and ceftazidime and the infant died. Recent work has shown that *E. sakazakii* can grow very well in expressed breastmilk (Lenati *et al*., 2005).

2.2 UPDATE ON MICROBIOLOGICAL ASPECTS

2.2.1 Characterization of the microorganism

E. sakazakii strains collected from clinical, food and environmental sources have been further characterized using molecular methods. Detailed DNA sequence analyses of the 16S rDNA and hsp60 genes indicate that there is considerable diversity between strains. Further analysis of the DNA sequences show that there are four cluster groups and all cluster groups have been found to contain clinical *E. sakazakii* isolates (Iversen *et al*., 2004). The majority of *E. sakazakii* strains including the type strain ATCC 29544 and the white or non-pigmented strains are within cluster 1. The Preceptrol™ (quality control) strain ATCC 51328 is in cluster 3. It is important to note that DNA-DNA hybridization studies demonstrate that these strains are from one species (S. Forsythe, personal communication, 2006). Genetic fingerprinting methods, such as pulsed-field gel electrophoresis, have been used for epidemiological analysis of the 2004 *E. sakazakii* outbreaks in France and New Zealand described above, as well as in the analysis of sporadic and outbreak strains of *E. sakazakii* in the USA.

The growth range of six *E. sakazakii* strains in PIF has been reported to be between 6° and 45°C, the optimum being 37° to 43°C (Iversen, Lane and Forsythe, 2004). *E. sakazakii* has been shown to form biofilms on latex, polycarbonate, silicon rubber and glass (Iversen, Lane and Forsythe, 2004; Lehner *et al*., 2005). Biofilm formation could lead to increased resistance to cleaning and result in clumps of cells occurring in sampled material. Capsule formation does vary between *E. sakazakii* strains, and capsule formation correlates with biofilms with higher cell counts (up to 10^4 cfu/cm^2). Capsule formation is an important consideration from production through to consumption of PIF due to its link with biofilm formation.

Additional data on the decimal reduction times (D-values) for *E. sakazakii* strains in PIF have recently been reported by Jung and Park (2006) which are pertinent with respect to thermoprocessing and recognition of the physiological diversity of *E. sakazakii* strains. *E. sakazakii* strains isolated from PIF exhibited D-values in the range of 3.52 to 3.58 minutes at 60°C. Strains isolated from brown rice had D-values of 3.79 to 3.86 minutes in PIF, while some other strains isolated from agricultural produce had D-values in the range of 4.40 to 4.79 minutes at 60°C. The identification of protein markers associated with strains with increased thermal resistance was reported by Williams *et al.* (2005), including the development of a PCR probe that can be used to identify resistant strains. Induced acid resistance at pH 3 has been demonstrated (Edelson-Mammel, Porteus and Buchanan, in press). This may be relevant with respect to the organisms' survival during passage through the neonate stomach, and the use of acidified infant formula products, which are currently used in some parts of Africa.

2.2.2 Isolation and identification

Table 4 provides an overview of a number of surveys for *E. sakazakii* in PIF. Positive isolates were obtained from between 2.9 and 14.2% of samples. The most recent study quantified the levels of *E. sakazakii* in PIF at 0.22 to 1.61 per 100 g. (Santos, 2006). In addition, *E. sakazakii* has been isolated from various non-PIF sources including dried baby food, milk powder, cheese, dry food ingredients, rice, herbs and spices (Iversen and Forsythe, 2004; Kandhai *et al*., 2004; Jung and Park, 2006). This confirms the ubiquity of the organism in food and the environment.

Several media have been developed specifically for the detection of *E. sakazakii*, rather than general Enterobacteriaceae, in PIF. A summary of the main features of these media are given in Table 5. They are based on the inclusion of chromogenic or fluorogenic substrates. An ISO (TS 22964) method has been adopted for PIF, based upon resuscitation in buffered peptone water, enrichment in modified lauryl sulphate broth (45°C) and a chromogenic selective agar (ESIA at 44°C). This method has been adopted within the EU microbiological criteria (EC 2073/2005)[1] for dried infant formula and dried dietary foods for special medical purposes intended for infants below the age of 6 months.

It should be noted that there are a number of apparent discrepancies with the current *E. sakazakii* selection procedures. Firstly, Farmer *et al*. (1980) reported that not all strains of *E. sakazakii* are yellow-pigmented. Additionally, pigment production in some strains is temperature dependent. Subsequently the FDA method incubates the TSA (tryptic soy agar) plates at 25°C for 48 to 72 hours. Pigment production is enhanced by light exposure and hence the use of artificial white light by Guillaume-Gentil *et al*. (2005), albeit at 37°C. A number of selection methods use raised incubation temperatures of 44° to 45°C. However, Nazarowec-White and Farber (1997) reported that only 3 out of 11 *E. sakazakii* strains grew at 44° to 45°C. The maximum growth temperature for 4 out of 11 strains was 41°C; this included the type strain ATCC 29544.

The correct identification of *E. sakazakii* based on biochemical profile kits has been reported to be problematic (Iversen, Druggan and Forsythe, 2004; Weiss, Becker and Holzapel, 2005). For example, Weiss, Becker and Holzapel (2005), in a survey of ready-to-eat salads for *E. sakazakii* obtained conflicting results when both the API20E and the DNA probe-based BAX system (DuPont Qualicon) were used to confirm *E. sakazakii* isolates. Thus it was considered that the issue of correct identification warrants further investigation. An artificial neural network has been applied to identify factors which discriminate *E. sakazakii* from similar, closely related organisms (Iversen *et al*., 2006). This predicted that tests for the metabolism of glucose-1-phosphate, sucrose and arginine gave the highest discrimination.

[1] Commission Regulation EC No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, L338/1. Available at http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_338/l_33820051222en00010026.pdf.

Table 4. Surveys of *E. sakazakii* in powdered infant formula.

Reference	Method	Volume tested (*g*)	Sample number	*E. sakazakii* positive (*%*)	Enumeration (*cfu/100 g*)
Muytjens, Roelofs-Willemse and Jasper, 1988	BPW, EE, VRBGA	333-555	141	20 (14.2)	0.36-66
Nazarowec-White and Farber, 1997	DW, EE, VRBGA	333	120	8 (6.7)	0.36
FDA, 2003	FDA	333	22	5 (22.7)	0.36
Heuvelink *et al.*, 2003	BPW, EE, VRBLA	25	101	2 (2)	nd
Kress *et al.*, 2004	BPW, EE, ESIA or DFI	300	40	5 (12.5)	nd
Iversen and Forsythe, 2004	BPW, EE, DFI	25	102	3 (2.9)	nd
Santos, 2006	FDA and BAX	5x100	98	12 (12.2)	0.22-1.61

Table 5. Methods for the detection of *E. sakazakii* in powdered infant formula.

Method	Pre-enrichment (37°C)	Enrichment	Primary isolation	Presumptive identification
FDA[a]	Sterile de-ionized water	EE broth 36°C	VRBG agar (36°C)	Purple colonies surrounded by a purple halo of precipitated bile acids. Streaked onto TSA (25°C for 48-72 hours) – yellow pigmented colonies
DFI[b]	BPW	EE broth (37°C)	DFI agar (37°C)	Blue/green colonies
NES[c]	BPW	mLST+van (45°C)	TSA (37°C with enhanced light exposure)	Yellow, α-glucosidase-positive colonies
ISO[d]	BPW	mLST+van (45°C)	ESIA (44°C)	Blue/green colonies
R&F ESPM-ESSM[e]	Sterile de-ionized water	EE broth (35°-37°C)	ESPM (35°-37°C) followed by screening on ESSM biplate (35°-37°C for 4-6 hours)	Blue-back raised to domed colonies 1-2 mm diameter 6-hour screening (bi-sugar plate with melibiose and sucrose) – yellowing around bacterial growth

[a] US Food and Drug Administration, 2002.
[b] Iversen, Druggan and Forsythe, 2004.
[c] Guillaume-Gentil *et al.*, 2005.
[d] ISO TS 22964.
[e] Restaino *et al.*, 2006.

Recently there have been a number of DNA probe-based schemes developed for the detection of *E. sakazakii* (Lehner, Tasara and Stephan, 2004; Malorny and Wagner, 2005; Seo and Brackett, 2005; Hassan *et al.*, 2006). Seo and Brackett (2005) were able to detect 100 cfu/ml reconstituted infant formula in 50 cycles of PCR (polymerase chain reaction) without enrichment.

2.2.3 Persistence in a desiccated state

Long-time persistence of pathogens in the desiccated state is of considerable importance. A comparative study has been reported which compared the survival of ten strains of *E. sakazakii* with nine strains of *C. koseri* and single strains of *C. freundii*, *Pantoea* spp. *Salmonella*, *K. oxytoca*, *K. pneumoniae*, *E. coli* and *E. vulneris* (Caubilla-Barron, Iversen and Forsythe,

2004). The organisms were desiccated in infant formula and stored for up to 2 years. The least persistent organisms were *C. koseri*, *C. freundii* and *E. cloacae*, followed by *E. coli*, *Salmonella* and *K. pneumoniae*. The most persistent organisms were the ten *E. sakazakii* strains, together with *K. oxytoca, E. vulneris* and *Pantoea* spp. The latter two organisms are not recognized pathogens. Hence, it is recognized that *E. sakazakii* can persist in the finished PIF product.

2.2.4 Pathogenicity and virulence

Advances have been made in terms of some of the pathogenesis research where non-primate animal models are being developed. For example, Lenati *et al*. (2006) are currently looking at developing non-primate animal models in order to gain a better understanding of the mechanisms of pathogenesis and dose response of *E. sakazakii*. Young pigs, chicks, rabbits, guinea pigs and gerbils were orally dosed with 10^9 cfu of three different strains of *E. sakazakii* isolated from different sources. Although in all cases colonization was observed – in that the organism was isolated from the stools of the animals for various periods of time and from various organs of chicks – no mortality was observed. Further work will be done using animal models to try and simulate as closely as possible the symptoms observed in high-risk human neonates. Research is also being carried out in the United States of America at the University of Georgia using various suckling mouse models.

In vitro studies have also been done in the United Kingdom; bacterial attachment and invasion of mammalian intestinal cells, macrophage survival and serum resistance for *E. sakazakii* were comparable with other Enterobacteriaceae (e.g. *E. cloacae* and *C. freundii*), but less than for *Salmonella* Typhimurium (Hurrell, Townsend and Forsythe, 2006; Townsend *et al*., 2006a; Townsend *et al*., 2006b). Strains from cluster 1 (see section 2.2.1) showed higher Caco-2 invasion rates than the other clusters. Similarly, the survival of *E. sakazakii* in macrophages varied, with strains from cluster 1 persisting or replicating, whereas those from cluster 3 were killed. In addition, strains from cluster 4 were serum sensitive. Hence, *E. sakazakii* clusters 1 and 2 showed greater virulence potential than clusters 3 and 4. Visualization in both macrophages and Caco-2 culture cells showed the uptake of *E. sakazakii* into phagosomes. Additional research has shown that *E. sakazakii* may contain genes that are similar genetically to the heat-stable, heat-labile and CDT toxin genes of *E. coli*. *E. sakazakii* may also contain genes present in a high-molecular-weight plasmid found in *Shigella flexneri* strains and associated with the capacity to invade and multiply within epithelial cells (A. Cravioto, personal communication, 2006).

2.3 DOSE RESPONSE

While there is work ongoing on the virulence and pathogenicity of *E. sakazakii*, there was not, at the time of the meeting, any new information available to further describe the dose-response relationship for this pathogen and it was not possible to update the findings of the 2004 FAO/WHO meeting in this regard. Therefore, the meeting considered the approach to describe the dose-response relationship developed in 2004 (FAO/WHO, 2004) to be relevant despite the limited data. A further description of the approach adopted to model the dose-response relationship is provided in section 3.

2.4 CONCLUSIONS

Since the 2004 FAO/WHO meeting, a number of research groups have started to work on various aspects of *E. sakazakii*. Considerable progress has been made in terms of the ecology and methodology related to this organism. Limited progress, however, has been made in the area of pathogenesis and virulence factors. Although there have been a couple more outbreaks linked to *E. sakazakii* and PIF, none of the additional data allow a much more specific definition of the groups of neonates and/or infants that are at particular risk of acquiring *E. sakazakii* infection. However, based on a review of 45 cases, it appears that there are two distinct infant risk groups – premature infants who develop bacteraemia outside of the neonatal period, with most, but not all, cases occurring in infants under 2 months and term infants who develop meningitis during the neonatal period. However, it was noted that the difference in timing of infection may be related to differences in timing of exposure to *E. sakazakii* rather than differences in susceptibility. Nonetheless, any infant may develop either syndrome at any age. The meeting therefore reiterated the findings of the 2004 FAO/WHO meeting that infants appear to be the group at particular risk. Neonates and infants under 2 months are at greatest risk. Furthermore, because cases occur in both hospital and outpatient settings and in a variety of infant populations, prevention efforts must be multifaceted, directed at healthcare as well as home settings, and must take into consideration the risk to infants both within and beyond the neonatal period.

The review of the most recent epidemiological and outbreak data highlighted the importance of good outbreak investigations both as a source of information and as a contribution to risk management. The meeting concluded that prompt, thorough investigations involving the cooperation of clinicians, laboratory professionals, and public health and regulatory officials should be promoted.

3. QUANTITATIVE RISK ASSESSMENT MODEL FOR *ENTEROBACTER SAKAZAKII* IN POWDERED INFANT FORMULA

3.1 INTRODUCTION

This section presents the quantitative risk assessment model developed for *E. sakazakii* in PIF, which was initiated at the first FAO/WHO meeting in 2004 and further developed in the interim. In elaborating this risk assessment model, the aim was to develop a useful tool to describe the factors leading to *E. sakazakii* infection in infants and identify potential risk mitigation strategies. The risk assessment achieves this by facilitating the comparison of different levels of product contamination and different preparation, handling and feeding scenarios. In addition, it provides a means to evaluate microbiological criteria and sampling plans in terms of the risk reductions and percentage of product lot rejected. Developing this model as a risk assessment tool means that the user can interact with the tool by means of a user-friendly interface. This interface allows the user to select some of the inputs to the model from either a predefined range or by the addition of a new value in order to better describe the scenario to be evaluated.

The risk assessment model estimates the risk of illness from *E. sakazakii* posed to infants from intrinsically contaminated PIF and does not consider contamination or recontamination from the preparation environment or other sources post-manufacture or the impact thereof. Therefore, effects of risk reduction can be smaller than predicted since a parallel route might not always be influenced by interventions. Experimental studies suggest that *E. sakazakii* contamination of PIF is at low levels, with reports in the literature suggesting contamination levels of less than 1 cfu/g (Muytjens, Roelofs-Willemse and Jasper, 1988). *E. sakazakii* has an observed growth range in brain heart infusion and UHT (ultra-heat treated) milk of between 5.5°C and (for some strains) 47°C (Nazarowec-White and Farber, 1997). The growth range of six *E. sakazakii* strains in PIF has been shown to be between 6° and 45°C (Iversen, Lane and Forsythe, 2004) (see section 2.2.2). These growth characteristics provide the opportunity for growth of any contaminating populations during the preparation of infant formula, resulting in higher levels of *E. sakazakii* at feeding. The model describes the effect that each of the preparation and storage stages have upon the microbiological quality of the reconstituted PIF, in terms of *E. sakazakii*, and examines the risks of different preparation and handling scenarios in terms of the relative risk posed to infants.

3.2 OVERVIEW OF THE RISK ASSESSMENT MODEL

The components of the risk assessment model are summarized in Figure 1. The risk assessment was developed on a modular basis using Analytica® software. The risk assessment has three main components:

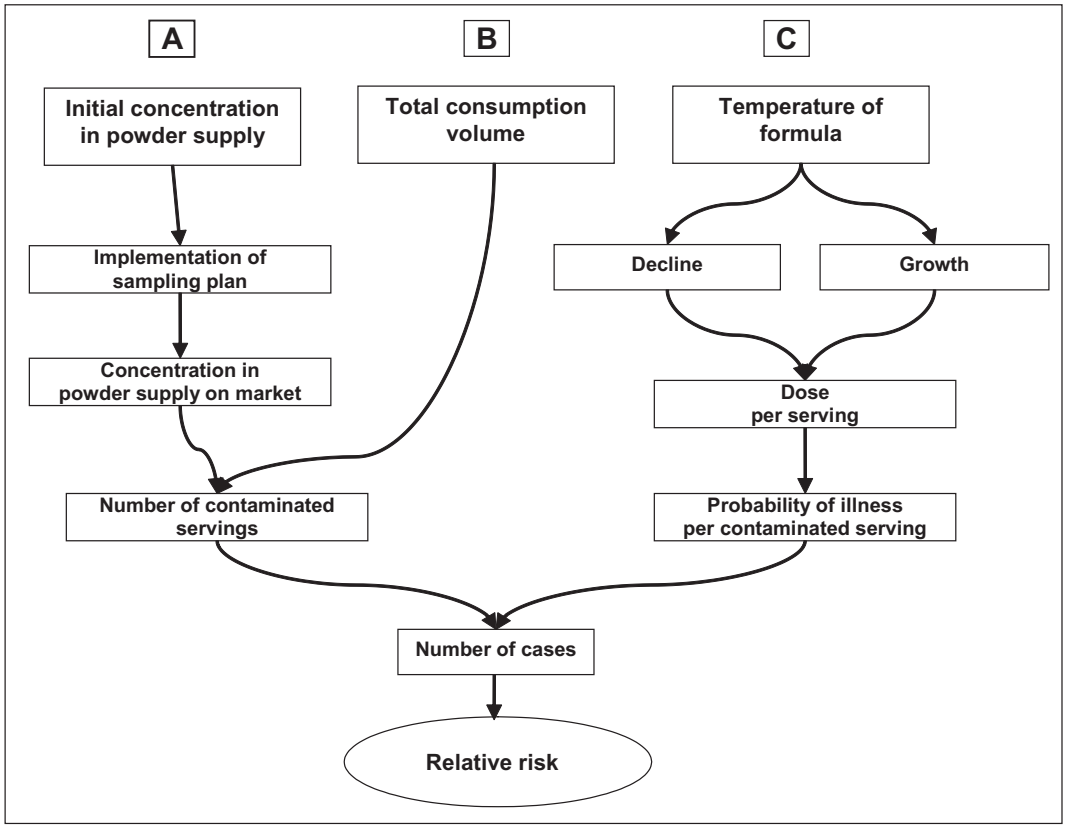


Figure 1. Schematic representation of the components of the risk assessment model.

- Component A addresses the level of *E. sakazakii* in the PIF at the point of preparation (initial level of contamination).

- Component B addresses consumption of PIF estimating the amount of powder consumed per million infant days or per million infants per day.

- Component C estimates the magnitude of the change in contaminating *E. sakazakii* (given a contaminated serving) that may occur as a result of preparation, holding and feeding practices. This includes growth and inactivation modules.

These components are combined to give an estimate of the number of cases per million infants per day, which in turn is translated into an estimate of the relative risk enabling a comparison of, for example, different preparation and feeding scenarios compared to a defined baseline scenario.

In understanding the schematic model, it is important to note that the model was not built to evaluate individual risk but risk at population level. Therefore, for example, consumption volume (component B) does not refer to the amount consumed by an individual infant but the amount of PIF consumed by an infant population. The underlying model assumption is that, if a serving is contaminated, the level of contamination in powder is low such that there is only 1 cfu per serving (prior to storage and growth). Component C of the schematic model calculates the increases and decreases from this initial level (i.e. 1 cfu/serving) that may occur during preparation, holding and feeding of the reconstituted formula to provide an estimate of the dose per serving and the probability of illness per serving. The number of contaminated servings is given by the combination of the "actual" or selected level of contamination in the powder supply (from component A) and the volume consumed (from component B). This number of contaminated servings is then combined with the risk per contaminated serving (from component C) to estimate the number of cases of illness on a population basis, and subsequently the relative risk.

3.2.1 Hazard characterization

Due to data limitations, the approach taken to estimate the dose-response relationship for *E. sakazakii* was to assume that illness results from 1 cfu per serving of *E. sakazakii* in dry PIF at the time of preparation. This is described by the exponential dose-response model, specifically:

$$P_I = 1 - \exp^{(-rd_c)}$$

where r is the exponential dose-response parameter and d_c is the dose at consumption that results from an initial contamination level of 1 cfu of *E. sakazakii* per serving (prior to storage and growth) in the dry product. As indicated above, this initial level of 1 cfu per serving is adjusted to take into account any growth or decline that may occur due to the conditions of preparation, holding and feeding to give an estimate of the dose ingested. The exponential model was chosen mainly due to the simplicity of the model and ease of interpretation of model parameters as there are no data available to provide a basis for model selection. The exponential model is a non-threshold model which is linear at low doses. The model is described by a single parameter r which can be interpreted as the probability that a single cell causes illness.

There were no data available to estimate the value of the dose-response parameter which is likely to be specific for each of the infant groups considered in the model. Therefore, six options are presented in the model as some baseline values. The options available range from 1×10^{-5} to 1×10^{-10}. Once selected, multipliers of this baseline value of r are also entered, thus enabling this baseline value of r to be adapted to represent the relative susceptibility of each of the infants. As a default, no pattern of susceptibility is assumed to apply across the infant groups and therefore values of 1 are implemented for the multiplier of the baseline value of the parameter r. Providing such options in the model enables a direct comparison of the impact of the assumptions regarding the value, and the relative susceptibility of the infant groups in terms of estimates of relative risk. This means that risk is not expressed in absolute terms, rather the risk associated with different scenarios or practices is expressed in comparison to a predefined baseline scenario or practice.

3.2.2 Exposure assessment

The level of exposure – or dose – at the point of consumption is a result of the level of contamination in the PIF at preparation and the overall effect of the conditions of preparation and holding of the prepared formula between preparation and storage on the size of any *E. sakazakii* populations contaminating the powder. As indicated in section 3.1 the model does not explicitly consider recontamination from other sources post-manufacture. The growth characteristics of *E. sakazakii* combined with the methods of chilling and holding used prior to consumption of prepared formula may allow the growth and/or decline of any *E. sakazakii* populations that may be contaminating the prepared PIF.

A number of options are provided for the user of the risk assessment model which define the variables and the specifics of the exposure pathway described by the model. These options constitute components of the model that the user may adapt or change to explore the impact on the model calculations. In each case, a range of options have been pre-programmed in the model; however, these options can be modified to reflect a particular interest of the user. The flexible components of the model are:

- Initial level of contamination of PIF
- Sampling plan employed
- Method of preparation employed

3.2.2.1 Estimating the E. sakazakii *concentration in dry product at preparation*

The level of *E. sakazakii* in PIF at preparation is a result of the initial level of *E. sakazakii* in the product, the impact of any microbiological criteria (and associated sampling plans and percentage of lots sampled) upon this level of contamination, and the decline in contamination that occurs during storage of the powder prior to preparation of the formula for feeding.

3.2.2.2 E. sakazakii *concentration in dry product*

As noted earlier, *E. sakazakii* has been reported to be present at low levels in powdered product. However, studies reporting levels in powder usually involve product testing in the marketplace as opposed to in the manufacturing environment prior to release for sale. While data were available from industry with regard to the levels of contamination (Appendix B), the true contamination level remained an area of uncertainty. Therefore, the available data were not used directly as an input on contamination level but guided the selection of a range of options on the level of contamination. The user is thus provided with the following three options and can select whichever is considered most appropriate:

- -5 log cfu/g (0.00001 cfu/g = 1 cfu/100 kg)
- -4 log cfu/g (0.0001 cfu/g = 1 cfu/10 kg)
- -3 log cfu/g (0.001 cfu/g = 1 cfu/1 kg)

If actual data are available on the initial level of contamination of PIF, they can be used to replace the above options.

One aim of the risk assessment was to examine the effect of microbiological criteria and their associated sampling plans upon estimates of relative risk. To do this, it is necessary to have an estimate of the level of contamination in the manufacturing environment which is analogous to the concentration at the point of sampling. Some of this type of data were submitted to FAO/WHO as part of the call for data and were used as a basis for the development of this function. There is interest in considering Enterobacteriaceae as an indicator of process hygiene in the manufacturing environment. Therefore, in addition to estimating the concentration of *E. sakazakii* in powdered product, it is also necessary to estimate the level of Enterobacteriaceae.

The model incorporates an approach to evaluate risk reduction associated with two types of sampling plans: a two-class sampling plan for *E. sakazakii* and a three-class sampling plan for Enterobacteriaceae, as well as a combination of both types of sampling plan. When it occurs, the model assumes random sampling from the mass of powdered infant formula at the end of manufacturing and prior to storage. As well as predicting the relative risk reduction associated with the sampling plan, the percentage of product lots rejected is also estimated.

3.2.2.3 Estimating the impact of storage on **E. sakazakii** *levels in PIF*

Experimental studies have demonstrated that *E. sakazakii* concentrations in PIF decline over time (Edelson-Mammel, Porteus and Buchanan, 2005). The reduction rate applied in the model is based on experimental data which indicated a slow decline rate of 0.001 log units per day (measured up to 687 days after the start of the experiment). Although a higher initial die-off rate (0.014 log units per day) has been demonstrated by the work of Edelson-Mammel, Porteus and Buchanan (2005), that work was undertaken using cells that had been pre-cultured in liquid media. As this is not representative of the manufacturing environment, it was considered more appropriate by the expert meeting to use the slow rate of decline (observed from day 153 to 697 days after the start of the experiment). Within the risk assessment, the implication of this assumption is that there is very limited reduction of *E. sakazakii* during storage. This is supported by the work of Caubilla-Barron, Iversen and Forsythe (2004), who studied the survival of ten strains of *E. sakazakii* and nine other species of Enterobacteriaceae (see section 2.2.3). It was noted that PIF can have a commercial shelf-life of up to two-and-a-half years, although much of the PIF is likely to be used within a much shorter time frame. The risk assessment model provides the user with the option to choose the storage period from between 0, 30, 100 and 365 days. For the analysis of various scenarios the storage time was fixed at 30 days. It was noted that, given the slow rate of decline, the decrease in *E. sakazakii* will be minimal even with longer storage periods.

3.2.2.4 Estimating the impact of preparation and holding on **E. sakazakii** *levels in reconstituted PIF*

During preparation, holding and feeding, formula will be subject to temperatures that provide the opportunity for both increases and declines in the concentration of contaminating *E. sakazakii*.

The model presents a range of options for the method of preparation and subsequent use (see section 4.2). Each of these preparation and use scenarios are described in terms of four main stages, specifically:

- Reconstitution of the powder
- Cooling or holding of prepared formula prior to feeding
- Warming of formula in preparation for feeding
- Feeding of the infant

For each of the preparation and use scenarios, these four stages vary in terms of the duration, ambient temperature and rate at which the formula is heated or cooled. The specifications for the scenarios and the values assigned to them for the purpose of this meeting are described in section 4.2. Note that the assigned values (e.g. room temperature) can be altered by the user of the model.

The temperature of the prepared formula during the course of time from preparation to the completion of feeding is estimated, providing a time-temperature profile of the prepared formula. Cooling rates for reconstituted powdered infant formula in both a bottle and a 1-litre container were used to inform this step. Considering holding as a time-sequenced event and assuming that the stages of preparation, cooling, warming and feeding occur consecutively (Figure 2), the temperature-time profile for the PIF is determined and the growth and decline of the population predicted using appropriate models. The process is divided into discrete time steps (e.g. 0.01 hour).

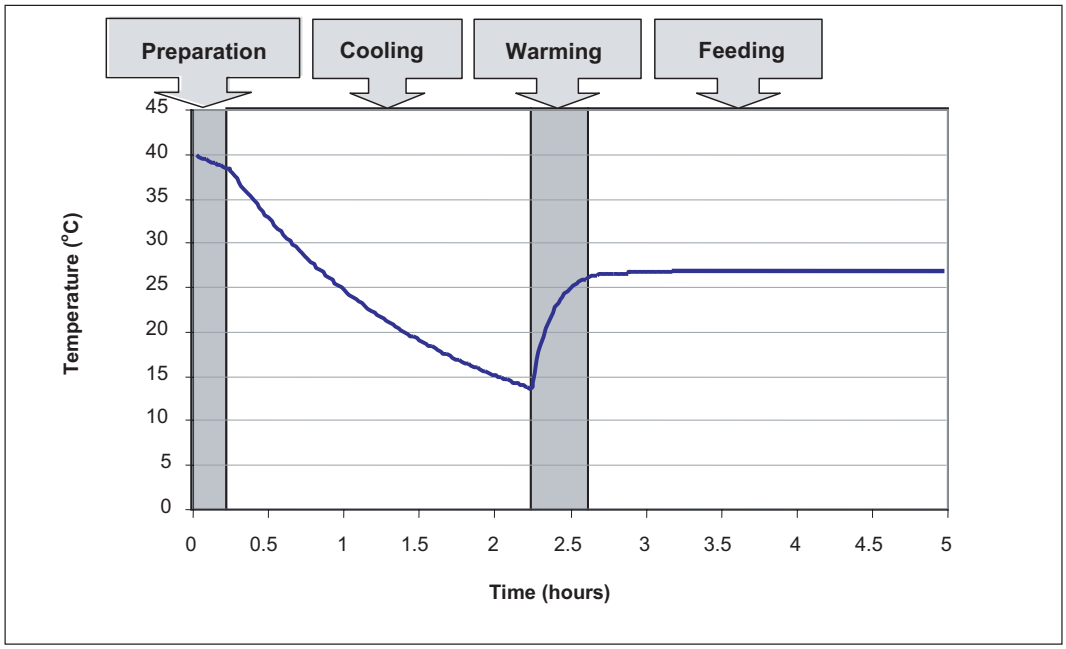


Figure 2. Example of a temperature-time profile generated by the risk assessment model for the stages of preparation, cooling, warming and feeding.

At each time interval the temperature of the PIF is predicted and the magnitude of growth or decline in any contaminating population is determined. The assumption is made that for each time interval, if the temperature of the PIF is less than the maximum permissible growth temperature for *E. sakazakii*, then growth occurs after an initial lag phase. If the temperature is greater than the maximum permissible growth temperature, then cell die-off occurs and population decline is predicted. This process provides an estimate of the level of *E. sakazakii* ingested.

3.2.3 Risk characterization

Through the consideration of the storage stages between preparation of the formula and feeding of the infant, the model predicts the level of contamination, and hence the ingested dose, resulting from feeding reconstituted PIF. The underlying assumption is that, if contaminated, the powder is contaminated at a level of 1 cfu per serving prior to any growth or decline which results during the preparation and feeding stages. The number of cases from PIF consumption per 1 million infant-days, defined as N_{Es}, is estimated from:

$$N_{Es} = \Theta.C_m.P_1$$

Here, Θ is the concentration of *E. sakazakii* in the dry product at the point of preparation of the PIF (thus taking into account the impact of any sampling strategies in place and any decline during storage); P_1 is the probability that illness results from the dry powder given an initial contamination level of 1 cfu of *E. sakazakii* per serving of the dry powder at the time of preparation (accounting for subsequent growth and inactivation during preparation and holding); and C_m is the daily powder consumption level per 1 million infants. The level of consumption of PIF is dependent upon the weight of the infant. There are seven classes of infants provided as options in the risk assessment, specified according to either the birthweight or age of the infant. For each of these classes a recommended daily intake of formula is specified in the model. The daily PIF consumption rate is given by converting the recommended ml/kg per day associated with body weight to million kg per day per infant. In order to do this conversion, the model assumed that 14 g of PIF was used to produce 100 ml of reconstituted formula. The daily PIF consumption rate per 1 million infants (C_m) is given by converting the recommended ml/kg associated with body weight per day to million kg per day per infant and multiplying by 1 million infants. The model predicts the number of cases for seven distinct groups of infant, across a range of preparation scenarios of PIF. The infant groups as defined by body weight and daily intake of PIF are given in Table 6.

In a model simulation, an initial concentration of *E. sakazakii* is selected and the concentration in the PIF at the point of preparation is estimated. The change of any contaminating *E. sakazakii* population from the beginning of preparation of the formula to completion of feeding is estimated using the model inputs to specify the components of the preparation scenarios. The preparation scenarios are defined by the preparation duration and temperature, and associated cooling rate of the prepared formula during preparation, re-warming of the formula, and cooling and feeding of the formula. At each time step, the temperature of the formula is calculated, the associated lag-phase duration and growth rate are estimated and any resulting increase or decrease in contamination

Table 6. Infant group definitions presented as options in the risk assessment model.

Infant group	Definition	Weight (*g*)	Daily intake (*ml/kg/day*)
Extremely low birthweight	Birthweight <1 000 g	800	150
Very low birthweight	Birthweight <1 500 g	1 250	200
Low birthweight	Birthweight <2 500 g	2 000	200
Premature neonate	Prior to 37 completed weeks	2 250	150
Term non-low-birthweight neonate	0 to 28 days of age	3 600	150
Young infant	29 days to 6 months of age	5 000	150
Older infant	6 to 12 months of age	9 000	55[a]

[a] This number assumes that PIF is not the sole source of nutrition for older infants (6-12 months of age).
Note: Data in the weight and daily intake columns in this table are based on discussions and expert opinion expressed at the FAO/WHO expert meeting in 2004.

calculated. The number of illnesses per million infants per day is then calculated and converted to a relative estimate of risk across the scenarios considered. Relative estimates of risk can then be readily compared across infant groups and preparation scenarios in order to determine which scenarios present both desirable and achievable levels of risk mitigation for the infant group(s) of interest.

3.3 REVIEW OF THE MODEL

In the course of the meeting, much time was spent reviewing the mechanics of the model, the correctness of the mathematics, the interpretation of data and the assumptions made therein. In general, the review was positive and the model changes were minimal. A more detailed description of the review and the proposals and modifications made are included in Appendix C. These changes were affected immediately and the meeting agreed that the resulting model was sound and fit for purpose. This updated model as described above was used for evaluating the risk associated with a number of different scenarios which are presented in section 4.

3.4 CONCLUSIONS

The meeting agreed that the model as modified based on the review and discussion was accurate and valid in terms of the approach taken, the interpretation of data etc., and was an appropriate tool for evaluating various risk-reduction scenarios. Therefore, this version of the model was used as a tool for the evaluation of risk-reduction scenarios as outlined in the following sections. As this tool will eventually be made available by FAO and WHO in a user-friendly format it will be possible for risk managers at various levels to use it to assist them in their decision-making processes. The model can also be useful to evaluate pertinent guidelines, educational messages and product labels, regarding the preparation, storage and handling of PIF, under development by WHO and FAO.

The full technical documentation of the model is available on the JEMRA Web pages at http://www.fao.org/ag/agn/jemra/enterobacter_en.stm and http://www.who.int/foodsafety/en/.

4. EVALUATION OF RISK MANAGEMENT INTERVENTIONS FOR *ENTEROBACTER SAKAZAKII* IN POWDERED INFANT FORMULA

4.1 IMPACT OF CONTROL MEASURES IN THE MANUFACTURING PLANT

On the basis of the available data, it was not possible to develop a risk assessment model to assess the impact of specific interventions and control measures in the manufacturing plant to minimize the levels of *E. sakazakii* in PIF.[1] However, it was possible to review the available scientific data from the industry to build on the findings of the first FAO/WHO meeting on this issue (FAO/WHO, 2004), identify any particular factors that contribute to the presence of this hazard in PIF at the manufacturing level and consider the impact of available control measures.

4.1.1 Current industry practices

Details on the different types of processes applied to the manufacture of PIF are outlined in the report of the 2004 FAO/WHO expert meeting (FAO/WHO, 2004). The processing conditions (time/temperature) as well as the characteristics of the liquid intermediary product (in terms of total solids) may vary depending on the manufacturer and the product formulation. Heat-treatments at temperatures above 75°C for 30 seconds, for example, will provide a reduction in excess of 10 log units of vegetative microorganisms such as *Salmonella* or Enterobacteriaceae, including *E. sakazakii*, and treatments above 100°C will lead to a reduction in excess of several hundred log units. These heat treatments are considered as CCPs (critical control points) and therefore procedures are in place to detect deviations (e.g. temperature drops) and to take appropriate corrective measures such as the redirection of the product to waste or reprocessing.

Additional steps such as the application of preheating before drying produce a further killing effect. Drying may also bring about reduction, albeit difficult to predict given the increase in resistance of microorganisms at lower water activities and the varying residence times in the drying tower. Neither pre-heating nor drying are considered to be controlled killing or inactivation steps.

The presence of Enterobacteriaceae, including *E. sakazakii*, in packaged PIF is thus not considered to be due to survival of the organism but to recontamination of the product after drying and during the subsequent processing steps (e.g. conveying, tipping and mixing with additional ingredients) up to the point of filling/packaging. Recontamination is related to the following three factors, the first two of which are linked:

[1] Throughout section 4.1, the term PIF (powdered infant formula) does not include follow-on formula.

1. The presence of these microorganisms in the processing environment (i.e. external parts of equipment and surroundings of the processing lines), making it possible for them to get into the processing lines.

2. The presence of these microorganisms originating from the processing environment (1) on internal surfaces of equipment that are in direct contact with the product.

3. The presence of these microorganisms in ingredients added and mixed into the dry base powder after the heat-processing step.

4.1.2 Processing environment

At present, the probability and extent of recontamination from the processing environment cannot be quantified and modelled. Nevertheless, it can be shown that the likelihood of the presence and the level of microorganisms in the finished product will depend on their level in the processing environment. Additional factors, such as the design of the equipment and premises, will influence the probability of recontamination.

In order to reduce the presence of Enterobacteriaceae, and in particular of *E. sakazakii*, in the finished product, it is necessary to reduce their levels in the processing environment. The control measures to address Enterobacteriaceae and *E. sakazakii* are based on good manufacturing and good hygiene practices developed and applied to control *Salmonella* in the manufacture of dry dairy-based products including PIF. Such measures have been implemented and improved over the past 30 years (see section 5.4).

Enterobacteriaceae, including *E. sakazakii*, are part of the normal flora in any type of processing environment, including food factories and homes. In the case of processing environments, experience and data have shown that it is indeed possible to drastically reduce the levels present in high-hygiene areas. On the other hand, it is not currently possible to completely eliminate this group of microorganisms from the environment. Thus, they are consistently present at low levels in processing environments and may sporadically gain access into the processing line and product.

Reduction of these microorganisms in the production environment is achieved through the combination of two measures:

- minimizing their entry into high-hygiene zones; and
- preventing proliferation of those that are already present.

Measures to minimize their entry into high-hygiene zones are basically the same as those applied to control the entry of *Salmonella* (see section 5.4). Depending on the situation of the factory and considering that the occurrence of *Salmonella* is rare in comparison to that of Enterobacteriaceae and *E. sakazakii*, which are ubiquitous and thus widely distributed, these measures need to be implemented even more rigorously or reinforced to ensure success. Increases in the levels of Enterobacteriaceae and *E. sakazakii* in processing environments can be due to an extensive and unanticipated entry of microorganisms (as a result of, for example, poorly planned construction or

maintenance activities) or more commonly to the occurrence of conditions which allow proliferation of the low number of microorganisms already present in the environment. Growth is only possible in the presence of water, which must, therefore, be eliminated to the extent possible. The presence of water in the processing environment can be a result of wet-cleaning of environments or equipment, where there is formation of condensation spots, or occasionally due to water infiltration following heavy rains or the use of water showers in fire emergencies. The different situations leading to the presence of water need to be managed and controlled in specific ways and the measures may vary accordingly, for example, limiting cleaning to dry-cleaning procedures or reviewing and managing the procedures where wet-cleaning is unavoidable and where other hazards such as allergens need to be controlled as well.

The impact of such control measures can be assessed through sampling and testing programmes for Enterobacteriaceae and/or *E. sakazakii* in relevant samples. Such sampling plans typically include relevant environmental samples (processing environments and equipment) and food contact surfaces, as well as finished products to demonstrate compliance to existing regulatory criteria. Detailed comments on the design of such environmental sampling and testing programmes have been provided by ICMSF (ICMSF, 2002). The benefits of such integrated sampling programmes are highlighted in the first report on microorganisms in PIF issued by FAO/WHO (2004).

The optimal application of enhanced control measures requires a thorough review and assessment of the actual situation on individual premises in order to identify:

- whether the basic control measures (as per section 5.4.) are well implemented and effective; and
- to what extent specific additional or reinforced measures as outlined above are in place or are needed.

On the basis of such an assessment, the required improvements need to be defined and implemented. Certain measures, such as training of personnel, may be implemented quite rapidly. Others may require much more time: for example, improvements requiring a significant investment of resources and time (e.g. modification of facilities and/or processing lines and equipment). The impact of such measures, i.e. consistent low levels of Enterobacteriaceae and *E. sakazakii* in finished products, will thus only become visible and tangible after several months (Figure 3).

As already outlined in the 2004 meeting report (FAO/WHO, 2004), it is necessary to implement monitoring procedures covering all relevant elements including the processing environment, product contact surfaces and finished products. This allows the verification of the effectiveness of the measures taken and the identification of any areas of weakness where further strengthening of the measures is required to achieve the targets (i.e. microbiological requirements established to enable compliance with finished product criteria) or comply with existing or future criteria.

Monitoring for Enterobacteriaceae represents an ideal tool to assess the effectiveness of preventive measures and to detect the occurrence of recontamination. The methods are simple and they provide rapid results allowing for early corrections when needed. Such monitoring can be complemented by testing for *E. sakazakii* in relevant samples, such as in the finished product.

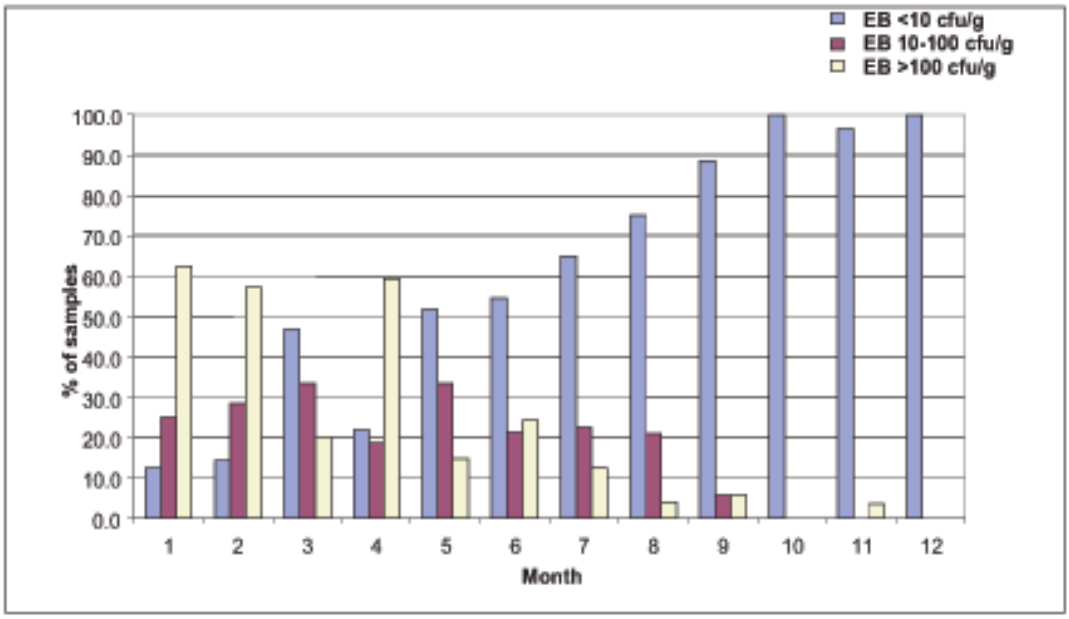


Figure 3. Reduction of Enterobacteriaceae (EB) in environmental samples from the dryer area in a plant manufacturing PIF following the implementation of enhanced control measures from 1 to 12 months after their implementation.[2]

Table 7. Analysis of finished product samples for coliforms (2 × 10 g samples) before and after the implementation of enhanced control measures in a plant manufacturing PIF.[2]

Period	Number of samples	Positive samples	Positive samples (%)
Sept.-Dec. 2003	434	2/434	0.45
Jan.-April 2004	328	0/328	0

Note: Data were submitted by industry in response to the FAO/WHO call for data.

Table 8. Analysis of finished product samples for *E. sakazakii* (1 × 333 g sample) before and after the implementation of enhanced control measures in a plant manufacturing PIF.[2]

Period	Number of samples	Positive samples	Positive samples (%)
Sept.-Dec. 2003	434	5/434	1.2
Jan.-April 2004	328	0/328	0

Note: Data were submitted by industry in response to the FAO/WHO call for data.

Such improvement programmes were initiated several years ago by some segments of the PIF industry. Specific improvements to address the levels of Enterobacteriaceae can lead to substantial reductions of these microorganisms in the processing environment and ultimately to a reduction of their occurrence, and consequently the occurrence of *E. sakazakii* in the finished PIF product (Figure 3, Tables 7 and 8).

[2] Figure 3 is based on one data set, Tables 7 and 8 on another.

Figure 3. Reduction of Enterobacteriaceae (EB) in environmental sar
manufacturing PIF following the implementation of enhanced control

4.1.3 Dry-mix ingredients

The ingredients used in dry-mixing operations represent a potential source of contamination of PIF. Since products are not subject to any further inactivation steps following their addition, these ingredients need to fulfil the same microbiological requirements as the finished product.

Since all these ingredients are submitted to heat treatments during their manufacture, the presence of Enterobacteriaceae, including *E. sakazakii*, is also due to recontamination during further processing and handling at the supplier level. As indicated in the first report on this issue (FAO/WHO, 2004), some of the ingredients are more likely to be contaminated than others. In order to obtain ingredients of the desired quality, and due to the limitations of sampling plans, it is not sufficient to determine their acceptability simply on the basis of the analysis of incoming ingredients. It is therefore important for manufacturers of PIF to work in close partnership with suppliers in order to explain their specific needs and to ensure that the desired quality can be achieved and guaranteed. The impact of such an approach is illustrated in the results of an unpublished industry survey of dry-mix ingredients before and after the implementation of improved control measures at supplier level and presented in Table 9 (J.L. Cordier, personal communication, 2006).

4.1.4 Conclusions

Although the risk assessment model cannot evaluate specific risk-reduction strategies that can be implemented at the processing level, except in the terms of comparing the risk associated with different levels of *E. sakazakii* in the finished product, an analysis of the data and information available from the industry lead to a number of conclusions. Control of recontamination of PIF with *E. sakazakii* from the processing environment following heat treatment is the critical activity required to minimize the risk associated with PIF. Achieving this requires the implementation of a number of measures modified according to the needs of individual manufacturing facilities. These measures include:

- The effective implementation of preventive measures (GMP/GHP and HACCP) as originally designed to control *Salmonella.*

Table 9. Example of the reduction of the occurrence of contaminated samples in dry-mix ingredients (unpublished industry data) over time and before and after the implementation of improved control measures at supplier level.

Ingredients	Number of positive samples					
	2002			2004		
	n	EB	Es	n	EB	Es
Lactose	2 219	70	0	2 418	22	0
Sucrose	1 691	28	2	1 812	21	0
Starch	1 389	155	40	687	4	3

n = total number of samples.
EB = Enterobacteriaceae.
Es = *E. sakazakii.*

mples from the dryer area in a plant
measures from 1 to 12 months

- The strengthening of these measures to further minimize entry of microorganisms and to avoid their multiplication by excluding water from the processing environment. The most effective means of achieving the latter is considered to be the implementation of systematic dry-cleaning.

- The selection of suppliers of dry-mix ingredients according to specified needs (e.g. microbiological requirements for dry-mix ingredients).

- The implementation of monitoring and environmental management programmes (environmental samples, product contact surfaces, finished products) based on Enterobacteriaceae, as indicators for process hygiene, and *E. sakazakii* in relevant samples to demonstrate control or to detect deviations and assess the effect of corrective actions. While the reduction of Enterobacteriaceae is desirable in terms of process hygiene, the data available did not allow the establishment of a statistical correlation between reductions in Enterobacteriaceae and *E. sakazakii*. This is discussed further in section 4.3.2.

4.2 PREPARATION, STORAGE AND HANDLING PRACTICES

This section describes the application of the risk assessment model to assess the relative risk associated with a range of consumer practices in terms of preparation and use of PIF.

4.2.1 Consumer practices (in hospital and at home)

In general there are few published data detailing how PIF is used. Therefore, prior to the meeting a questionnaire was developed and sent to hospitals and to contacts in consumer organizations with expertise on food and/or baby food issues. Information on practices in Bangladesh, Japan, Malaysia (with insights for Singapore, Thailand, Indochinese countries, Hong Kong, China and India), New Zealand, United States of America, Luxembourg, Slovenia, United Kingdom, the Netherlands, Iceland and southern Africa (examples from Swaziland, Tanzania, Botswana) was obtained from the questionnaires and other sources.

From the information examined, it was clear that there are a wide variety of methods used to prepare, store and use PIF, both in hospitals and other institutions and in the home. These include: mixing PIF in cold or hot water; immediate feeding or storage for short or extended times with or without refrigeration; re-warming or feeding without warming; and extended or short "feeding periods", defined here as the time after re-warming (or after storage, if no re-warming) until all of the prepared formula has been consumed. There may, for example, be several feedings from a single prepared container, or a single feeding may last an extended amount of time.

4.2.2 Selected scenarios

To explore the impact of different handling and use practices, eight scenarios were investigated with the conditions specified for cool, warm and very warm room temperatures, i.e. a total of 24 basic scenarios were investigated. These scenarios are illustrated in Figure 4 and cover the combinations of the following:

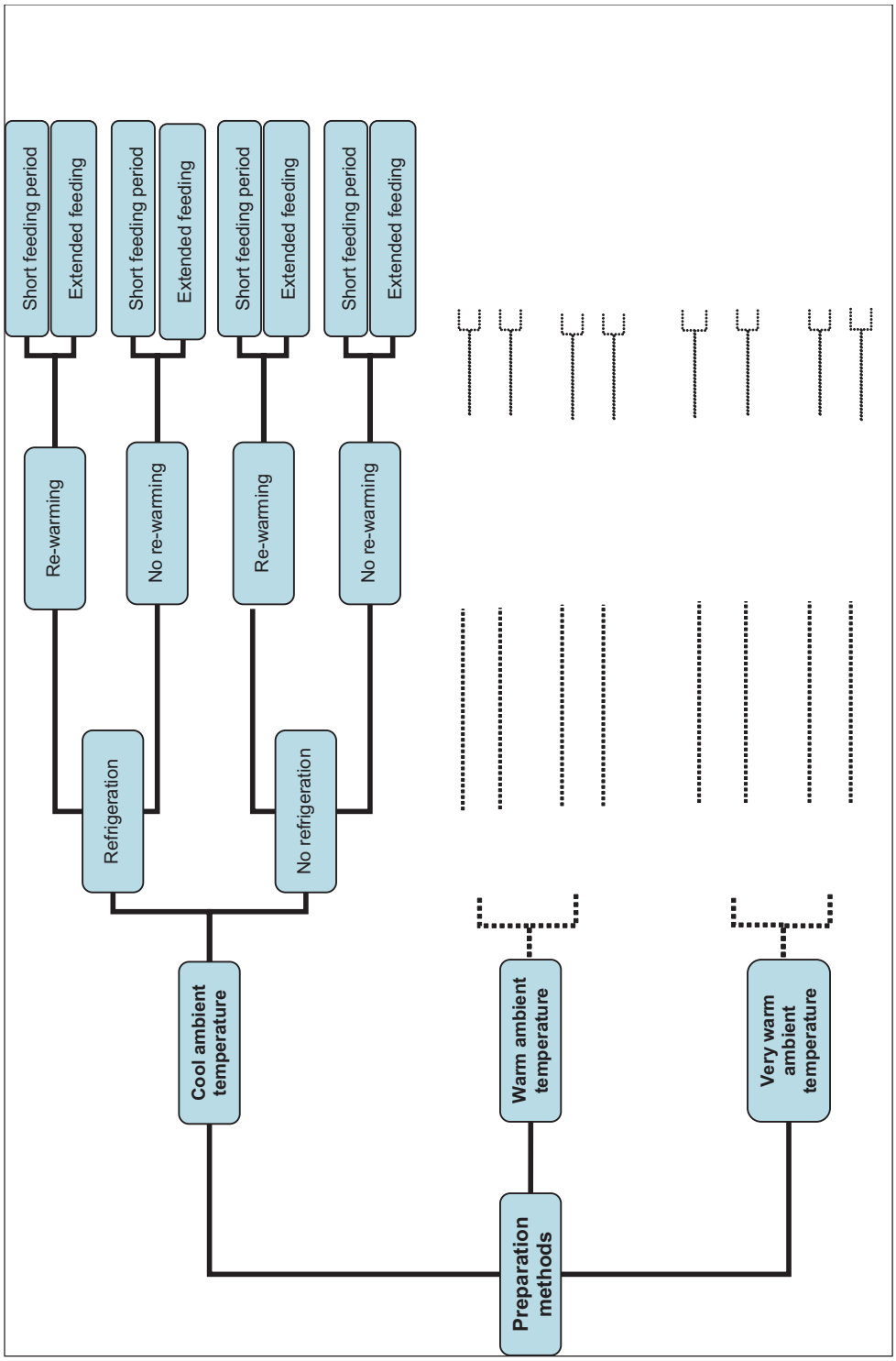


Figure 4. Flow diagram illustrating the 24 basic scenarios considered in the risk assessment. Each of the 8 scenarios illustrated for a cool ambient temperature were also undertaken for both warm and very warm ambient room temperatures.

- Cooling by refrigeration (4°C) or holding at room temperature.
- Inclusion or exclusion of an explicit re-warming action (for example through the use of a bottle warmer).
- Short or long feeding period (see definition above).

In addition, each of these scenarios was run at a series of different reconstitution temperatures using mixing water at 10°, 20°, 30°, 40°, 50°, 60° and 70°C and simulated preparation in an individual bottle (resulting in the comparison of 168 different preparation scenarios). Reconstitution temperatures greater than 70°C were not considered, but risk reductions will be greater than those predicted when formula is reconstituted at 70°C.

4.2.3 Relative risk: Results of modelling

For each of the scenarios evaluated, the PIF is considered to be pre-mixed in a small container and the ambient temperature is specified depending upon the room temperature (cool, warm or very warm). The small container, for example a feeding bottle or cup, is then either cooled in the refrigerator or held at room temperature for a specified amount of time. Prior to feeding, re-warming to 37°C may occur if specified, and then the formula is held at room temperature for the duration of the feeding period. Note that if the formula is predicted to be at a temperature greater than 37°C (i.e. reconstituted with hot water) the "re-warming" stage is a process of rapid cooling to achieve a feeding temperature of 37°C, for example by running under a cold tap or use of an ice bath). For the purposes of illustration of the scenario-associated risks, the following assumptions were made:

- The ambient temperature in a cool room is 20°C.
- The ambient temperature in a warm room is 30°C.
- The ambient temperature in a very warm room is 35°C (this could be representative of the ambient temperatures in a newborn nursery or a NICU [neonatal intensive care unit]).
- The temperature of a refrigerator is 4°C.
- Re-warming is implemented for 15 minutes and achieves a temperature of 37°C.
- The feeding period is at room temperature as specified by:
  - a short feeding period that lasts 20 minutes; or
  - an extended feeding period that lasts 2 hours (this could represent the situation whereby an infant is fed small amounts of formula from the same container over a period of time).
- For each period the rate at which the formula cools/warms is assigned based upon experimental research using formula cooled in feeding bottles (values for the cooling rate constants were obtained from M. Zwietering [personal communication, 2004]).
- Refrigerated holding time is 4 hours.
- Room temperature holding time is 1 hour.

The results of the risk assessment model for the scenarios are presented in terms of relative risk. To enable the calculation of relative risk, a baseline scenario is specified for each set of scenarios. The relative risk is given by dividing the risk estimate by the baseline risk estimate; therefore the baseline scenario has a relative risk value of 1. If the risk associated with a particular scenario is greater than the risk estimate for the baseline, it is expressed as an *x*-fold increase in risk, if it is

lower than the baseline it is expressed as an *x*-fold decrease in risk and if the risk is the same as that of the baseline scenario it is expressed as 1. Note that as results are based upon relative risk, results should not be compared between tables on a purely numerical basis, as different tables may be based upon different baseline scenarios.

4.2.3.1 Preparation and use of formula at a cool ambient room temperature

Relative risk estimates for the eight scenarios conducted at a cool room temperature are given in Table 10. The baseline scenario to which all other scenarios were compared was as follows:

- PIF reconstituted with water at a temperature of 30°C
- No refrigeration of the formula after mixing
- No re-warming of the formula
- Formula used 1 hour after preparation
- Short feeding time (20 minutes)

This baseline scenario is identified as such in Table 10. The relative risks associated with reconstitution with water at temperatures of between 10° and 70°C are described in Table 10.

Overview of findings

- The relative risks associated with reconstitution of PIF with 10° and 20°C water indicate that in terms of risk such scenarios are equivalent to the baseline scenario of reconstitution of PIF at 30°C.

Table 10. Relative risk of different preparation, storage and handling practices for formula prepared and used at a cool ambient room temperature (20°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Preparation, storage and feeding scenarios	Relative increase or decrease in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration, re-warming, extended feeding period	1	1	**+ 2.0**	**+ 8**	**+ 33**	**+ 1.8**	> - 100 000
Refrigeration, re-warming, short feeding period	1	1	1	1	**+ 2.3**	- 1.3	> - 100 000
Refrigeration, no re-warming, extended feeding period	1	1	1	1	1	- 1.3	> - 100 000
Refrigeration, no re-warming, short feeding period	1	1	1	1	1	- 1.3	> - 100 000
No refrigeration, re-warming, extended feeding period	1	1	**+ 2.8**	**+ 12**	**+ 41**	**+ 1.4**	> - 100 000
No refrigeration, re-warming, short feeding period	1	1	1	1	**+ 2.9**	- 1.3	> - 100 000
No refrigeration, no re-warming, extended feeding period	1	1	1	**+ 1.3**	**+ 15**	- 1.3	> - 100 000
No refrigeration, no re-warming, short feeding period	1	1	1 (Baseline)	1	**+ 1.5**	- 1.3	> - 100 000

Note: Note: The baseline scenario used here is different to the baseline scenarios used in other tables and therefore a direct comparison of the relative risks should not be made between this and other tables.

- For reconstitution of PIF at 40°C, those scenarios which include both re-warming of the formula and extended feeding periods (three scenarios) are associated with an increased risk compared to the baseline scenario where there is no re-warming of the formula and the feeding time is short.

- For reconstitution of PIF at 50°C, all of the scenarios in which the formula was not refrigerated were associated with an increased risk as well as the two scenarios where the formula was refrigerated and then re-warmed before use.

- The three highest risk scenarios involved reconstitution at 50°C.

- The combination of re-warming to 37°C and an extended feeding period was associated with increased risks for reconstitution temperatures of 30°, 40°, 50° and 60°C, as such a scenario provides the opportunity for *E. sakazakii* growth throughout the feeding period.

- A reconstitution temperature of 60°C provides risk reductions except in those scenarios which also include re-warming of the formula and an extended feeding period.

- For all scenarios, 70°C is associated with dramatic risk reductions as this has a significant bactericidal effect.

4.2.3.2 Preparation and use of formula at a warm ambient room temperature

Relative risk estimates for the eight scenarios conducted in a warm room temperature are given in Table 11. The baseline scenario to which all other scenarios were compared was as follows:

- PIF reconstituted with water at a temperature of 30°C
- No refrigeration of the formula after mixing
- No re-warming of the formula
- Formula used 1 hour after preparation
- Short feeding time (20 minutes)

This baseline scenario is also identified as such within Table 11. The relative risks associated with reconstitution of PIF with water at temperatures between 10° and 70°C are described in Table 11. The overall pattern of risk is generally similar in terms of the preparation scenarios that pose the greatest risks to that predicted for scenarios conducted in a cool ambient room temperature.

Overview of findings

- For reconstitution of PIF with 10°, 20° or 30°C water, the two scenarios which included both re-warming of the formula and an extended feeding period exhibited an increase in risk compared to the baseline scenario. In addition, PIF that was reconstituted with 30°C water, not refrigerated or re-warmed but used for an extended feeding period, exhibited a small increase in risk compared to the baseline scenario, which differed only in the length of the feeding period, which was short.

Table 11. Relative risk of different preparation, storage and handling practices for formula prepared and used at a warm ambient room temperature (30°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Preparation, storage and feeding scenarios	Relative increase or decrease in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration, re-warming, extended feeding period	**+ 2**	**+ 3.4**	**+ 8**	**+ 27**	**+ 83**	**+ 1.8**	> - 100 000
Refrigeration, re-warming, short feeding period	1	1	1	1	**+ 2.6**	- 1.3	> - 100 000
Refrigeration, no re-warming, extended feeding period	1	1	1	1	**+ 2.7**	- 1.3	> - 100 000
Refrigeration, no re-warming, short feeding period	1	1	1	1	1	- 1.3	> - 100 000
No refrigeration, re-warming, extended feeding period	**+ 3**	**+ 6**	**+ 15**	**+ 55**	**+ 161**	1	> - 100 000
No refrigeration, re-warming, short feeding period	1	1	1	**+ 1.7**	**+ 5**	- 1.3	> - 100 000
No refrigeration, no re-warming, extended feeding period	1	1	**+ 2.8**	**+ 22**	**+ 97**	- 1.3	> - 100 000
No refrigeration, no re-warming, short feeding period	1	1	1 (Baseline)	1	**+ 3**	- 1.3	> - 100 000

Note: The baseline scenario used here is different to the baseline scenarios used in other tables and therefore a direct comparison of the relative risks should not be made between this and other tables.

- For reconstitution with 40°C water, four of the eight scenarios were associated with an increased risk, three of which included an extended feeding period.

- PIF that was reconstituted with 50°C water was associated with an increased risk for all scenarios except in the case of no re-warming and a short feeding time where the risk was essentially the same as the baseline scenario.

- The combination of re-warming to 37°C and an extended feeding period was associated with increased risks for PIF reconstituted at temperatures of between 10° and 50°C.

- Reconstitution with water at a temperature of 60°C provided risk reductions except where the scenario included the combination of re-warming and an extended feeding period.

- For all scenarios, reconstitution at 70°C was associated with dramatic risk reductions.

4.2.3.3 Preparation and use of formula at a very warm ambient room temperature

To further explore the impact of room temperature, an ambient room temperature of 35°C was considered for all scenarios. Relative risk estimates for the eight scenarios conducted in a 35°C room temperature are given in Table 12. The baseline scenario to which all other scenarios were compared was as follows:

- PIF reconstituted with water at a temperature of 30°C
- No refrigeration of the formula after mixing

- No re-warming of the formula
- Formula used 1 hour after preparation
- Short feeding time (20 minutes)

This baseline scenario is identified in Table 12. The relative risks associated with reconstitution with water at temperatures between 10° and 70°C are described in Table 12. The overall profile of risk is generally similar to that predicted for scenarios conducted in a warm room temperature.

Overview of findings

- Of all the scenarios considered, no refrigeration combined with re-warming and an extended feeding period was the scenario associated with the highest degree of risk for PIF reconstituted with water at temperatures of between 10° and 50°C.

- The combination of re-warming and an extended feeding period was associated with increased estimates of risk compared to the baseline. It was noted that both re-warming of formula and extended feeding periods are common practice in NICUs, particularly when neonates or infants are fed via a feeding tube.

4.2.3.4 Influence of the refrigeration temperature

To explore the influence of refrigeration temperature on risk, the impact of refrigeration at temperatures in the range of 2° to 10°C was estimated. Following preparation, reconstituted formula

Table 12. Relative risk of different preparation, storage and handling practices for formula prepared and used at a very warm ambient room temperature (35°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Preparation, storage and feeding scenarios	Relative increase or decrease in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration, re-warming, extended feeding period	**+ 4**	**+ 6**	**+ 13**	**+ 38**	**+ 106**	**+ 1.3**	> - 100 000
Refrigeration, re-warming, short feeding period	1	1	1	1	**+ 3**	- 1.3	> - 100 000
Refrigeration, no re-warming, extended feeding period	1	1	1	1	**+ 4**	- 1.3	> - 100 000
Refrigeration, no re-warming, short feeding period	1	1	1	1	1.0	- 1.3	> - 100 000
No refrigeration, re-warming, extended feeding period	**+ 6**	**+ 11**	**+ 28**	**+ 93**	**+ 208**	- 1.3	> - 100 000
No refrigeration, re-warming, short feeding period	1	1	1	**+ 2.4**	**+ 5**	- 1.3	> - 100 000
No refrigeration, no re-warming, extended feeding period	1	**+ 2.0**	**+ 10**	**+ 54**	**+ 105**	- 1.3	> - 100 000
No refrigeration, no re-warming, short feeding period	1	1	1 (Baseline)	**1.4**	**+ 3**	- 1.3	> - 100 000

Note: The baseline scenario used here is different to the baseline scenarios used in other tables and therefore a direct comparison of the relative risks should not be made between this and other tables.

was refrigerated for 4 hours, then re-warmed and used in the course of an extended feeding period. Refrigeration at 2°C was considered the baseline. Tables 13 and 14 show the increase in relative risk associated with refrigeration temperature for formula prepared and used at cool and warm ambient room temperatures, respectively.

Overview of findings

- Compared with the 2°C baseline refrigeration temperature, as the temperature of refrigeration increased, the level of risk increased for PIF that was reconstituted at temperatures of between 30° and 50°C.
- Refrigeration temperature had no impact on formula reconstituted with water at 70°C. This holds for scenarios of preparation and use at both the cool and warm ambient room temperatures.
- In all cases explored, the increase in relative risk was less than 1.5-fold.

Table 13. Relative risk associated with refrigeration temperature for formula prepared and used at a cool ambient room temperature (20°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Temperature of water used for reconstitution of PIF	Temperature of refrigerator				
	2°C	4°C	6°C	8°C	10°C
10°C	1 (Baseline)	1.00	1.00	1.00	1.00
20°C	1 (Baseline)	1.00	1.00	1.00	1.00
30°C	1 (Baseline)	**+ 1.07**	**+ 1.14**	**+ 1.21**	**+ 1.29**
40°C	1 (Baseline)	**+ 1.07**	**+ 1.15**	**+ 1.26**	**+ 1.36**
50°C	1 (Baseline)	**+ 1.09**	**+ 1.17**	**+ 1.27**	**+ 1.39**
60°C	1 (Baseline)	**+ 1.10**	**+ 1.16**	**+ 1.23**	**+ 1.39**
70°C	1 (Baseline)	1.00	1.00	1.00	1.00

Note: The baseline scenarios used here are different to the baseline scenarios used in other tables and therefore a direct comparison of the relative risks should not be made between this and other tables.

Table 14. Relative risk associated with refrigeration temperature for formula prepared and used at a warm ambient room temperature (30°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Temperature of water used for reconstitution of PIF	Temperature of refrigerator				
	2°C	4°C	6°C	8°C	10°C
10°C	1 (Baseline)	**+ 1.02**	+ 1.04	**+ 1.06**	**+ 1.08**
20°C	1 (Baseline)	**+ 1.04**	+ 1.08	**+ 1.11**	**+ 1.15**
30°C	1 (Baseline)	**+ 1.04**	+ 1.09	**+ 1.16**	**+ 1.23**
40°C	1 (Baseline)	**+ 1.07**	+ 1.14	**+ 1.24**	**+ 1.33**
50°C	1 (Baseline)	**+ 1.07**	+ 1.17	**+ 1.25**	**+ 1.36**
60°C	1 (Baseline)	**+ 1.03**	+ 1.07	**+ 1.10**	**+ 1.16**
70°C	1 (Baseline)	1.00	1.00	1.00	1.00

Note: The baseline scenarios used here are different to the baseline scenarios used in other tables and therefore a direct comparison of the relative risks should not be made between this and other tables.

4.2.3.5 Influence of the duration of the feeding period

To further explore the impact of the duration of the feeding period, relative risk estimates were obtained for feeding periods ranging from 20 minutes to 6 hours. The baseline level to which all other scenarios were compared was a 20-minute feeding period. Scenarios both with and without a refrigeration step were considered. The relative risks associated with PIF reconstituted with water at temperatures of between 10° and 70°C for the different feeding durations for scenarios conducted at both cool and warm ambient room temperature are described in Tables 15 and 16, respectively.

Overview of findings

- In general, an increased risk was associated with an increase in the duration of the feeding period with a 6-hour period associated with the greatest increases in relative risk compared to the baseline scenario, except when PIF was reconstituted at 70°C. This pattern is seen for scenarios conducted at both cool (20°C) and warm (30°C) room temperatures.

- For PIF reconstituted with water at temperatures from 10° to 60°C, an increased risk was associated with feeding times of 4 and 6 hours at a cool ambient temperature and 2, 4 and 6 hours at a warm ambient temperature. It was noted that the hang-time of reconstituted PIF in NICUs may extend up to 4 hours.

Table 15. Effect of extended feeding periods at a cool ambient room temperature (20°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Preparation, storage and feeding scenarios	Relative increase or decrease in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration, re-warming, 0.33-hour feeding period	1.0	1.0	1 (Baseline)	1.0	**+ 3**	-2.4	> - 100 000
Refrigeration, re-warming, 1-hour feeding period	1.0	1.0	1.0	**+ 2**	**+ 10**	-1.2	> - 100 000
Refrigeration, re-warming, 2-hour feeding period	1.0	1.0	**+ 2**	**+ 8**	**+ 40**	**+ 3**	> - 100 000
Refrigeration, re-warming, 4-hour feeding period	**+ 3**	**+ 6**	**+ 16**	**+ 63**	**+ 309**	**+ 25**	> - 100 000
Refrigeration, re-warming, 6-hour feeding period	**+ 19**	**+ 33**	**+ 91**	**+ 365**	**+ 1 770**	**+ 146**	> - 100 000
No refrigeration, re-warming, 0.33-hour feeding period	1.0	1.0	1.0	1.0	**+ 3**	-2.4	> - 100 000
No refrigeration, re-warming, 1-hour feeding period	1.0	1.0	1.0	**+ 3**	**+ 11**	-1.4	> - 100 000
No refrigeration, re-warming, 2-hour feeding period	1.0	1.0	**+ 3**	**+ 11**	**+ 45**	**+ 3**	> - 100 000
No refrigeration, re-warming, 4-hour feeding period	**+ 4**	**+ 7**	**+ 21**	**+ 86**	**+ 349**	**+ 22**	> - 100 000
No refrigeration, re-warming, 6-hour feeding period	**+ 23**	**+ 42**	**+ 122**	**+ 493**	**+ 1 996**	**+ 126**	> - 100 000

Note: The baseline scenario used here is different to the baseline scenarios used in previous tables and therefore a direct comparison of the relative risks should not be made between this and previous tables.

- For PIF reconstituted at 50°C, an increase in risk was observed for all scenarios considered at both cool and warm ambient room temperatures.

- An increase in risk was predicted after 2 hours for PIF reconstituted at 30°C and after 1 hour for PIF reconstituted at 40°C at both cool and warm ambient temperatures.

- It was only when PIF was reconstituted with water at temperatures of 60° or 70°C that any risk reduction was predicted relative to the baseline. For PIF reconstituted at 60°C, these reductions are only observed for feeding times of 20 minutes and 1 hour. For PIF reconstituted with water at 70°C, there are large risk reductions compared to the baseline scenario irrespective of the duration of the feeding period.

4.2.3.6 Influence of the duration of cooling/holding period

According to information received via a questionnaire on consumer practices, in some cases PIF is mixed centrally and then distributed from this point without any refrigeration. The formula may stand at ambient temperature for several hours before being fed to infants, and in some cases leftover formula is not discarded but used in the next feeding which may take place several hours after preparation (Vilakati *et al*., 2005).

In order to explore the impact of these types of practices, the effect of the duration of the holding time (2-8 hours) of the reconstituted formula, both refrigerated and non-refrigerated, before

Table 16. Effect of extended feeding periods in a warm ambient room temperature (30°C) (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Preparation, storage and feeding scenarios	Relative increase or decrease in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration, re-warming, 0.33-hour feeding period	1	1	1 (Baseline)	1	**+ 3**	- 3	> - 100 000
Refrigeration, re-warming, 1-hour feeding period	1	1	1	**+ 4**	**+ 12**	- 2	> - 100 000
Refrigeration, re-warming, 2-hour feeding period	**+ 2**	**+ 4**	**+ 8**	**+ 27**	**+ 90**	**+ 3**	> - 100 000
Refrigeration, re-warming, 4-hour feeding period	**+ 111**	**+ 166**	**+ 381**	**+ 1 236**	**+ 4 049**	**+ 155**	> - 100 000
Refrigeration, re-warming, 6-hour feeding period	**+ 4 444**	**+ 6 667**	**+ 15 198**	**+ 48 544**	**+ 150 602**	**+ 6 211**	> - 100 000
No refrigeration, re-warming, 0.33-hour feeding period	1	1	1	**+ 2**	**+ 5**	- 3	> - 100 000
No refrigeration, re-warming, 1-hour feeding period	1	1	**+ 2**	**+ 7**	**+ 20**	- 3	> - 100 000
No refrigeration, re-warming, 2-hour feeding period	**+ 3**	**+ 6**	**+ 16**	**+ 53**	**+ 155**	**+ 2**	> - 100 000
No refrigeration, re-warming, 4-hour feeding period	**+ 150**	**+ 279**	**+ 724**	**+ 2 404**	**+ 6 993**	**+ 76**	> - 100 000
No refrigeration, re-warming, 6-hour feeding period	**+ 6 024**	**+ 11 173**	**+ 28 736**	**+ 92 593**	**+ 245 700**	**+ 3 040**	> - 100 000

Note: The baseline scenario used here is different to the baseline scenarios used in previous tables and therefore a direct comparison of the relative risks should not be made between this and previous tables.

re-warming and an extended feeding period was considered. In this set of scenario analysis, the baseline scenario to which all other scenarios were compared was:

- Re-warming of the formula prior to feeding
- Holding of formula for 2 hours after preparation (either refrigerated or non-refrigerated)
- Extended feeding time (2 hours)

Different baselines were established for formula reconstituted at different temperatures: PIF reconstituted at 10°C and held for 4, 6 and 8 hours prior to feeding was compared to a baseline scenario where the formula was reconstituted at 10°C but only held for 2 hours prior to feeding. Different baselines were used for refrigerated and non-refrigerated holding. Specifically, reconstituted PIF held for 4, 6 and 8 hours without refrigeration was compared to a baseline where the reconstituted PIF was held for 2 hours without refrigeration. The relative risks predicted for each duration of holding for PIF reconstituted at seven different temperatures (10°-70°C) and prepared and used at both cool and warm ambient room temperature are given in Tables 17 and 18, respectively.

Overview of findings

- For scenarios conducted at a cool ambient temperature, when refrigeration was used the risks associated with reconstitution at 10°, 20° and 70°C were not affected by holding time. For all other temperatures, an increase in risk was predicted in association with an increased refrigeration time. This occurs because in each case, as the refrigeration time increases, a greater proportion of the lag time is consumed during this phase. Note however that this increase was never more than 1.25-fold for the durations considered.

- When refrigeration was not used, and the reconstituted PIF was held at room temperature, all scenarios with the exception of 70°C were associated with significantly increased risks.

- In all cases the risk associated with reconstitution at 70°C was not affected by the holding times explored.

- For scenarios conducted at a warm ambient temperature, increased holding times were associated with increased risk; significantly increased risks were associated with holding at room temperature (i.e. no refrigeration), with the exception of formula reconstituted at 70°C, which is not affected by holding time.

- In general, increased holding times are associated with increased risks; the exception is PIF reconstituted using water at 70°C.

4.2.3.7 Additional scenarios

Several additional scenarios were evaluated at both cool and warm ambient temperatures as shown in Table 19. These scenarios attempted to evaluate some of the other practices not covered in the previous scenarios that were identified in the response to the questionnaire on consumer practices. The baseline scenario to which all other scenarios were compared was as follows:

- Mixing in a bottle
- Cooling and holding for 1 hour at ambient temperature
- No active re-warming
- A short feeding period (20 minutes)

Table 17. Comparison of the relative risk related to holding time in/out of refrigeration before extended feeding (2 hours) for scenarios conducted at a cool ambient room temperature (20°C) (+*x* means an increase in risk of *x*-fold).

	Time between preparation and feeding	Relative increase in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
		10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration	2 hour (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)
	4	1	1	**+ 1.12**	**+ 1.12**	**+ 1.14**	**+ 1.22**	1
	6	1	1	**+ 1.15**	**+ 1.16**	**+ 1.17**	**+ 1.24**	1
	8	1	1	**+ 1.18**	**+ 1.19**	**+ 1.19**	**+ 1.24**	1
No refrigeration	2 hour (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)
	4	**+ 3**	**+ 4**	**+ 4**	**+ 6**	**+ 10**	**+ 12**	1
	6	**+ 11**	**+ 12**	**+ 12**	**+ 33**	**+ 57**	**+ 74**	1
	8	**+ 30**	**+ 36**	**+ 64**	**+ 176**	**+ 300**	**+ 403**	1

Note: The baseline scenarios used here are different to the baseline scenarios used in previous tables and therefore a direct comparison of the relative risks should not be made between this and previous tables. In addition, in this table different baselines have been used for each reconstitution temperature and for refrigerated and non-refrigerated holding of reconstituted formula. Therefore, only the three scenarios in the column below each baseline scenario should be compared.

Table 18. Comparison of the relative risk related to holding time in/out of refrigeration before extended feeding (2 hours) for scenarios conducted at a warm ambient room temperature (30°C) (+*x* means an increase in risk of *x*-fold).

	Time between preparation and feeding	Relative increase in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
		10°C	20°C	30°C	40°C	50°C	60°C	70°C
Refrigeration	2 hour (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)
	4	**+ 1.06**	**+ 1.09**	**+ 1.09**	**+ 1.12**	**+ 1.17**	**+ 1.19**	1
	6	**+ 1.08**	**+ 1.11**	**+ 1.11**	**+ 1.14**	**+ 1.19**	**+ 1.22**	1
	8	**+ 1.11**	**+ 1.11**	**+ 1.13**	**+ 1.16**	**+ 1.19**	**+ 1.24**	1
No refrigeration	2 hour (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)	1 (Baseline)
	4	**+ 11**	**+ 16**	**+ 32**	**+ 46**	**+ 53**	**+ 63**	1
	6	**+ 354**	**+ 600**	**+ 1 189**	**+ 1 377**	**+ 680**	**+ 2 745**	1
	8	**+ 12 721**	**+ 18 548**	**+ 15 268**	**+ 2 793**	**+ 705**	**+ 65 502**	1

Note: The baseline scenarios used here are different to the baseline scenarios used in previous tables and therefore a direct comparison of the relative risks should not be made between this and previous tables. In addition, in this table different baselines have been used for each reconstitution temperature and for refrigerated and non-refrigerated holding of reconstituted formula. Therefore, only the three scenarios in the column below each baseline scenario should be compared.

This baseline scenario is also identified in Table 19. The other scenarios considered are as follows:

Scenario 1: Mixing in a bottle, cooling and holding for 1 hour at room temperature, no re-warming followed by a feeding period of 20 minutes (this process combined with reconstitution of the PIF with water at a temperature of 30°C is used as the baseline scenario).

Scenario 2: Mixing in a bottle, cooling with running water within 5 minutes to 37°C, feeding for 20 minutes (37°C). This process using 50°C water to reconstitute the PIF is a recommended scenario in Japanese homes.

Scenario 3: Mixing in a very large container (25 litres), no refrigeration, holding at room temperature for 1 hour followed by feeding over a 20-minute period. Anecdotal evidence suggests that this practice occurs.

Scenario 4: Mixing in a very large container (25 litres) with preparation and holding lasting 1 hour at ambient temperature followed by refrigeration for 6 hours, re-warming and feeding for 20 minutes. The formula is dispensed as individual feeds just prior to feeding. Anecdotal evidence suggests this practice occurs.

Scenario 5: Mixing in a large container (1 litre), with preparation and holding lasting 1 hour at ambient temperature, no refrigeration and a feeding period of 20 minutes.

Scenario 6: Mixing in a large container (1 litre), with preparation and holding lasting 1 hour at ambient temperature, followed by refrigeration for 6 hours, re-warming and feeding for 20 minutes.

These scenarios were analysed for both a cool (20°C) and warm (30°C) ambient temperature.

Overview of findings

- For all scenarios analysed, reconstitution of PIF with water at temperatures of 60° and 70°C was associated with risk reductions compared to the baseline scenario.

- Scenario 2: There was no increase in risk compared to the baseline scenario for PIF reconstituted at all the temperatures considered and reconstitution with water at 60° and 70°C resulted in risk reductions compared to the baseline scenario.

- Scenario 3: Reconstitution with 40° and 50°C water poses an increased risk as compared to the baseline. All other temperatures are either equivalent to the baseline or lead to risk reductions compared to the baseline scenario.

- Scenario 4: Of the set of six scenarios considered, this scenario poses the situation associated with greatest risk. This is as a result of holding for 1 hour at room temperature prior to cooling, and the subsequent slower cooling of the prepared formula in a 25-litre compared to a 1-litre container of formula.

Table 19. Relative risks associated with additional scenarios with reconstitution at different temperatures (+*x* means an increase in risk of *x*-fold, -*x* means a decrease in risk of *x*-fold).

Scenarios	Relative increase in risk compared to the baseline scenario of 1 at different temperatures of reconstitution of PIF						
	10°C	20°C	30°C	40°C	50°C	60°C	70°C
Cool room temperature (20°C)							
1. Mix in bottle, 1 hour cooling and holding, no re-warming, 20 minutes feeding	1	1	1 (Baseline)	1	**+ 2.9**	-1.33	> - 100 000
2. Mix in bottle, 5 minutes cooling to 37°C, 20 minutes feeding (37°C)	1	1	1	1	1	-1.33	> - 100 000
3. 25-litre container, no refrigeration, 1 hour holding, 30 minutes feeding	1	1	1	**+ 1.8**	**+ 2.6**	-1.37	> - 100 000
4. 25-litre container, 1 hour preparation, 6 hours refrigeration, re-warming, 30 minutes feeding	1	1	**+ 24**	**+ 9 090**	**+ 84 745**	-1.37	> - 100 000
5. 1-litre container, no refrigeration, 1 hour holding, 30 minutes feeding	1	1	1	1	**+ 2.0**	-1.33	> - 100 000
6. 1-litre container, 1 hour preparation, 6 hours refrigeration, re-warming, 30 minutes feeding	1	1	1	**+ 2.0**	**+ 14**	-1.33	> - 100 000
Warm room temperature (30°C)							
1. Mix in bottle, 1 hour cooling and holding, no re-warming, 20 minutes feeding	1	1	1 (Baseline)	**+ 1.7**	**+ 5.0**	-1.34	> - 100 000
2. Mix in bottle, 5 minutes cooling to 37°C, 20 minutes feeding (37°C)	1	1	1	1.0	1.0	-1.34	> - 100 000
3. 25-litre container, no refrigeration, 1 hour holding, 30 minutes feeding	1	1	1	**+ 2.4**	**+ 1.6**	-1.37	> - 100 000
4. 25-litre container, 1 hour preparation, 6 hours refrigeration, re-warming, 30 minutes feeding	1	1	**+ 56**	**+ 17 699**	**+ 72 463**	-1.37	> - 100 000
5. 1-litre container, no refrigeration, 1 hour holding, 30 minutes feeding	1	1	1	**+ 1.1**	**+ 4.2**	-1.34	> - 100 000
6. 1-litre container, 1 hour preparation, 6 hours refrigeration, re-warming, 30 minutes feeding	1	1	**+ 1.2**	**+ 5.0**	**+ 45**	-1.34	> - 100 000

Note: The baseline scenarios used here are different to the baseline scenarios used in previous tables and therefore a direct comparison of the relative risks should not be made between this and previous tables.

- Scenario 5: This scenario is essentially equivalent to the baseline scenario in terms of risk except for an increase in risk when PIF is reconstituted at 50°C.

- Scenario 6: This scenario is similar in pattern to scenario 3 in terms of risk, with further increased risk associated with reconstitution with 40° and 50°C water as compared to scenario 3 and the baseline scenario.

- With the exception of scenario 2, an increased risk was predicted for all other scenarios when PIF was reconstituted at 50°C.

These findings hold for both cool and warm room temperatures, with the exception of three combinations of preparation method and reconstitution temperature which were associated with increased risk compared to the baseline scenario when conducted at warm ambient temperatures. These combinations were:

- Scenario 1, PIF reconstituted with water at 40°C
- Scenario 5, PIF reconstituted with water at 40°C
- Scenario 6, PIF reconstituted with water at 30°C

4.2.4 Conclusions

4.2.4.1 Reconstitution liquid temperature/temperature of reconstitution

- When the temperature of mixing water is 10° or 20°C, minimal growth and inactivation is observed, and therefore in many scenarios risk is either equivalent or reduced compared to the baseline scenario. However, subsequent holding for long periods at room temperature can result in growth and increased risk.

- When the temperature of mixing water is 30° or 40°C, no inactivation of *E. sakazakii* will occur; however, conditions are favourable for growth. Therefore, in cases where the formula is not consumed immediately, quick cooling to lower temperatures is required to minimize growth. Re-warming followed by holding or holding at room temperature for an extended period (even in the absence of re-warming) may result in growth and therefore increased risk.

- When the temperature of the mixing water is 50°C, no appreciable inactivation occurs and risk is determined by the amount of growth that can occur. As a result, rapid cooling to lower temperatures is required to minimize growth.

- In the case of the 60°C mixing water temperature, some initial inactivation occurs, but depending on the particular preparation scenario this inactivation can be overwhelmed by the magnitude of growth that may occur if temperatures permit, for example during extended feeding times.

- With reconstitution using 70°C mixing water, significant inactivation occurs. This inactivation was not outweighed by any growth that may have occurred in any of the scenarios.

4.2.4.2 Holding times

- Increased holding times at room temperature result in large increases in risk, as a result of the growth that occurs. This effect is exaggerated for warmer room temperatures.

- The same holding times at refrigeration temperatures indicated less than a 1.3-fold risk increase.

4.2.4.3 General

- Scenarios that involve periods of holding at room temperatures (both cool and warm) are associated with greatest risk.

- Reconstitution with liquid of 70°C is an effective risk mitigation strategy for all scenarios investigated.

- In general, the highest risk scenarios are associated with reconstitution at temperatures of 40° and 50°C when the formula is not consumed immediately.

- The use of larger containers for preparation and cooling of the formula is associated with increased risk as a result of the slower cooling rate of the larger volume of formula; therefore formula should be cooled in small containers if possible.

These outputs of the risk assessment model serve to illustrate those practices which should be minimized or avoided in the preparation, holding and use of PIF as well as those practices which can be used to minimize the risk of illness from contaminated PIF. The evaluation of scenarios at different ambient temperatures shows the importance of considering this in the establishment of good practices, particularly as ambient temperatures in facilities for neonates or young infants such as NICUs may be warm or very warm – temperatures favourable for the growth of *E. sakazakii*.

Several international reference documents specify that labelling should include adequate information to enable safe use of the product. For example, in the International Code of Marketing of Breast milk Substitutes, article 9 on Labelling states (in section 9.2) that manufacturers and distributors of infant formula should include "instructions for appropriate preparation, and a warning against the health hazards of inappropriate preparation". The General Principles of Food Hygiene (CAC/RCP 1-1969, REV. 4-2003) states in section 9.3 on Labelling: "Pre-packaged foods should be labelled with clear instructions to enable the next person in the food chain to handle, display, store, and use the product safely."

Considering the results of the model and taking into account these international reference documents, measures to provide appropriate messages to users, including consumer education activities and product labels, will need to be reviewed and revised as appropriate. For example, many labels call for mixing PIF at temperatures around 50°C, which, according to this risk assessment generally results in the greatest risks for the range of scenarios considered, unless, as is sometimes recommended, the reconstituted formula is consumed immediately. The model indicates that reconstituting PIF at 70°C provides the greatest risk reduction. However, it is recognized that not all PIF products are formulated to be mixed at 70°C. Some of the other considerations associated with reconstitution at this temperature are outlined in Appendix D. Guidelines to be developed in response to the WHA resolution could be informed by the outcome of this series of scenario analysis.

In addition, to comply with the International Code of Marketing of Breast milk Substitutes, consideration should be given to updating labels and consumer education programmes to provide a warning against the health hazards of inappropriate preparation (e.g. scalding as well as risks from pathogens). Providing information on the consequences if users do not follow instructions has been shown to be effective in motivating users to comply with the instructions (FDA, 2001; Wogalter, 2005).

4.3 MICROBIOLOGICAL CRITERIA FOR POWDERED INFANT FORMULA

The implementation of microbiological criteria is one of the control measures that can be employed to reduce the risk of *E. sakazakii* infection associated with PIF. Microbiological criteria include:

- microbiological limit to be implemented;
- testing method to be employed;
- sampling plan (i.e. size and number of samples to be examined); and
- actions to be taken when the microbiological limit is exceeded.

Microbiological criteria can be used either as a tool for verifying that other control measures have been applied effectively or as a means of directly identifying unacceptable lots, i.e. not complying with an established limit, and thus preventing their release into domestic or international trade. The focus of the current evaluation is the latter application: the elimination of unacceptable lots. Thus, a base assumption in the scenarios developed in the course of the meeting was that all lots of PIF would be tested against the microbiological criteria. If a lesser degree of testing was undertaken (e.g. only every other lot tested), the ability of the testing programme to remove unacceptable lots would decrease proportionally.

The stringency of a microbiological criterion is established by the combination of the microbiological limit selected, the number of samples analysed and the size of those samples. The stringency of a microbiological criterion can be increased by increasing the number of samples and/or the size of the samples or, in sampling plans where enumeration is performed, by decreasing the microbiological limit. The traditional application of microbiological criteria is to individual product lots, with the goal of eliminating those lots that have an unacceptably high level of contamination. However, it is important to note that, depending on the limit chosen, the variability of the contamination in the lot and the characteristics of the sampling plan chosen, there is a calculable probability of discarding lots that do not exceed the stated or implied microbiological limit (Type II error) and accepting other lots that actually should have been excluded (Type I error).

There are several different types of sampling plans that can be employed for the microbiological testing of foods. The most common approach is "attribute testing" which is used to determine "presence/absence" of a specific microorganism (i.e. +/- data) or to quantify it according to different classes (e.g. <100 cfu/g vs. ≥100 cfu/g). Both "two-class" and "three-class" attribute sampling plans will be discussed below, with particular focus on two-class plans.

4.3.1 Two-class attribute sampling plans for *Enterobacter sakazakii* in powdered infant formula

The effect that a PIF sampling plan has on relative risk reduction is a function of several variables. For any single lot of PIF, the level of contamination and the within-lot variability will determine the likelihood that a sample will be positive for *E. sakazakii* and thus accepted or rejected. In addition, the average of the concentrations of *E. sakazakii* across all lots and the between-lot variability among lots will determine the percentage of all lots rejected for a microbiological limit/sampling plan combination. The effectiveness of any potential sampling plan to reduce the relative risk of

E. sakazakii infection is then determined based on the number of contaminated servings that are prevented from entering commercial distribution.

It was decided that, at the current time, the most effective means for this meeting to provide guidance to Codex, national governments and industry on how a microbiological criterion could be used to reduce relative risk was to provide examples of how effectively different sampling plans were able to reject lots with elevated levels of contamination and the corresponding predicted reduction in relative risk. Calculation of both rejection rates and relative risk reduction requires three measurements to characterize current PIF production: the mean log concentration (MLC) (cfu/g) of *E. sakazakii* across all lots of PIF, the between-lot standard deviation (σ_b) across all PIF lots, and the within-lot standard deviation (σ_w) for individual PIF lots. Three values for the MLC: -3, -4 and -5 log[cfu/g]; and two for σ_b: 0.5 and 0.8, were selected on the basis of an estimation of MLC based on data acquired in the scientific literature and submitted in response to the FAO/WHO call for data. The estimated concentrations based on these data ranged from -2.79 to -5.24 log[cfu/g], with a mean of -3.84 log[cfu/g] and a σ_b of 0.696 (Table 20). The explanation of how these values were derived is summarized in Box 1. No data were available upon which the σ_w could be estimated; therefore three values (0.1, 0.5 and 0.8) were considered, representing a wide range of values.

The risk assessment model was used to evaluate nine sampling plan scenarios (Table 21) compared to a baseline scenario of absence of sampling and zero lot rejection. One of the outputs is the relative risk reduction associated with a specific sampling plan compared to no sampling plan. In order to assist in understanding and interpreting relative risk reduction, Table 22 provides an overview of some of the different ways of expressing risk reduction.

In order to achieve the aforementioned objective, the model was set up to run different sampling plan scenarios with various levels of assumed contamination. A total of 162 sampling plan scenarios were performed, encompassing the variable combinations of three values for MLC (-5, -4 and -3 log[cfu/g]) × 2 σ_b (0.5 and 0.8) × 3 σ_w (0.1, 0.5 and 0.8) × 9 sample plans (1 and 10 g sample sizes and 3, 5, 10, 30 or 50 samples). The outputs are expressed in terms of the relative risk reduction compared to no sampling plan and the estimated rejection rate associated with a particular sampling plan. Some of these outputs are presented in Table 23.

The impact of the nine sampling plans on risk reduction for lots with a mean log concentration of -3, -4 or -5 log[cfu/g] and between-lot standard deviations (σ_b) of 0.5 and 0.8 is depicted graphically in Figure 5. This illustrates that for product with a very low mean concentration of *E. sakazakii*, the level of risk reduction for consumers as a result of implementing a sampling plan is low. However, as the mean concentration of *E. sakazakii* in the product increases, the impact of the sampling plan is also greater. Thus the implementation of a sampling plan for product with an MLC of -3 log[cfu/g] will lead to a greater level of risk reduction for consumers of that product compared to the level of risk reduction achieved through the implementation of a sampling plan for product that already has a very low mean concentration of *E. sakazakii*, such as -5 log[cfu/g]. The between-lot variation also has an impact on the effectiveness of sampling plans to reduce risk. As the between-lot variation increases, the impact of the sampling plan in terms of risk reduction also increases.

Table 20. Calculated concentration values based on published studies in the scientific literature and unpublished studies provided to FAO/WHO on the frequency of *E. sakazakii* contamination of PIF.

Company/Study	Year	Sample size (*g*)	Samples tested	*E. sakazakii* +	$C_{Poisson}$	$Log_{10}C$
A	2004	50	37	0	nd	
A	2004	50	281	5	0.000359	-3.44481
B	2004	10	497	1	0.000201	-3.69592
B	2004	10	2 018	9	0.000447	-3.34971
B	2004	10	1 286	*0.5*	3.89E-05	-4.41019
B	2004	10	437	*0.5*	0.000114	-3.94126
B	2004	10	2 114	1	4.73E-05	-4.325
B	2004	100	293	*0.5*	1.71E-05	-4.76753
C	2004	333	11 558	26	6.76E-06	-5.16986
D	2004	333	12	0	nd	
D	2004	333	12	0	nd	
E	2004	333	67	3	0.000138	-3.86149
F	2004	25	30	0	nd	
G	2004	333	518	1	5.8E-06	-5.23635
G	2004	333	571	8	4.24E-05	-4.37293
H	2004	100	320	7	0.000221	-3.65526
I	2004	250	6	2	0.001622	-2.78999
I	2004	250	121	27	0.00101	-2.99569
I	2004	250	198	23	0.000494	-3.30634
J	2004	65	20	2	0.001621	-2.79024
K	2004	333	316	20	0.000196	-3.70698
L	2004	333	434	5	3.48E-05	-4.45845
L	2004	333	328	*0.5*	4.58E-06	-5.33902
L	2004	100	940	13	0.000139	-3.85616
L	2004	100	888	16	0.000182	-3.74035
L	2004	100	908	2	2.21E-05	-4.65658
L	2004	100	921	4	4.35E-05	-4.36125
L	2004	100	255	15	0.000606	-3.21735
L	2004	100	2 523	25	9.96E-05	-4.00182
L	2004	100	756	8	0.000106	-3.97312
Muytjens, Roelofs-Willemse and Jasper	1988	333	141	20	0.000459	-3.33784
Iversen and Forsythe	2004	25	82	2	0.000988	-3.00537
FDA	2003	1 332	22	5	0.000194	-3.71317
Leuschner *et al.* [a]	2004	450	58	8	0.00033	-3.48172
Nazarowec-White and Farber	1997	333	120	8	0.000207	-3.68364
Heuvelink *et al.*	2002	25	40	1	0.001013	-2.99451
Heuvelink *et al.*	2003	25	101	2	0.0008	-3.0969
					Mean log concentration (MLC)	**-3.84051**
					Standard deviation	**0.695961**

[a] Following the meeting it came to the attention of the Secretariat that this paper had been retracted at the request of the chief editor and authors.

Note: To maximize the use of available data an estimated concentration was determined by arbitrarily assuming "0.5" positives, only in those studies where no *E. sakazakii* was isolated from more than 100 samples analysed (see Box 1 for further information).

A-L = Data received from industry in response to the FAO/WHO call for data.

C = Estimated concentration (cfu/g).

nd = Not determined, since no positives found and total amount of samples smaller than 100.

Box 1

Estimating the concentration of *Enterobacter sakazakii* in powdered infant formula

Relating an attribute sampling plan to its predicted relative risk reduction requires the availability of an estimate of the mean and standard deviation of concentration of *E. sakazakii* among PIF lots. While specific data were not available, these values were estimated using the prevalence data in the literature and provided through the FAO/WHO call for data (Table 20). These data were converted to likely concentration values based on the assumption that sampling is a Poisson process, and that the concentration in a dataset can be described using the relationship:

$$C_{Poisson} = \frac{-\ln(1-P)}{s}$$

where $C_{Poisson}$ is the estimated concentration (cfu/g), P is the reported prevalence, and s is the sample size (g). No attempt was made to differentiate samples taken at retail or manufacture, although there is a possibility that the prevalence at manufacture would be higher due to: (1) industry data being taken before rejection of lots and (2) the potential for a decline in levels during retail storage.

The data presented in each row of Table 20 were considered as one "unit"; the prevalence and sampling size were used to calculate a concentration for that group of samples. Studies where no *E. sakazakii* were detected among the lots sampled were not used. After being transformed to log values, the concentrations from the 29 available studies were used to calculate the MLC and its standard deviation, i.e. MLC = -3.73 and σ_b = 0.647.

So as to maximize the use of the data available, in those instances where a study did not isolate *E. sakazakii* and $\geq$100 samples were analysed, an estimated concentration was determined by arbitrarily assuming "0.5" positives in these units and calculating the concentration based on that prevalence.

For example:

Sample size (g)	Samples tested	*E. sakazakii* positive	$C_{Poisson}$	$\log_{10}C$
100	293	0.5 (was 0)	1.71E-05	-4.77

The inclusion of these studies decreased the mean (MLC = -3.84) and increased the standard deviation (σ_b = 0.696) slightly.

The meeting considered that the values generated through this process can only be considered crude estimates of the concentrations and variability of *E. sakazakii* among PIF lots. Ideally, the data would have been gathered in a manner that would have allowed the concentration of each lot sample to be determined (e.g. an MPN of each lot conducted); however, most data currently available are based on presence/absence testing only. The meeting considered that the accumulated data were sufficient to identify a range of MLC and σ_b values that would be appropriate for consideration through the analysis of scenarios using the risk assessment model.

Table 21. Two-class sampling plan scenarios evaluated using the risk assessment model.

Plan	Number of samples examined (n =)	Size of samples taken (s =) (*g*)
A	3	1
B	5	1
C	10	1
D	50	1
E	3	10
F	5	10
G	10	10
H	30	10
I	50	10

Table 22. Understanding relative risk and examples of different ways of expressing risk reduction.

Baseline risk	Relative risk reduction	% reduction in risk	New level of risk
100	1	0	100
100	1.2	16.7	83.3
100	2	50	50
100	4	75	25
100	8	87.5	12.5
100	10	90	10

Note: Baseline risk is set at 100 units (e.g. cases of illness in a population per year). A relative risk reduction of 1 is equivalent to the baseline. A relative risk reduction of 2 is equivalent to a two-fold reduction compared to the baseline or a 50% reduction in risk. This table is for illustrative purposes only and not as output of the risk assessment model.

It is apparent from the results of the simulations that increases in the MLC or σ_b result in a higher reject rate within any specific sampling plan. The results of the simulation for sampling plans A to I for lots with an MLC of -3, -4 and -5 log[cfu/g], a between-lot standard deviation (σ_b) of 0.8 and a within-lot standard deviation (σ_w) of 0.5 are depicted in Figure 6, which shows that the higher the MLC of *E. sakazakii*, the greater the reject rate. While the MLC of the lot has the greatest impact on rejection rate, σ_b also has an impact, albeit to a lesser extent than the MLC (Figure 7). These results can be interpreted as indicating that to reduce the potential for rejection of product (and thus increase risk reduction), manufacturers should focus on ensuring that the overall mean log concentration is low and the variation between lots is controlled. It was noted that no impact was achieved by attempting to change the homogeneity in any single lot.

As expected, increasing the number of samples and the sample size increases the likelihood of detecting lots with elevated levels of *E. sakazakii*, thus increasing the reject rate. For example, Figure 8a depicts the effect of sample number and 1 g and 10 g sample sizes on rejection rates for MLC = -4, $\sigma_b = 0.8$ and $\sigma_w = 0.5$. These simulations are also depicted together as a function of total sample mass examined on Figure 8b, demonstrating the proportionality between total sample mass and rejection rate.

The risk associated with any specific PIF lot is a function of the number of contaminated servings it will yield, and the ability of a microbiological criterion to reduce that risk in an effective manner is based on correctly identifying those lots with the highest levels of contamination. When

Table 23a. Rejection rates and relative risk reductions predicted by simulation analysis of nine two-class sampling plans for PIF, with $\sigma_b = 0.5$ and $\sigma_w = 0.5$.

Mean log (*cfu/g*)	Sampling plan code	Sampling plan	Probability of rejection of lot	Relative risk reduction
-5	A	n=3, s=1	5.8 E-05	1
-5	B	n=5, s=1	9.5E-05	1
-5	C	n=10, s=1	1.9 E-04	1
-5	D	n=50, s=1	9.7 E-04	1
-5	E	n=3, s=10	5.8 E-04	1.002
-5	F	n=5, s=10	9.5 E-04	1.002
-5	G	n=10, s=10	1.9 E-03	1.005
-5	H	n=30, s=10	5.8 E-03	1.02
-5	I	n=50, s=10	9.6 E-03	1.03
-4	A	n=3, s=1	5.9 E-04	1.001
-4	B	n=5, s=1	9.9 E-04	1.003
-4	C	n=10, s=1	2.0 E-03	1.006
-4	D	n=50, s=1	9.6 E-03	1.03
-4	E	n=3, s=10	5.8 E-03	1.01
-4	F	n=5, s=10	9.6 E-03	1.02
-4	G	n=10, s=10	0.019	1.05
-4	H	n=30, s=10	0.053	1.13
-4	I	n=50, s=10	0.083	1.21
-3	A	n=3, s=1	5.7 E-03	1.02
-3	B	n=5, s=1	9.4 E-03	1.03
-3	C	n=10, s=1	1.9 E-02	1.05
-3	D	n=50, s=1	8.4 E-02	1.21
-3	E	n=3, s=10	5.0 E-02	1.12
-3	F	n=5, s=10	8.0 E-02	1.19
-3	G	n=10, s=10	0.15	1.34
-3	H	n=30, s=10	0.33	1.83
-3	I	n=50, s=10	0.44	2.23

Note: The rejection rate and risk reduction apply to the situation where every lot is tested. Rejection rates and risk reduction would need to be pro-rated accordingly, if sampling plans were not applied to every lot. The results presented in this table are based on the assumptions that a) the average contamination concentration is lognormally distributed *between* lots, b) local contamination *within* lots is lognormally distributed with $\sigma_w = 0.5$, and c) between-lot standard deviation is $\sigma_b = 0.5$ (see Table 22b for $\sigma_b = 0.8$). The baseline scenario to which all sampling plan scenarios are compared is a no sampling plan scenario with zero rejection rate.

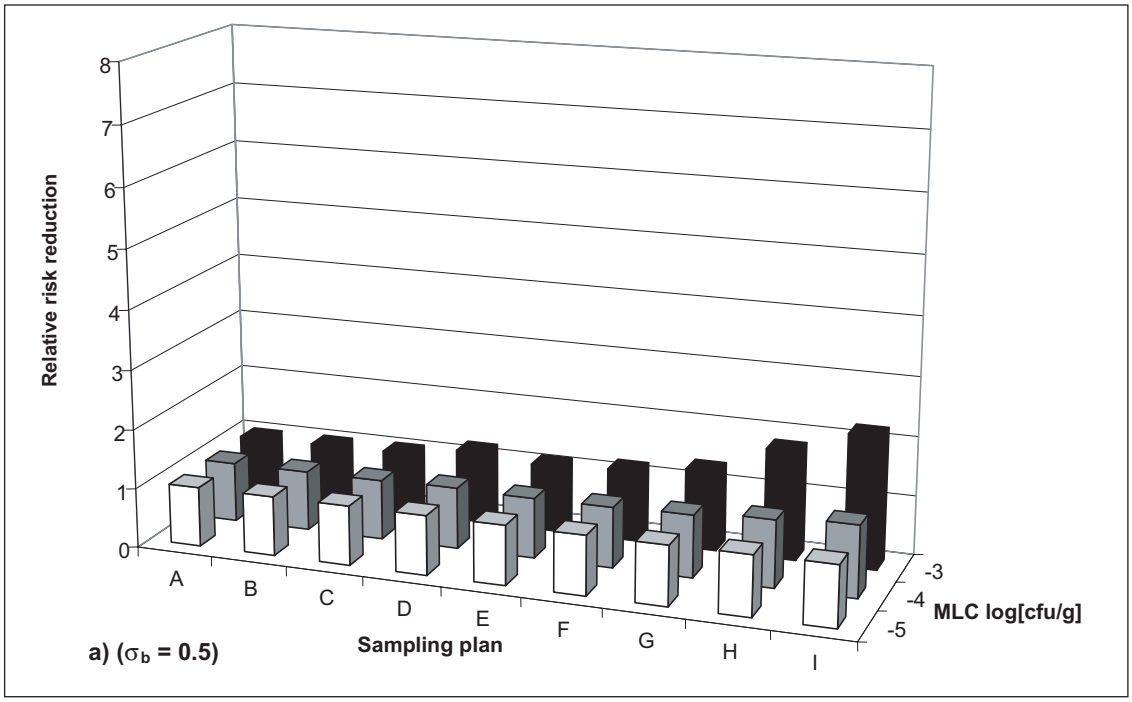
the rejection rates across all sampling plan simulations were compared against the predicted relative reduction rates (Figure 9), a pattern indicating two relationships was evident. These two relationships reflect the two simulated σ_b values, with $\sigma_b = 0.8$ yielding the high relative risk reductions when the MLC is high (i.e. MLC = -3.0 log[cfu/g]). Thus, a higher proportion of the lots rejected in the $\sigma_b = 0.8$ simulations had a higher level of *E. sakazakii*, thus producing a greater relative reduction in risk. This relationship between relative risk reduction and rejection rate again points out that

Table 23b. Rejection rates and relative risk reductions predicted by simulation analysis of nine two-class sampling plans for PIF, with $\sigma_b = 0.8$, $\sigma_w = 0.5$.

Mean log (*cfu/g*)	Sampling plan code	Sampling plan	Probability of rejection of lot	Relative risk reduction
-5	A	n=3, s=1	1.6 E-04	1.003
-5	B	n=5, s=1	2.7 E-04	1.005
-5	C	n=10, s=1	5.3 E-04	1.009
-5	D	n=50, s=1	2.6 E-03	1.05
-5	E	n=3, s=10	1.6 E-03	1.03
-5	F	n=5, s=10	2.6 E-03	1.05
-5	G	n=10, s=10	5.1 E-03	1.08
-5	H	n=30, s=10	0.014	1.21
-5	I	n=50, s=10	0.023	1.32
-4	A	n=3, s=1	1.6 E-03	1.03
-4	B	n=5, s=1	2.7 E-03	1.04
-4	C	n=10, s=1	5.2 E-03	1.09
-4	D	n=50, s=1	0.023	1.32
-4	E	n=3, s=10	0.014	1.19
-4	F	n=5, s=10	0.023	1.30
-4	G	n=10, s=10	0.041	1.51
-4	H	n=30, s=10	0.096	2.07
-4	I	n=50, s=10	0.14	2.50
-3	A	n=3, s=1	0.014	1.22
-3	B	n=5, s=1	0.022	1.3
-3	C	n=10, s=1	0.040	1.49
-3	D	n=50, s=1	0.14	2.49
-3	E	n=3, s=10	0.087	1.86
-3	F	n=5, s=10	0.13	2.27
-3	G	n=10, s=10	0.20	3.14
-3	H	n=30, s=10	0.37	5.71
-3	I	n=50, s=10	0.46	7.76

Note: The rejection rate and risk reduction apply to the situation where every lot is tested. Rejection rates and risk reduction would need to be pro-rated accordingly, if sampling plans are not applied to every lot. The results presented in this table are based on the assumptions that a) the average contamination concentration is lognormally distributed *between* lots, b) local contamination *within* lots is lognormally distributed with $\sigma_w = 0.5$, and c) between-lot standard deviation is $\sigma_b = 0.8$ (see Table 22a for $\sigma_b = 0.5$). The baseline scenario to which all sampling plan scenarios are compared to is a no sampling plan scenario with zero rejection rate.

when manufacturers attempt to decrease their rejection rates, initial gains may be achieved by reducing the variability between lots, but as the variability between lots decreases, more meaningful (and probably cost-effective) reductions in rejection rates will require reducing the MLC of the lots through the implementation of new practices or technologies.



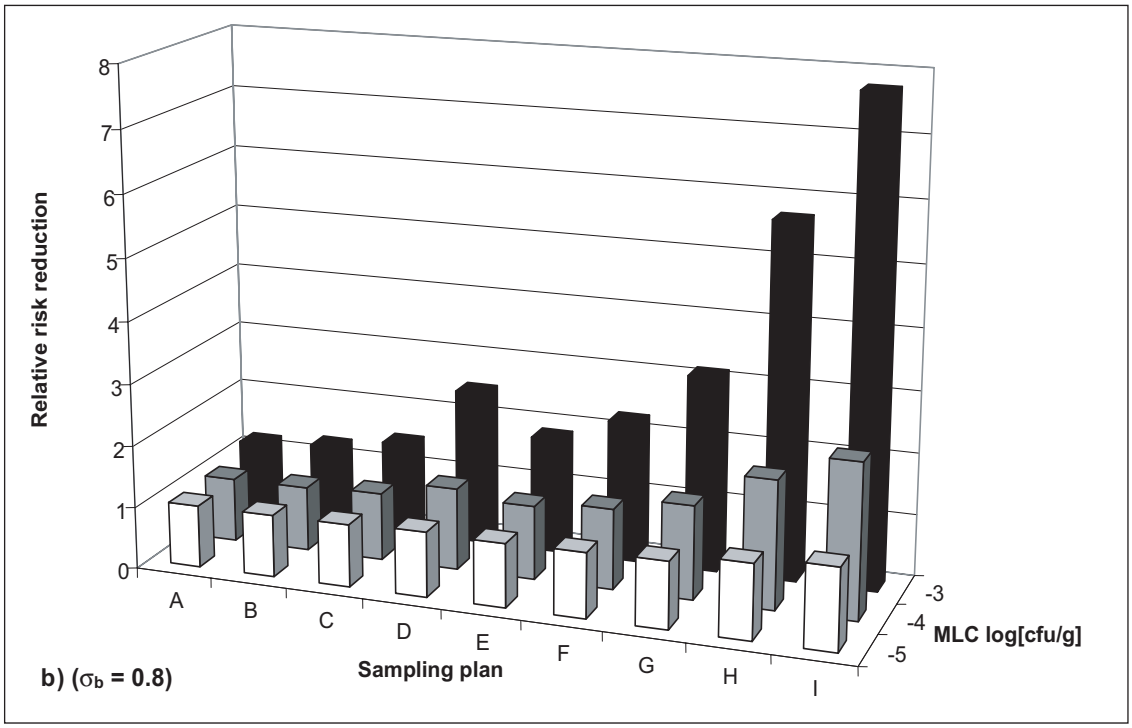


Figure 5. Impact of sampling plan, assumed mean log concentration of *E. sakazakii* and between-lot standard deviation on relative risk reduction. a) σ_w = 0.5, b) σ_b = 0.8.

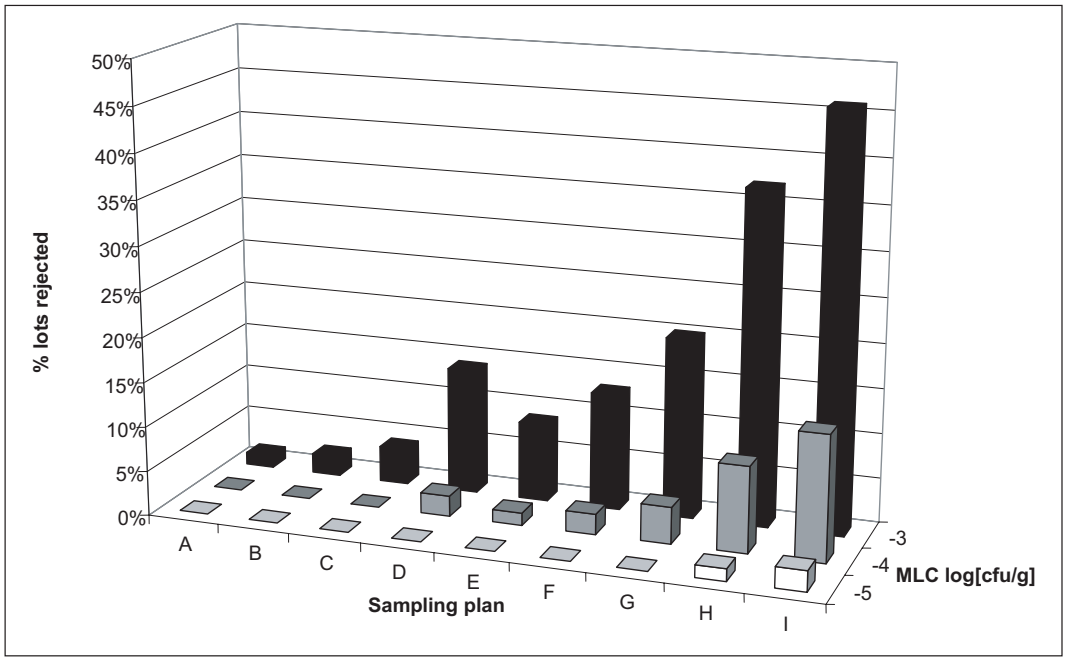


Figure 6. The effect of mean log concentration of *Enterobacter sakazakii* (σ_b = 0.8, σ_w = 0.5) on the simulated rejection rate for lots evaluated using a range of two-class attribute sampling plans as described in Table 21.

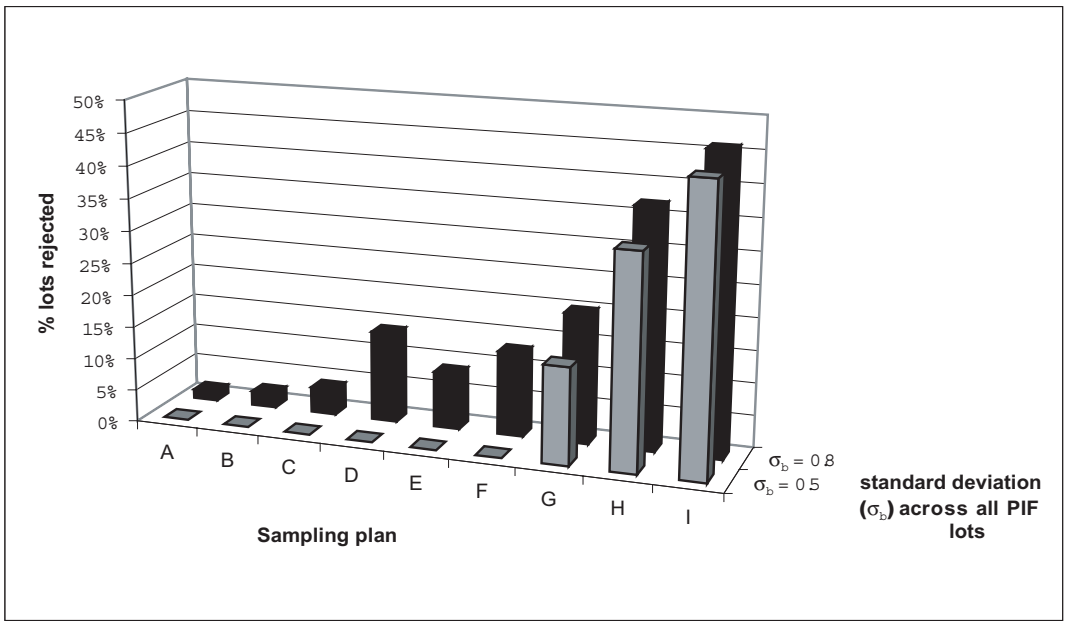


Figure 7. The effect of between-lot standard deviation (σ_b) on the simulated rejection rate for lots with a mean log concentration of *Enterobacter sakazakii* of -3 log[cfu/g] and σ_w = 0.5 evaluated using a range of two-class attribute sampling plans as described in Table 21.

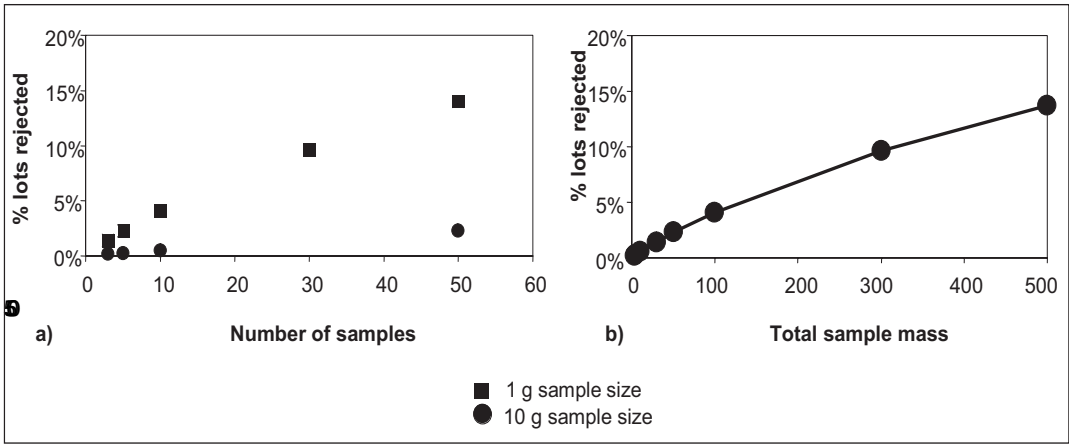


Figure 8. Effect of sample size and sample number (a) and total sample mass (b) on simulated rejection rate for a population of PIF lots that have MLC = -4, σ_b = 0.8 and σ_w = 0.5.

4.3.2 Sampling plans for Enterobacteriaceae

In addition to the two-class plans for *E. sakazakii*, the meeting discussed the possibility of utilizing a two-class or three-class plan for Enterobacteriaceae in PIF as an indicator microorganism based on either presence/absence testing or quantification. As in the discussion above, the establishment of a sampling plan would require the availability of values for MLC, σ_b and σ_w for Enterobacteriaceae in PIF. In addition, estimating the impact of the sampling plans developed for Enterobacteriaceae on the relative risk reduction of *E. sakazakii* would require the availability of a means for relating the rejection of lots based on the presence of unacceptable levels of Enterobacteriaceae to a

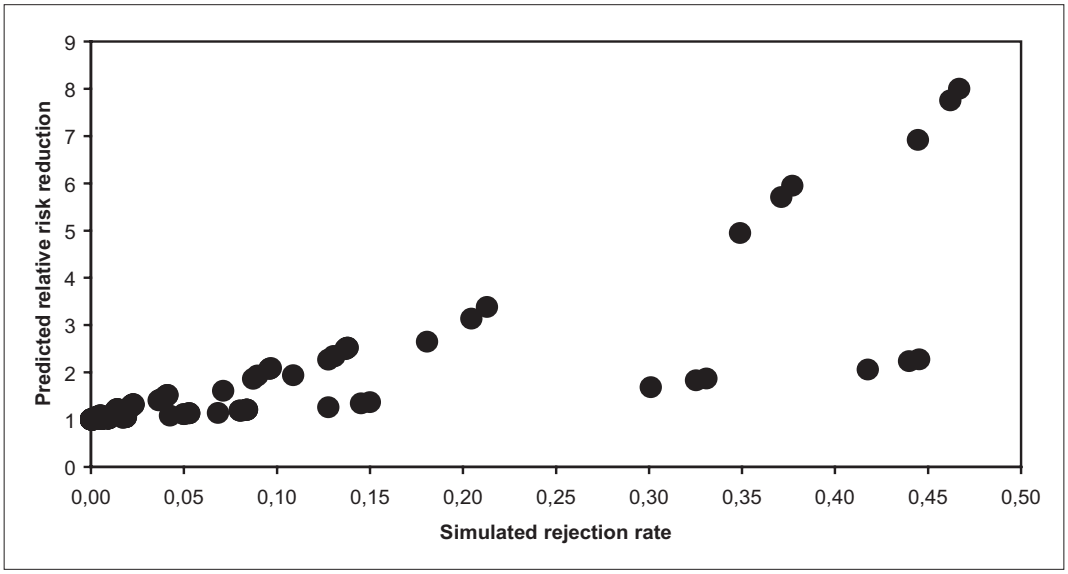


Figure 9. Relationship between simulated rejection rate and predicted relative risk reduction across all of 162 simulations of the nine sampling plans as a function of MLC, σ_b and σ_w.

rejection rate for *E. sakazakii*. However, on the basis of the information available to the meeting, the extent of the statistical relationship between Enterobacteriaceae and *E. sakazakii* could not be determined. While it was included in the risk assessment model, the meeting participants were unconvinced that they could develop a meaningful set of scenario evaluations incorporating sampling plans for Enterobacteriaceae because of the nature of the problem and currently available but inconclusive data. However, the meeting also noted that knowledgeable industry scientists feel that controlling Enterobacteriaceae is an effective tool to help control *E. sakazakii*. It may be possible to develop such a sampling plan through an expert elicitation of individuals with extensive practical experience in the specific application of this indicator test for the control of *E. sakazakii* or, alternatively, by issuing a call for data that more specifically identifies the type of data needed for establishing more definitively any relationship between the likely presence of *E. sakazakii* and Enterobacteriaceae in PIF.

4.3.3 Consideration of the cumulative impact of sampling plans through changes in production management and lot rejection

A sampling plan is designed to detect and eliminate lots that are contaminated above a specific detectable level. In the previous examples, the relative risk reductions and the proportion of product rejected were estimated for an overall distribution of contamination that was meant to represent all the PIF being produced and sampled. There will also be a more long-term effect of a sampling plan above this direct effect, due to changes in either the mean concentration or variability. This phenomenon is explained by considering that the overall distribution is likely to be made up of several other distributions. These could be disaggregated down from a global level, to perhaps a country level, or further to a company level, and ultimately down to a factory level. To illustrate this point, panel A of Figure 10 shows a broad distribution that could represent the overall distribution of contamination in PIF. Panel B shows how that distribution could come about as part of three distributions representing three discrete groups of producers.

In the current example we assume that the PIF coming into the market comes from three types of process:

- Best (left curve): Process producing product with a low mean concentration of *E. sakazakii.*
- Moderate (middle curve): Process producing product with a moderate mean concentration of *E. sakazakii.*
- Poor (right curve): Process producing product with a high mean concentration of *E. sakazakii.*

This grouping into three categories is arbitrary and is only intended to set the stage for how a sampling plan could produce long-term results that are different from the immediate potential risk reduction that results from lot rejection.

Figure 11 illustrates the situation when a sampling plan is applied in this type of environment. Those manufacturers classified as “best” were producing PIF with a low mean concentration even before the sampling plan, and the consumers of the product coming from these manufacturers were originally exposed to a very low risk. As a result of the sampling plan, which is designed to pick up lots with higher contamination, very few lots from this category of manufacturer are

rejected (very low lot rejection rate). Since the manufacturer was originally making product that was contaminated at a very low mean concentration, the relative risk reduction for consumers of product from these manufacturers is also low.

Those manufacturers in the "moderate" category were producing PIF with a moderate mean concentration, and consumers of product from these manufacturers were exposed to a higher risk than those consuming product from manufacturers in the "best" category. When the sampling plan is implemented, it results in a moderate portion of the lots from this category being rejected. Those lots produced by this category of manufacturer that are highly contaminated have a high probability of being detected by the sampling plan and being rejected. As a result, while the risk to consumers of product from this category of manufacturer was not very high, the rejection of highly contaminated lots translates to a moderate relative risk reduction.

Finally, those manufacturers classified as "poor" were producing product with a high mean concentration. Consumers exposed to product coming from these manufacturers were at higher risk than those exposed to product from manufacturers in the other categories. Large portions of the lots coming from manufacturers in this category have higher contamination rates. As a result, the sampling plan has a higher probability of detecting and rejecting these lots. Since the higher risk lots are removed from the market, a significant relative risk reduction for the consumers of product from these manufacturers would be expected.

So far, the implementation of a hypothetical sampling plan as illustrated in Figure 11 has provided some risk reduction as intended. It penalizes "poor" manufacturers by detecting and rejecting higher contaminated lots, and impacts public health by producing large relative risk reductions for consumers of product from these manufacturers. In addition, better manufacturers who are not producing higher contaminated lots do not have to reject large portions of product.

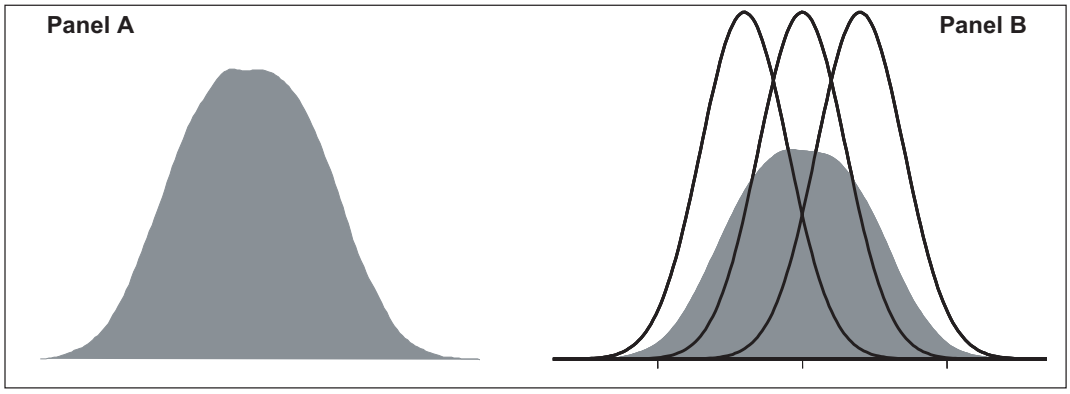


Figure 10. Illustration of how a distribution of PIF contamination could be made up by several discrete distributions.

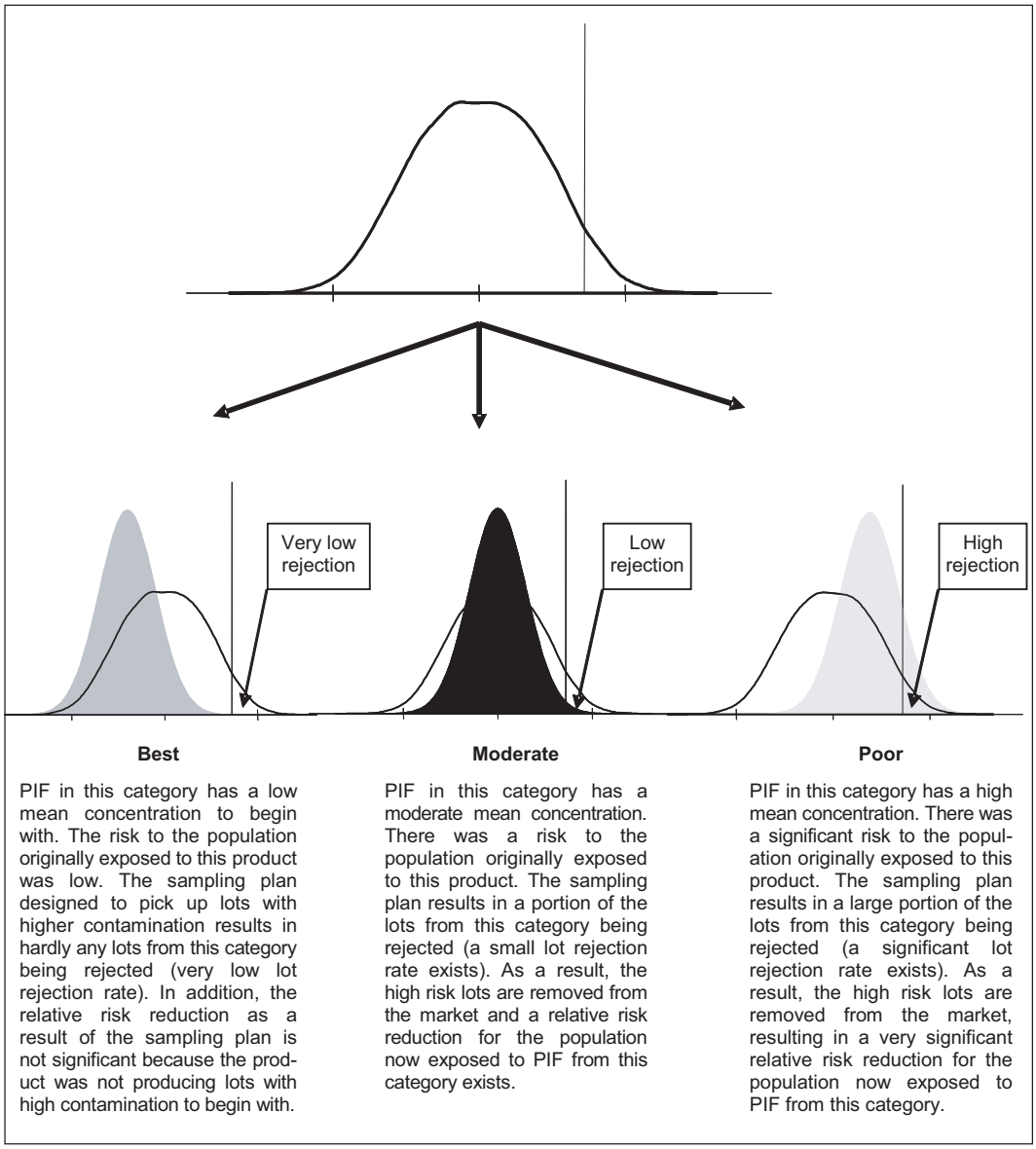


Figure 11. Illustration of the impact of a sampling plan in an environment in which the overall distribution of PIF contamination is driven by three types of producers: Best, Moderate and Poor.

Since manufacturers such as the ones classified as "poor" in Figure 11 will have to reject large portions of their product, there will be a greater and greater incentive to try to improve their process and move into the "best" or "moderate" category. In the long term, the overall relative risk reductions would be expected to be even greater than those realized immediately after implementation of the sampling plans.

4.3.4 Illustrative example of the cumulative impact of sampling plans through changes in production management and lot rejection

This section illustrates this concept further using estimates generated by the risk assessment model to first show the lot rejection rates and relative risk reductions soon after the sampling plans are implemented. Two future scenarios are then generated to show the effect on both relative risk-reduction and lot-rejection rates, as more and more manufacturers move out of the "poor" and "moderate" categories and into the "best" category. The scenario parameters and the sampling plan risk reductions are presented in Tables 24 and 25, respectively.

In this example, when the sampling plan is implemented, the "best" category has a 0.5% lot rejection rate with a corresponding risk reduction of 1.1 compared to the "poor" category, which has a lot rejection rate of 20% and a corresponding risk reduction of 3.1. As described earlier, these categories represent a larger overall distribution of PIF in the market. If 80% of the producers are in the "best" category, 15% in the "moderate" category and 5% in the "poor" category, then across all manufacturers there will be a 2% lot rejection rate and overall relative risk reduction of 2.2.

In the future, the rejection rates experienced by some of the manufacturers are likely to translate into a greater incentive to try to move their processes into the better categories so as to reduce their lot rejection rates. Table 26 summarizes the assumed baseline and future proportions of manufacturers existing in the different categories.

In future stage 1, the proportion of production in the "poor" category drops to 2% and then to 0% in future stage 2, while the proportion of production in the "best" category increases from 80% to 90% in future stage 1 and to 95% in future stage 2.

Table 24. Scenario parameters used in the risk assessment model to estimate lot reduction rates associated with sampling plans.

Between-lot means (log [*cfu/g*])	-5, -4, -3
Between-lot standard deviation of log means	0.8
Within-lot standard deviation of log concentration	0.5
Number of samples	10
Sample size (*g*)	10

Table 25. Sampling plan risk reductions and percentage product rejected associated with sampling plans applied to manufacturers in the "Best", "Moderate" and "Poor" categories.

Group	Mean log assumed	Proportion rejected	Risk reduction within this group
Best	-5	0.5%	1.1
Moderate	-4	4%	1.5
Poor	-3	20%	3.1
Overall scenario (80% best, 15% moderate, 5% poor):		2%	2.2

Table 26. Share of PIF production volume per group as effect of sampling plan encourages producers to move into "better" categories over time.

Stage	Best	Moderate	Poor
Baseline	80%	15%	5%
Future stage 1	90%	8%	2%
Future stage 2	95%	5%	0%

Note: This is a hypothetical scenario, for the purpose of illustration, with respect to the proportions of PIF production coming from the three production groups.

Table 27. Relative risk reduction associated with different future scenarios as described in Table 26.

Stage	Overall lot rejection rate	Producer management risk reduction	Sampling plan risk reduction	Combined risk reduction
		X	Y	Z = X * Y
Baseline	2.0%	1	2.2	2.2
Future Stage 1	1.2%	2.0	1.9	3.8
Future Stage 2	0.7%	5.0	1.2	6.0

X = Relative to baseline scenario (before sampling).
Y = Relative to risk without sampling in same time frame.
Z = Combined impact of management risk reduction and sampling plan risk reduction relative to risk without sampling in baseline time frame.

In the future scenarios, the impact on risk reduction is a result of two distinct phenomena. The first is the effect of production shifting into better categories (producer management risk reduction) and the second is the direct result of the sampling plan detecting and rejecting contaminated lots. In future stage 1, the impact of changes in the production of PIF across the industry (producer management risk reduction) due to the shift in the proportions of production (Table 26) results in a twofold risk reduction. The sampling plan in this new environment contributes an additional 1.9 relative risk reduction, for an overall risk reduction of 3.8, compared to the baseline situation of 2.2 (Table 27).

In future stage 2, the impact of further changes in the production of PIF across the industry results in a risk reduction by a factor of 5 (Table 27). The sampling plan in this new environment has a lot rejection rate of 0.7%, which translates to an additional risk-reduction factor of 1.2. The net result in this case is a cumulative risk reduction by a factor of 6.

As the scenarios proceed, an increasing proportion of the risk reduction relative to the baseline is due to the changes brought about by production management, with the application of sampling plans eventually contributing a decreasing proportion of the accumulated risk reduction.

4.3.5 Conclusions

The risk assessment module on microbiological criteria and sampling plans provided useful insights into the effectiveness of different sampling plans in terms of risk reduction. Using the risk assessment to provide information on the proportion of lots rejected produces a realistic picture in terms of the

impact of not meeting the established criteria. By allowing a comparison of different scenarios, the risk assessment illustrated that the better the process in terms of producing a product with a low mean log concentration of pathogen, the lower the level of losses due to lot rejection.

The expert meeting did not recommend any specific criteria and sampling plans for *E. sakazakii* in PIF. Rather it focused on illustrating how the risk assessment tool could be used to evaluate sampling plans. However, by looking at a total of 162 sampling plan scenarios, 54 of which are presented in Table 22, it was considered that some of these could be useful for the purposes of Codex. If Codex has other sampling plans that they would like to have evaluated, this can be done relatively easily using this risk assessment tool. The tool also has the capacity to evaluate sampling plans for Enterobacteriaceae. However, this was not carried out in the course of the meeting as it was considered that there was not adequate scientific data available at this time to correlate the reductions in Enterobacteriaceae with those for *E. sakazakii*.

5. *SALMONELLA* IN POWDERED INFANT FORMULA

5.1 EPIDEMIOLOGY AND PUBLIC HEALTH

5.1.1 Epidemiology of *Salmonella* infections

At 139.4 cases per 100 000 infants, the incidence of salmonellosis (from all sources) among infants was reported to be more than eight times greater than the incidence across all ages in the United States of America in 2002 (CDC, 2004). Salmonellosis incidence patterns are similar in the United Kingdom. Infants experience the highest rate of infection, with 181 cases per 100 000 infants (Skirrow, 1987). It is unclear whether the increased rate among infants results from greater susceptibility, or whether infants are simply more likely than persons in other age groups to seek medical care or have stool cultures performed for symptoms of salmonellosis. However, infants are more likely to experience severe illness or death from salmonellosis, and infants with immunocompromising conditions are particularly vulnerable. In multiple case-control studies, breastfeeding has been inversely associated with *Salmonella* infection. The largest and most recent study demonstrated that infants with *Salmonella* infection were less likely to have been breastfed (OR+0.5, 95% CI=0.3-0.6)[1] (Jones *et al*., in press). Transmission of *Salmonella* via expressed breastmilk has also been described in a few case reports (Quatishat *et al*., 2003; Chen *et al*., 2005).

5.1.2 Recent outbreaks of salmonellosis associated with powdered infant formula

Between January and April 2005, 104 infants (i.e. children <12 months of age) developed *Salmonella* Agona infections in France (InVS, 2005). At least 38 of them (37%) were hospitalized. A case-control study was conducted to identify possible vehicles of infection. All 23 case infants and none of the 23 control infants consumed powdered infant formula from one manufacturer during the week before illness. During an environmental investigation of the implicated manufacturing plant, *Salmonella* Agona was isolated from seven samples obtained from the production line and from PIF; pulsed-field gel electrophoresis profiles of these isolates and the clinical isolates were indistinguishable. Seven lots of formula were recalled on 7 April 2005; four cases subsequently occurred among infants whose parents were unaware of the recall.

At least five outbreaks of salmonellosis involving approximately 150 infants were associated with PIF between 1985 and 2000 (Table 28). Most of these outbreaks involved unusual *Salmonella* serotypes, which likely aided in the recognition of the outbreaks. In many regions of the world where *Salmonella* serotyping is not routinely performed, identification of geographically or temporally diffuse outbreaks would be difficult. Further, since most infant diarrhoea is caused by viral pathogens

[1] OR = odds ratio; CI = confidence intervals.

Table 28. Historical salmonellosis outbreaks associated with PIF.

Salmonella serotype	No. infants affected	Vehicle	Location	Year	Reference
Ealing	48	Powdered infant formula	UK	1985	Rowe *et al.*, 1987
Tennessee	≥3	Powdered infant formula	USA, Canada	1993	CDC, 1993
Virchow	48	Powdered infant formula	Spain	1994	Usera *et al.*, 1996
Anatum	17	Powdered infant formula	UK, France	1996-7	Threlfall *et al.*, 1998
London	30	Powdered infant formula	Republic of Korea	2000	Park *et al.*, 2004
Total	**146**				

(Lieberman, 1994), stool cultures may not be collected routinely even in regions with laboratory surveillance capacity. Consequently, outbreaks of salmonellosis due to contaminated PIF are likely to be under-reported.

5.2 DOSE RESPONSE

A dose-response model from outbreak data was developed for the FAO/WHO risk assessments of *Salmonella* in eggs and broiler chickens (FAO/WHO, 2002). This was the first *Salmonella* dose-response relationship to be developed using "real world" outbreak data, as previous dose-response models had been based on data from feeding trials using *Salmonella* or surrogate pathogens. However, among the outbreak data there were only two single and separate infant cases: a 9-month-old child with cystic fibrosis who fell ill after receiving an estimated daily dose of 44.8 *Salmonella* Schwarzengrund cells in pancreatin, and a 1-year-old child who fell ill after a one-off dose of 200 cells of *Salmonella* Schwarzengrund, also via pancreatin. It was noted that no dose-response data for infants under 9 months were included among the data from which the outbreak dose-response model was developed. Therefore, the model is limited in its application to young infants. In one outbreak that involved more than 1 500 people, it was estimated that the dose was <10 cells of *Salmonella* Typhimurium in servings of cheese. While there are no dose-response data or a model for infants – or different susceptibility groups among infants – the existing dose-response models indicate that illness can result from very low doses of *Salmonella* cells. Thus, the existing dose-response curve for gastroenteritis may be sufficient considering the biological endpoints of *Salmonella* infection in infants and if it is to be used in the evaluation of interventions on a relative risk basis only.

5.3 UPDATE ON MICROBIOLOGICAL ASPECTS

The *Salmonella* genus has been well studied for many years, and there are established surveillance schemes in some developed and few (if any) developing countries. Detection methods have been validated by ISO (International Organization for Standardization) and BAM (Bacteriological Analytical Manual); standardized serotyping schemes are established, as is molecular characterization (PulseNet). The resuscitation of stressed *Salmonella* cells has been a focus of recovery and detection methods for many years. It is well known that *Salmonella* is a diverse genus, recognized with the use of parallel detection methods (i.e. two enrichment broths, two selective agars) for each sample being analysed. However, it is important to note that while lactose-positive fermenting

strains of *Salmonella* are very uncommon, they will not easily be picked up using routine *Salmonella* detection media such as XLD (xylose lysine deoxycholate) and BGA (brilliant green agar).

Therefore, laboratory specialists need to use appropriate isolation/diagnostic media to avoid missing lactose-positive strains which have been responsible for outbreaks of salmonellosis associated with PIF; further research is warranted in this area.

5.4 RISK-REDUCTION STRATEGIES

5.4.1 Current industry practices

The implementation of specific preventive measures to prevent the contamination of dehydrated dairy products with *Salmonella* goes back 30 or 40 years, and the effect of these measures is illustrated in the results of a survey performed in the United States of America over several years (Table 29; Mettler, 1989). Further systematic improvements were triggered after two outbreaks in Australia in 1977 (Forsyth *et al*., 2003) and guidelines have been published by different organizations such as the International Dairy Federation (IDF).

The control measures are based on four principles:

1. Avoidance of entry of *Salmonella* into processing facilities and in particular in the zones from drying to filling, considered as high-hygiene areas.
2. Avoidance of multiplication of *Salmonella* in case of entry.
3. Hygienic design of high-hygiene zones and the equipment located in such zones.
4. Use of dry-mixed ingredients which are free of *Salmonella*.

It must be emphasized that the control measures are not specific for PIF, but are applicable for other types of dried dairy products, milk powders and dry-mixed ingredients, such as lactose or other similar dry products (e.g. soy-based products). For all these products, *Salmonella* is recognized as the most significant hazard.

Preventing the entry of the pathogen into the high-hygiene areas is based on the implementation of appropriately designed

Table 29. Effect of the implementation of preventive measures to control *Salmonella* as illustrated by the outcome of a USDA Salmonella Surveillance programme for skim milk powder carried out over a 20-year period.

Year	No. samples tested	% positive
1967	7 843	0.7
1968	17 496	0.2
1969	12 822	0.3
1970	11 254	0.36
1971	25 321	0.27
1972	28 736	0.17
1973	16 652	0.31
1974	12 058	0.52
1975	10 423	0.71
1976	14 418	1.90
1977	16 517	1.08
1978	17 224	0.6
1979	13 036	0.3
1980	9 720	0.1
1981	13 061	0.06
1982	16 624	0.02
1983	23 622	0.01
1984	23 455	0.11
1985	26 479	0.14
1986	31 912	0.03
1987	19 431	0.03
1988	14 058	0.01

Source: Mettler, 1989 (based on).

good hygiene practices. These are mainly based on the principles of zoning aimed at segregating the high-hygiene areas from the rest of the factory. This is achieved through physical separations (walls) and the creation of well-controlled access points both for personnel and for goods, such as the ingredients used in dry-mixing operations, packaging material, pieces of equipment and others. This has to be supported by appropriate procedures to minimize pathogen entry such as clothing changes for personnel and the stripping of ingredient bags before entry to the high-hygiene areas. The zoning is further reinforced through the appropriate control of utilities, such as air (e.g. filtration of external air) and the creation of overpressure (i.e. elevated air pressure so that the movement of air is out of the high-hygiene areas).

Avoiding the multiplication of *Salmonella* if present is achieved through the reduction or elimination of water, in particular wet-cleaning procedures, in high-hygiene areas. The presence of water will, in such cases, lead to a dramatic increase in *Salmonella* in the processing environment and thus to an important increase in the risk of recontamination of the product.

An additional important element is the hygienic design of processing areas (walls, ceilings, floors) and equipment, allowing the maintenance of a high standard of hygiene and cleanliness. Such elements are also key, in cases where the pathogen is present in order to enable its rapid eradication and prevent its establishment in the premises. The establishment of *Salmonella* strains within the processing environment will lead to recurrent problems, its dissemination throughout the whole plant and an increase every time favourable conditions occur, i.e. in the presence of water.

The effectiveness of the preventive measures can be verified through the application of sampling and testing programmes using environmental samples and samples from product contact surfaces as outlined by ICMSF (ICMSF, 2002). These programmes are targeted specifically against *Salmonella* and are supported by programmes targeting Enterobacteriaceae, which are always present in such processing environments. Industry experience has indicated that Enterobacteriaceae are an excellent hygiene indicator providing valuable information on deviations (increase in levels) and thus allowing the rapid correction of situations, which, if left uncorrected, could lead to problems with *Salmonella*. It must be pointed out that low levels of Enterobacteriaceae are not a guarantee of the absence of *Salmonella* and for this reason the programmes targeting Enterobacteriaceae and *Salmonella* are only useful if run in parallel. Such programmes, if well designed and implemented, will allow the detection of the presence of *Salmonella* in high-hygiene areas at the earliest possible stage and, if detected, will rapidly trigger corrective actions to eliminate the pathogen. These programmes are complemented with end-product testing in order to achieve compliance with regulatory requirements. Practical experience and data show that it is possible to control *Salmonella* in processing environments to an extent where it will be virtually completely absent. Under these conditions, the risk of recontamination is extremely low and it is possible to manufacture products fulfilling the most stringent microbiological requirements, e.g. absence in 60×25 g as recommended by the Codex Alimentarius.

The absence of *Salmonella* (i.e. the compliance to regulatory requirements) in dry-mixed ingredients cannot be achieved through the selection (acceptance/rejection) of lots based upon product testing alone. This can only be achieved through the careful selection of suppliers and close collaboration to guarantee the delivery of the required quality. This is of particular importance

for high-risk ingredients as identified by manufacturers depending on their products and their composition.

5.4.2 Consideration of current microbiological criteria

The current Codex sampling plan (ICMSF case 15) for *Salmonella* was considered by the FAO/WHO 2004 meeting to probably be adequate in terms of current methodology and the limit of detection for *Salmonella*. The current meeting concurred with this finding. However, no sampling and testing plan can provide evidence of the total absence of *Salmonella* cells. It is possible that low level and sporadic contamination by *Salmonella* cells would not be detected even by the stringent case 15 sampling plan (n=60, c=0, m=0, sample size=25 g).

5.4.3 Preparation and use of powdered infant formula by consumers

While the absence of a risk assessment for *Salmonella* meant that it was not possible to evaluate the relative risk of salmonellosis associated with various preparation and use scenarios, the meeting noted that those scenarios that decreased the risk of *E. sakazakii* infection were also likely to decrease the risk of salmonellosis. Infant formula may become contaminated with *Salmonella* during preparation and handling in hospital and home settings. In addition to environmental sources, those preparing and handling infant formula are a potential source of contamination and may introduce the hazard if hygienic practices are not followed. Thus the meeting reiterated the importance of good hygienic practices in the preparation and handling of PIF, including hand-washing with soap and the careful cleaning of work surfaces and equipment. With regard to storage of reconstituted PIF, it was noted that temperatures of less than 5°C are necessary to prevent the growth of *Salmonella* during refrigerated storage. Yet surveys of domestic refrigerators indicate that a significant proportion of refrigerators (approximately 10%) operate at temperatures above 10°C (Azevedo *et al*., 2005) indicating the potential for growth of *Salmonella* in reconstituted PIF in some refrigerators.

5.4.4 Need for risk assessment?

The current risk assessment does not consider *Salmonella* and this meeting did not develop any quantitative risk assessment for *Salmonella* in PIF. However, the meeting did become aware of a draft risk assessment that was underway in Australia and an overview of this work was presented. This draft risk assessment is not yet publicly available and so could not be critically reviewed by the meeting. However, some of the general observations from this work were considered in evaluating the need to develop a specific risk assessment on *Salmonella* in PIF as a basis for provision of scientific advice to Codex. The risk assessment under development in Australia was a probabilistic process simulation model which examined the influence of the temperature of water used for the reconstitution of PIF on *Salmonella* cell numbers (S. Dobson, personal communication, 2006, Confidential Draft Report to Dairy Australia, 2005).

As with any risk assessment, a future risk assessment of *Salmonella* in PIF could be enhanced if better data were to become available (e.g. with regard to the distribution of contaminating cells in PIF and the length of the lag phase for cells recovering from a long period of storage in dry

conditions). Those preparing and handling infant formula are a potential source of contamination in addition to environmental sources. Therefore, incorporation of contamination at the point of reconstitution or during handling and feeding would be valuable scenarios to include in a future risk assessment. The outcomes from a future risk assessment may result in the further refinement of advice to inform risk management decisions. In the meantime, the risk assessment model for *E. sakazakii* in PIF could be adapted for *Salmonella* through the use of *Salmonella*-specific parameters for growth, thermal inactivation etc. Additionally the risk process model for *Salmonella* in PIF developed for Dairy Australia and currently in draft form may be made publicly available in the future.

5.5 CONCLUSIONS

The meeting concurred with the conclusion of the previous FAO/WHO meeting that the current Codex-recommended criteria for *Salmonella* are appropriate given the current methodology with regard to testing and the limit of detection.

The meeting concluded that risk management decisions with regard to the management of *Salmonella* in PIF could be made without a further risk assessment. It considered that there was adequate information about *Salmonella* with regard to dose response and thermal inactivation to provide a basis for risk management decisions related to *Salmonella* in PIF. The meeting noted, for example, that at least some *Salmonella* serotypes have the potential to cause illness at very low doses, which may be a specific concern for infants, particularly those in the high-susceptibility category (premature, low birthweight, immunocompromised). However, given the thermal resistance of *Salmonella*, reconstituting PIF at a temperature of $\geq$70°C or using commercially sterile formula or fortifiers, would provide a high level of protection against *Salmonella* infection from these food sources. Nevertheless, the assessment of more complex risk management options may require a quantitative MRA, but the meeting agreed that the final decision to do a risk assessment should be a risk management (Codex) one.

The meeting also noted the importance of using the appropriate methodology to detect the strains of *Salmonella* that were causing illnesses and concluded that laboratories need to use appropriate media and methods to correctly identify atypical *Salmonella* (e.g. lactose-positive strains).

6. RESPONSE TO CODEX AND RECOMMENDATIONS

6.1 RESPONSE TO CODEX

As outlined in section 1, Codex requested that the expert meeting address a number of specific questions. While the details of how these questions were addressed are provided in the proceeding sections, the response to each of the questions is summarized below.

> *i) Taking into consideration any existing and new information on* E. sakazakii *and existing and new data on* Salmonella,[1] *identify if possible the distribution of cases linked to the different types of powdered formula*[2] *as a function of age, and define specifically the age groups and other groups of infant and young children at greatest risk.*

The meeting reviewed the information available on the most recent outbreaks of *E. sakazakii* illness in infants and also considered information on, and further analysis of, previous cases and outbreaks of *E. sakazakii*-related illnesses. In noting that *E. sakazakii* has caused invasive infection in all age groups, the meeting reiterated the findings of the 2004 FAO/WHO meeting that infants appear to be the group at particular risk. Neonates and infants under 2 months of age are at greatest risk. The meeting noted that there do appear to be two distinct infant groups in terms of the syndrome they develop – premature infants who develop bacteraemia outside of the neonatal period with most cases occurring in infants under 2 months and term infants who develop meningitis during the neonatal period. This difference in timing of infection may however be related to differences in timing of exposure to *E. sakazakii* rather than differences in susceptibility; it was also noted that any infant can develop either syndrome at any age. The meeting pointed out that infections have occurred in both hospital and outpatient settings and it was noted that as older infants generally live at home in the community, infections in such infants may be more likely to be under-reported. Considering that cases of *E. sakazakii* illnesses have occurred in a variety of settings and infant populations, prevention efforts must be multifaceted. This issue is addressed in section 2.1.

With regard to *Salmonella*, data from the United States of America in 2002 indicated that the incidence of salmonellosis was more than eight times greater in infants than in the general population. It was unclear whether the increased rate among infants results from greater susceptibility, or whether infants are more likely than persons in other age groups to seek medical care or have stool cultures performed for symptoms of salmonellosis. However, the meeting concluded that infants

[1] The need for any risk assessment work on *Salmonella* will be reviewed following an initial literature review and consideration of available data.

[2] "Powdered formula" is used here to describe powdered infant formula, follow-up formula, formula for Special Medical Purposes (FSMP) intended for infant, and human milk fortifiers, as described in section 6.1 of the 2004 Meeting Report "*Enterobacter sakazakii* and other microorganisms in powdered infant formula" (FAO/WHO, 2004).

are more likely to experience severe illness or death from salmonellosis, and infants with immunocompromising conditions are particularly vulnerable. This issue is addressed in section 5.1.

ii) Review the dose-response and growth models of E. sakazakii*, using new data that is becoming available.*

Although studies are underway in relation to pathogenicity and virulence of *E. sakazakii* and despite two outbreaks of *E. sakazakii* illness in infants since the first FAO/WHO expert meeting, there was no new information available to better define the dose-response relationship. Therefore, the meeting considered the approach to describe the dose-response relationship developed in 2004 (FAO/WHO, 2004) to still be relevant. The meeting also noted that it was unlikely that an accurate picture of the dose response for neonates will ever really be possible and, while any animal model data generated will provide better estimates than are currently available, it will only be at best an extrapolated estimate. This is addressed in sections 2.3 and 2.4.

Any information on the growth of *E. sakazakii* that was available either in the published literature or provided in response to the FAO/WHO call for data was considered in the development of the risk assessment model. Details on the elaboration of the model are provided in section 3.

iii) Evaluate specific control measures for different manufacturing operations (depending on data provided by manufacturers of powdered formula), which could minimize product contamination by E. sakazakii *and evaluate how microbiological criteria for Enterobacteriaceae can be used as an indication of process hygiene.*

While data were provided by industry sources in response to the call for data and used to inform the development of the risk assessment model, there were not suitable data on the impact of specific control measures within the manufacturing environment to facilitate the development of a component in the risk assessment model to assess such measures. However, an analysis of the available industry data led to a number of conclusions by the expert meeting. It was noted that the control of recontamination of PIF with *E. sakazakii* from the processing environment following heat treatment was the critical activity required to minimize the risk associated with PIF. Achieving this required the implementation of a number of measures modified according to the needs of individual manufacturing facilities. Such measures include:

- The effective implementation of preventive measures (GMP/GHP and HACCP).
- The strengthening of these measures to further minimize entry of the microorganisms and to avoid their multiplication by excluding water from the processing environment, the most effective means of which was considered to be the implementation of systematic dry-cleaning.
- The selection of suppliers of dry-mix ingredients according to specified needs.
- The implementation of monitoring and environmental management programmes.

Further descriptions of industry practices are provided in section 4.1.

Monitoring for Enterobacteriaceae is considered by industry to be a good means of monitoring process hygiene. However, in the case of *Salmonella* it was pointed out that low levels of

Enterobacteriaceae are not a guarantee of the absence of *Salmonella* and for this reason the programmes targeting Enterobacteriaceae and *Salmonella* are only useful if run in parallel. It is likely that the situation with *E. sakazakii* is similar. The expert meeting considered the application of criteria for Enterobacteriaceae as an indicator microorganism for *E. sakazakii*. Such an application would require the establishment of a correlation between Enterobacteriaceae and *E. sakazakii* levels. The meeting considered the data available on this and used a correlation function to try and establish whether or not such a correlation existed. However, the meeting concluded that the available data did not indicate any correlation between Enterobacteriaceae and *E. sakazakii*. Yet neither was it possible with the available data to rule out a possible correlation. Without such a correlation, the meeting was unable to develop a meaningful set of scenario evaluations. However, the meeting did note that industry scientists feel that controlling Enterobacteriaceae is an effective tool to help control *E. sakazakii*. Therefore, the availability of additional information either through expert elicitation or data collection efforts may allow further consideration of this in the future. This issue is addressed in section 4.3.2.

iv) a) In light of new data submitted by ISDI/industry request that the risk assessment be updated to take into consideration this new information and make the output available to the Working Group (in charge of redrafting the proposed draft Code) for the development of microbiological criteria; b) Use the risk assessment to evaluate the risk reduction associated with various control measures, microbiological criteria and sampling plans.

a) The data available from industry were considered in the development of the risk assessment. While this included some information on the levels of contamination, the true contamination level is an area of uncertainty. Therefore, the available data were not used directly as an input on contamination level, but guided the selection of a range of options on the level of contamination. The user of the risk assessment is thus provided with the three options: -5 log cfu/g (0.00001 cfu/g), -4 log cfu/g (0.0001 cfu/g), -3 log cfu/g (0.001 cfu/g), and can select that which is considered most appropriate, or can replace them with a figure based on actual data where available. More information on the elaboration of the model and the data used therein are provided in section 3.

b) The risk assessment model has the capacity to evaluate the risk reduction associated with sampling plans for *E. sakazakii* as well as a range of measures relevant to the preparation and use of PIF. As there were no specific criteria and sampling plans proposed for evaluation, the meeting selected a range of 162 different sampling scenarios with the aim of illustrating how this module of the risk assessment works and to provide insights into the effectiveness of different sampling plans in terms of risk reduction. The model can also estimate the proportion of lots rejected in association with the implementation of a specific sampling plan. This means that the risk assessment also provides a picture in terms of the impact of not meeting the established criteria. By allowing a comparison of different scenarios, the risk assessment illustrated that the better the process in terms of producing a product with a low mean concentration of pathogen, the lower the level of losses due to lot rejection.

The expert meeting did not recommend any specific criteria and sampling plans for *E. sakazakii* in powdered infant formula. However, by looking at a total of 162 sampling plan scenarios, it was considered that some of these could be useful for the purposes of Codex. This tool can be made

available to the relevant Codex Committees and working groups to evaluate any additional sampling plans not considered in the course of the meeting. This issue is addressed in detail in section 4.2.

v) Request that the aspects of the risk assessment model addressing preparation, storage and handling of powdered formula be revisited to ensure that all currently used preparation procedures are evaluated.

An important component of the risk assessment model is its ability to assess the relative risk associated with various consumer practices in terms of the preparation, storage and use of powdered infant formula. As there was not much published information on such practices, a questionnaire was developed to collect some information through hospitals, consumer groups and others. This information was used as a basis for the 574 different preparation and use scenarios considered and evaluated by the risk assessment in the course of the meeting. Details of all the scenarios evaluated are provided in section 4.2. In general, scenarios that involve periods of holding at room temperature are associated with greatest risk. This effect is exaggerated for warmer room temperatures. The same holding times at refrigeration temperatures indicated less than 1.3-fold risk increase. Reconstitution of PIF with liquid of 70°C was evaluated to be an effective risk mitigation strategy for all scenarios investigated. The highest risk scenarios were associated with reconstitution at temperatures of 40° and 50°C, when the formula is not consumed immediately. As a result, quick cooling to lower temperatures to minimize growth is essential. When PIF reconstituted at temperatures of 10° or 20°C was evaluated, minimal growth and inactivation was observed, but subsequent holding for long periods at room temperatures, including extended feeding periods, can result in growth and therefore increased risk. In the case of reconstitution at 60°C, some initial inactivation occurs but, depending on the particular preparation scenario, this inactivation can be overwhelmed by the magnitude of growth that may occur if temperatures permit, for example with extended feeding times. It was observed that extended feeding periods of more than 1 hour, particularly at warm ambient temperatures, were associated with increasing relative risk. Also the use of larger containers for cooling of the formula was associated with increased risk as a result of the slower cooling rate of the formula, indicating that formula should be cooled in small containers if possible. It was noted that some of the scenarios evaluated which exhibited increasing relative risk, such as extended feeding periods at a warm or very warm ambient room temperature, may be similar to those in hospitals and intensive care units. Also the higher relative risk associated with holding of reconstituted formula at room temperature for long periods highlights the importance of providing good guidance and educational messages on the safe preparation and use of powdered infant formula.

Several international reference documents specify that labelling should include adequate information to enable safe use of the product. For example, in the International Code of Marketing of Breast milk Substitutes, article 9 on Labelling states (in section 9.2) that manufacturers and distributors of infant formula should include "instructions for appropriate preparation, and a warning against the health hazards of inappropriate preparation". The General Principles of Food Hygiene (CAC/RCP 1-1969, REV. 4-2003) states in section 9.3 on Labelling: "Pre-packaged foods should be labelled with clear instructions to enable the next person in the food chain to handle, display, store, and use the product safely."

Considering the results of the model, and also the Codex requirement for clear labelling with regard to safe use of pre-packaged foods, the meeting considered that measures to provide appropriate messages to users, including consumer education activities and product labels, will need to be reviewed and revised as appropriate. For example, many labels call for mixing PIF at temperatures around 50°C, which, according to this risk assessment, generally results in the greatest risks for the range of scenarios considered, unless, as is sometimes recommended, the reconstituted formula is consumed immediately. The model indicated that reconstituting PIF at 70°C provides the greatest risk reduction. However, it also recognized that not all PIF products are formulated to be mixed at 70°C. Some of the other considerations associated with reconstitution at this temperature are outlined in Appendix D. In addition, the meeting considered that the development of international guidelines for users of PIF, as requested by the WHA, could be informed by the outcome of this series of scenario analysis.

The CCFH also noted that "the need for any risk assessment work on Salmonella *will be reviewed following an initial literature review and consideration of available data". The meeting therefore also considered the issue of* Salmonella *in powdered infant formula.*

The meeting considered the industry practices currently in place – or at least available for implementation – for the control of *Salmonella*, and concurred with the conclusion of the previous FAO/WHO meeting that the current Codex-recommended criteria for *Salmonella* are appropriate given the current methodology with regard to testing and the limit of detection.

The meeting considered the need for a quantitative risk assessment on *Salmonella* in PIF and noted that one was under development in Australia and also that, if needed, it would be possible to adapt the *E. sakazakii* risk assessment model for PIF to assess the risk associated with *Salmonella* in PIF. However, the meeting did not consider that a quantitative risk assessment for *Salmonella* in PIF was needed at the present time and concluded that risk management decisions with regard to the management of *Salmonella* in PIF could be made based on existing information. The meeting concluded that there was adequate information about *Salmonella* with regard to dose response and thermal inactivation to provide a basis for risk management decisions related to *Salmonella* in PIF. The meeting noted, for example, that at least some *Salmonella* serotypes have the potential to cause illness at very low doses, which may be a specific concern for infants, particularly those in the higher susceptibility category (premature, low birthweight, immunocompromised). However, given the thermal resistance of *Salmonella,* reconstituting PIF at a temperature of ≥70°C, or using commercially sterile formula or fortifiers would provide a high level of protection against *Salmonella* infection from these food sources. Nevertheless, the assessment of more complex risk management options may require a quantitative MRA, but the meeting agreed that the final decision to do a risk assessment should be a risk management (Codex) one.

6.2 OTHER KEY OUTCOMES

Before addressing the questions from the Codex Committee on Food Hygiene, the expert meeting reviewed the risk assessment model developed for *E. sakazakii* in PIF. The meeting agreed that the model as modified based on the review and discussion that took place during the meeting was appropriate, accurate and valid in terms of the approach taken, the interpretation of data etc., and

was an effective tool for the evaluation of various risk-reduction scenarios. Therefore, this version of the model was used as a tool for evaluation of risk-reduction scenarios as outlined above.

The meeting also noted the intention of FAO/WHO to make this risk assessment tool eventually available in a user friendly format so that it could be used by risk managers at various levels to assist them in their decision-making processes. While such an approach was welcomed, the meeting advised that care be taken to ensure that the final product would be used in an appropriate manner.

In reviewing the categorization of the microorganisms or microbial toxins of concern in powdered infant formula, based on the strength of evidence of a causal association between their presence in PIF and illness in infants (as developed by the first FAO/WHO meeting on this issue in 2004), and considering information available since then, the meeting noted that *E. sakazakii* and *Salmonella* continued to be the only pathogens in category A. Recent reports led to the inclusion of some additional organisms in categories B and C.

Based on research findings reported since 2004, the meeting noted that *E. sakazakii* is now recognized as a genotypically and phenotypically diverse bacterial species. Further studies are underway to determine optimal methods of isolation and identification and to address the virulence and the ecology of this pathogen. The meeting reiterated the importance of good hygienic practices in the preparation and handling of PIF, including hand-washing with soap and the careful cleaning of work surfaces and equipment.

Salmonella has been considered a hazard in PIF and related milk-based products for several decades. A review of recent and historical outbreaks of salmonellosis linked to intrinsically contaminated PIF confirmed its public health importance and the need for risk management vigilance. Outbreaks were only identified by laboratory-based food-borne disease surveillance systems and usually by some unique quality of the epidemic strain, e.g. a rare serotype or the ability to ferment lactose. The meeting recognized that salmonellosis due to contaminated PIF was probably under-reported. The meeting also noted the importance of using the appropriate methodology to detect the strains of *Salmonella* that were causing illnesses and concluded that laboratories need to use appropriate media and methods to correctly identify atypical *Salmonella,* such as lactose-positive strains.

6.3 RECOMMENDATIONS

In considering the recommendations to be made, the meeting firstly re-endorsed those that had been made by the 2004 FAO/WHO meeting on this issue.

The expert meeting made the following additional recommendations:

6.3.1 To member countries, FAO and WHO

- Develop prevention strategies for *E. sakazakii* infections caused by contaminated PIF that address the different stages of production and preparation and use of PIF, taking into consideration the risk to infants – both within and beyond the neonatal period and of any immune status.

- Develop educational messages on the safe handling, storage and use of powdered infant formula, including the health hazards of inappropriate preparation and use; target healthcare workers, parents and other caregivers in both hospitals and the community, since *E. sakazakii* infections have occurred in hospital and home settings.

- Review and revise product labels, as appropriate, to enable caregivers to handle, store and use the product safely, and to make clear the health hazards of inappropriate preparation.

- Encourage member countries to establish surveillance and rapid response networks, and facilitate coordinated investigation by clinicians, laboratorians and public health and regulatory officials, to enable the timely recognition and cessation of outbreaks of illness associated with *E. sakazakii* and the identification of contaminated PIF.

- Encourage countries to enhance laboratory-based surveillance, including reporting to Salm-Surv, the WHO salmonellosis worldwide surveillance network, since laboratory-based surveillance is the only way in which past outbreaks of salmonellosis associated with intrinsically contaminated PIF have been recognized.

- Encourage laboratories conducting surveillance for *Salmonella*, and manufacturers and regulators testing for *Salmonella* in PIF, to use isolation and diagnostic methods which can identify lactose-fermenting strains of this organism, since these have been the cause of some of the outbreaks of salmonellosis associated with PIF.

- Encourage scientists to determine the optimal isolation and identification methods for *E. sakazakii*, taking into account the new research data demonstrating genetic and phenotypic diversity in the species.

- Encourage research to determine ecological niches and virulence factors for *E. sakazakii* to better target risk mitigation strategies and control measures.

- Develop and review international guidelines – as requested by the 2005 WHA – educational messages and product labels regarding the preparation, storage and handling of PIF, considering the results of the *E. sakazakii* risk assessment model presented in this report.

- At this time, the meeting did **not** recommend that FAO/WHO conduct a quantitative risk assessment for *Salmonella* in PIF.

6.3.2 To Codex

- Make risk management recommendations based on the outputs of the JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment) risk assessment for *E. sakazakii* in PIF and the estimates of potential risk reductions of proposed control measures. In particular, give consideration to the elaboration of sampling plans and microbiological criteria for *E. sakazakii* and Enterobacteriaceae in PIF, and labelling recommendations, specifically in the revision of CAC/RCP 21-1979.

6.3.3 To member countries

- Apply the risk assessment in developing national risk management strategies for the reduction of risks associated with PIF, such as appropriate educational programmes.

- Encourage industry to effectively implement preventive measures and to strengthen those measures that further minimize entry of the microorganisms of concern into the manufacturing environment and avoid their multiplication therein.

6.3.4 To industry

- Effectively implement, to the extent possible and feasible, preventive measures, including the strengthening of those measures that further minimize entry of the microorganisms and avoid their multiplication, such as the exclusion of water from the processing environment to the extent possible and feasible. The most effective means of achieving the latter is considered to be the implementation of systematic dry-cleaning.

- Support research that allows further evaluation of the effectiveness of Enterobacteriaceae as an indicator organism pointing to conditions in the manufacturing environment or final product that have increased potential for harbouring *E. sakazakii* or *Salmonella*.

6.3.5 To FAO and WHO

- In future "calls for data", provide more specific details with regard to the type and format of data needed in order to enable data providers to target their efforts towards the provision of data which can be effectively used in the risk assessment process.

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Appendix A

LIST OF BACKGROUND PAPERS AND PRESENTATIONS

Hospital practices related to powdered infant formula in developing countries	Alejandro Cravioto
Epidemiology of *Enterobacter sakazakii* infection among infants	Anna Bowen
Infant feeding practices in industrialized countries	Anna Bowen
Enterobacter sakazakii infections and colonisations in neonates associated with the use of powdered infant formula – France, 2004	Bruno Coignard
Does the model incorporate the most recent and appropriate information regarding clinical, industry, scientific, hospital practices?	Emma Hartnett and Greg Paoli
Risk assessment for *E. sakazakii* in powdered infant formula	Emma Hartnett and Greg Paoli
Calculation of risk reduction through the use of microbiological criteria for *Enterobacter sakazakii* in powdered infant formula	Emma Hartnett and Greg Paoli
Update on industry activities and progress	Jean Louis Cordier
Update on the pathogenesis of *Enterobacter sakazakii*	Jeffrey Farber
Illnesses linked to consumption of powdered infant formula intrinsically contaminated by *Salmonella*	Kaye Wachsmuth
Consumer practices related to the preparation and use of powdered infant formula	Lisa Lefferts
Current studies being carried out on *Enterobacter sakazakii* by researchers at the Center for Food Safety and Applied Nutrition, USFDA	Robert Buchanan

Recent ICMSF activities related to *Enterobacter sakazakii*	Robert Buchanan
Update on the microbiological aspects of *Enterobacter sakazakii* including genetics, growth studies and diagnostic advances	Stephen Forsythe
Assessment of *Salmonella* in powdered infant formula in Australia (Draft)	Sue Dobson

Appendix B

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

Source	Information/Data received
China – China CDC	Surveillance for *Enterobacter sakazakii* in powdered infant formula in China
France – Agence Française di Sécurité Sanitare des Aliments	Hygiene recommendations for the preparation, handling and storage of feeding bottles
Indonesia – Bogor Agricultural University	Molecular identification of *Enterobacter sakazakii* using PCR targeting the gene of the 16S rRNA and the 16S-23S rDNA intergenic spacer region *Enterobacter sakazakii* in infant food manufactured in Indonesia and Malaysia
International Formula Council	*Enterobacter sakazakii* in powdered infant formula – Summary of product data, environmental data, methodology and product deposition
International Special Dietary Foods Industries	Compilation of data on the presence of Enterobacteriaceae in the environment of powdered infant formula in some European plants A simple flow chart of powdered infant formula production ISDI background paper on *Enterobacter sakazakii* ISDI position paper on *Enterobacter sakazakii*
Japan – National Institute of Health Sciences	Annual production of powdered infant formula Infant population, feeding practices and consumption patterns of PIF Guidance for the preparation and use of PIF Survey of *E. sakazakii* in PIF
Netherlands – Wageningen University	Data on the growth of *Enterobacter sakazakii* in powdered infant formula Effects of preculturing conditions on lag time and specific growth rate of *Enterobacter sakazakii* in reconstituted powdered infant formula (now published in *Appl. Environ. Microbiol.*, 72: 2721-2729)

Source	Information/Data received
Switzerland – Nestlé Nutrition	Information on manufacture and preparation and use of PIF Data to improve the hazard characterization Surveillance of hospital-acquired bacteraemia in English hospitals 1997-2002 The bacteriological quality of hospital-prepared infant food (Rowan, N.J., Anderson, J.G., & Anderton, A. 1997. *Journal of Hospital Infections*, **35**: 259-267) Growth of *E. sakazakii* at different pH values in reconstituted infant formula Décontamination des poudres alimentaire; revue bibliographique et nouvelles perspectives
Thailand – Food and Drug Administration	Survey of occurrence of *Enterobacter sakazakii* in milk-based powdered infant formula
Consumer groups	*Enterobacter sakazakii* in formula, Netherlands International recall notices for powdered infant formula

Appendix C

REVIEW OF RISK ASSESSMENT MODEL

The risk assessment model was reviewed by a small group of risk assessment experts, both before and during the meeting. One day of the meeting was dedicated entirely to reviewing the mechanics of the risk assessment model. Following an overview presentation by the model developers, the experts went step by step through the model. In particular, comments focused on the scope of the model, the general model structure, and the modules on growth and inactivation, preparation and holding, contamination and consumption. A component which was developed to estimate risk reductions and percentage of product rejected associated with sampling plans was also examined in detail.

Scope

The importance of clearly outlining the scope of the model was emphasized so that the user was immediately aware of the application and limitations of the model. In particular, the following need to be highlighted:

- The model only considered intrinsically contaminated powdered infant formula.
- The model does not consider recontamination with *E. sakazakii* from the preparation equipment or environment. However, the implications of this should be explained in the risk assessment documentation.
- The model assumes that contamination of the product is homogenous and does not consider the phenomenon of clumping. There were no data available to clearly indicate that clumping was an issue to be considered. However, if at a later date data become available to indicate this, there would be implications for the model and in particular the module on sampling. If clumping is a common occurrence, then sampling would not be an effective risk mitigation strategy. Text should be included in the risk assessment documentation to describe this. It may also be a stimulus for research on this issue.
- The current risk assessment only addresses *E. sakazakii* in powdered infant formula and does not consider *Salmonella*. It was noted that the model could also have an application for *Salmonella* but several modifications would be required to address microorganism-specific components of the model, e.g. growth and inactivation models, dose response.

General issues

The sampling component of the model has been verified using a second software – "R" – as a means of ensuring that there are no errors in the model specifically related to the software. The calculations for the other parts of the model have been successfully reproduced in Excel.

Given that the ultimate objective with this risk assessment model is to make it publicly available as a user-friendly tool, a number of issues pertinent to such a broad release were discussed. The most appropriate means of making the model available would be via a web-based platform. Careful consideration will need to be given as to how this is done and issues to be addressed include the use of disclaimers, provision of careful guidance to the users, inclusion of default scenarios, ensuring that the format in which outputs are produced include the associated assumptions and provision of user selections. In relation to this issue, it was noted that the Analytica model structure as currently presented to the user may not be very intuitive, in particular for users not familiar with this software and at whom this tool is ultimately aimed. It was suggested to consider decreasing embedding at the top level to facilitate this change. A concern was raised as to whether the user-selectable options provided by the model could be open to abuse and misuse, e.g. select baselines to artificially make the case for predetermined options. However, it was pointed out that with the way the model is structured, it would be transparent if indeed this was done.

Growth, inactivation, preparation and holding

The basis in terms of the data used and assumptions made for the components of the model addressing growth and inactivation of *E. sakazakii* and preparation practices and holding times were considered in detail. Some of the specific issues considered are summarized below:

- It was noted that the model currently considers that PIF is stored for a period of 30 days before use. However, storage of dry products such as PIF is often for much longer periods and could be up to two-and-a-half years for this product. It was noted that die-off of *E. sakazakii* in PIF decreases considerably after 153 days (Edelson-Mammel, Porteus and Buchanan, 2005). The issue of product shelf-life was considered and it was noted that while some product may be used within a few months of manufacture, some product may not be used for up to 2 years. It was agreed that the model should allow for longer storage periods and this was achieved by making this input modifiable by the user. The die-off function used in the model was also adjusted to the more conservative rate of 0.001 log/cfu per day which is more typical in PIF stored for 5 months or more.

- The reviewers requested that the growth model in the risk assessment include the most recent data available on growth rates of *E. sakazakii.*

- It was noted that the data for cooling rates used in the model were based on single bottle-cooling experiments. Information as to the bottle size and reconstituted volume used in these experiments and as a basis for this component of the model should be clearly stated.

- It was noted that a conservative value for thermal resistance was used in the model. Considering that different strains of *E. sakazakii* exhibit different thermal resistance characteristics, this was considered appropriate but needs to be clearly noted in the risk assessment model documentation.

- It was noted that the risk assessment documentation would benefit from more clear explanation as to how the issue of re-warming was considered in the risk assessment model.

- There were several issues considered with regard to temperature of the formula during feeding. The temperature currently considered in the risk assessment model is based on the temperature suggested by industry and included on the labels of PIF. It was noted that in some situations a higher temperature may be more appropriate. It was also noted that the temperature profile of PIF fed to hospitalized infants via a tube is likely to be different and such feeding may occur over a longer period of time.

- With regard to preparation scenarios, it was recommended that a broader range of scenarios be considered. As follow-up to this, the expert meeting described a range of scenarios to be considered in the risk assessment model. Such scenarios included end-point pasteurization for very young infants and consumers not re-heating product but taking it out of the fridge and allowing it to reach room temperature.

Dose response

In reviewing the dose-response component of the model, it was recommended that the range of r-values be expanded to include more susceptible population groups, considering that there appears to be some evidence for increased susceptibility in certain infant subgroups. Consideration was also given to coupling of r-values with susceptibility measures. In conclusion, it was agreed to add a couple of additional baseline values and add relative susceptibility to that baseline level.

Microbiological criteria and sampling plans

In discussing the approach taken to assess the risk reduction associated with different two- and three-class sampling plans, it was considered that the approach taken was sound. This issue was addressed further in the course of the meeting to determine the most appropriate way in which to use this model component. Some of the specific issues considered are as follows:

- Fractional organisms – this is sometimes an issue in models where organisms are "created" artificially due to mathematical treatment of concentration, reduction and growth. However, it was considered that this issue was appropriately handled in the current model.

- Correlation between Enterobacteriaceae and *E. sakazakii* – the data available on this were explicitly considered and the subsequent calculations reviewed. A calculation error in the formula used to describe the correlation was identified. This was corrected and led to the conclusion that the available data do not indicate any correlation between Enterobacteriaceae and *E. sakazakii*. However, neither is it possible with the available data to rule out a possible correlation.

- The need for some specific information related to product testing from industry was identified in the course of the review with regard to what happens to a lot that tests positive and whether this needs some consideration in the risk assessment model.

- The issue of homogeneity of contamination on the impact of criteria and sampling was also discussed. In the event of dry powder becoming contaminated with pathogens at some point in

the manufacturing plant following production of powder in the spray-drying tower, it is unlikely that there will be a completely homogeneous distribution of cells in the powder at the point when it is packaged. The distribution of contaminating cells in a powder lot will influence the number of servings contaminated with cells and the variability of cell numbers among contaminated servings at the point of reconstitution. This has implications for risk assessment and sampling plans. The distribution of cells will be influenced by the physical processes involved in contamination including the point in the manufacturing line at which contamination occurs, the extent of clumping of cells and the extent to which cells are mixed in PIF prior to the powder being put into cans or other packages. It is possible that distribution patterns will vary between lots and between manufacturing plants. There are no data available to adequately describe the distribution of bacteria that may contaminate PIF following powder formation in manufacturing plants. Distribution patterns in PIF could be described by large-scale sampling and enumeration of individual lots. Given the rarity and typically low level of contamination of lots of PIF pathogens, it may be difficult to determine cell distribution patterns; however, obtaining these data for bacteria which may contaminate PIF in manufacturing plants post-pasteurization would provide valuable insights for risk assessment and sampling plan design. However, the current level of data means that for the purposes of this work the distribution of contaminating cells was considered to be homogenous.

Conclusion

It was considered that the model was generally well constructed and the approach sound. Only one calculation error was identified and this was immediately corrected. Some additional modifications to the model as suggested above were also immediately implemented in order to facilitate the use of the model in the course of the meeting. The model documentation requires further refinement which will be undertaken following the expert meeting.

Appendix D

IMPACT OF RECONSTITUTION AT HIGH TEMPERATURES ON OTHER CHARACTERISTICS OF POWDERED INFANT FORMULA

It was noted that some concerns have been raised about recommending the use of very hot water for reconstituting PIF. This is water that has only been slightly cooled after boiling (i.e. at a temperature of >70°C). The concerns raised were over: loss of heat-sensitive nutrients in the food; scalding risk to the infant and the person preparing the formula; activation of *B. cereus* or other bacterial spores in the formula; and clumping of the powder. Data on reduction in vitamin levels in PIF prepared with boiling water from one study indicate that the only vitamin that is significantly affected is vitamin C (Table D1). The reduction in vitamin C levels was in the range 5.6 to 65.6% in four powders that were tested; however, prior to the addition of water, all four powders had actual levels of vitamin C that were higher than the can label indicated, and for three of the four powders, the level in the formula after preparation with boiling water was still higher than the level indicated on the can label. In the fourth powder in which the percentage reduction in vitamin C was more than twice the level of percentage reduction in the other powders, the level after the boiling water treatment was 9 mg/100 cal and the can label was 12 mg/100 cal. Though the level of 9 mg/100 cal was lower than the can label for this formula, it was within the range of the can labels for the other three powders (8-9 mg/100 cal). Thus, this particular study would appear to indicate that the reduction in vitamin levels from the use of water >70°C is not significant. As these results are the basis of only one study, the meeting did not agree to make any specific recommendations on this issue. However, it was noted that the option of fortifying formula to accommodate any reduction in vitamins could be possible if the practice of preparing formula with very hot water was recommended.

In relation to a scalding risk to infants and the person preparing the formula, it was noted that parents and caregivers are either trained or are aware of the sensitivity of the skin of infants to heat, and of the care needed for example in ensuring that water used for bathing is at a suitable temperature. It is feasible that such training and information could easily be extended to the use of infant formula prepared with very hot water, and that a warning could further be included in the labelling on the packaging. To avoid scalding the person preparing the formula, a mixing method other than shaking would be needed to ensure the formula was dissolved properly and that any contaminating pathogens were exposed adequately to the heat. This could be achieved with the use of a long-handled spoon (sterilized) with the base of the spoon the size of a teaspoon or smaller.

B. cereus or other spores may be activated by the use of very hot water to reconstitute PIF; therefore, it remains important to advise caregivers that once the formula has cooled to a suitable

temperature for feeding, it should be used immediately or, if the formula is to be stored for later use, it should be stored in a refrigerator operating at a temperature <5°C. In relation to the possibility of powder clumping on addition of very hot water, several powders currently available do not clump on addition of very hot water. This depends on the product formulation and it is likely that current technology could be applied to address this issue for any other products.

It was also noted in the course of the meeting that some PIF products may contain added probiotics; these would be killed by reconstitution at 70°C.

Table D1. Vitamin content of infant formulas reconstituted according to label instructions and with boiling water (Results of a study undertaken by FDA in 2003).

Vitamin (units/100 cal) Label declaration Reconstitution procedure		Products			
		Similac with Iron	Enfamil ProSobee Soy	CVS Infant Formula with Iron	Carnation Good Start with Iron
Sample no.:		147984	209219	209220	209221
"Use by" date:		1 Nov. 2005	1 Sept. 2005	28 Oct. 2004	Oct. 2004
Vitamin A (*IU/100 cal*)	Label	300	300	300	300
Per label		712±7.4	677±8.6	597±11.3	451±14.8
Boiling water		686±1.2	678±30.5	613±2.52	459±7.55
Change (%)		-3.7	+0.1	+2.68	+1.77
Vitamin D (*IU/100 cal*)	Label	60	60	60	60
Per label		71.2±1.4	57.8±2.72	56.5±1.99	56.7±1.59
Boiling water		73.2±8.4	52.8±1.19	62.6±6.17	55.4±1.27
Change (%)		+2.8	+0.1	+10.80	-2.29
Vitamin E (*IU/100 cal*)	Label	1.5	2	1.4	2
Per label		2.9±0.09	5.16±0.07	3.12±0.07	3.68±0.16
Boiling water		2.9±0.09	4.44±0.11	3.02±0.04	3.55±0.05
Change (%)		0	-14.0	-3.21	-3.532
Vitamin K (µg*/100 cal*)	Label	8	8	8.3	8.2
Per label		17.8±2.5	9.4±1.11	11.8±0.29	15.4±0.25
Boiling water		17.9±2.6	9.7±0.97	11.9±0.17	14.8±0.45
Change (%)		+0.56	+3.2	+0.85	-3.896
Thiamine (µg*/100 cal*)	Label	100	80	100	60
Per label		223±6.1	168±3.1	164±19.3	141±3.06
Boiling water		218±4.5	17.±11.0	178±8.72	141±1.73
Change (%)		-2.2	+1.2	+8.54	0
Riboflavin (µg*/100 cal*)	Label	150	90	150	135
Per label		266±12.2	154±2.5	247±19.1	337±22.9
Boiling water		261±8.7	143±17.0	257±6.81	308±16.7
Change (%)		-1.9	-7.1	+4.05	-8.61
Vitamin B_6 (µg*/100 cal*)	Label	60	60	62.5	75
Per label		86.3±9.2	114±6.5	136±1.15	118±1.62
Boiling water		80.8±3.8	116±4.6	139±3.06	120±1.31
Change (%)		-6.4	±1.8	±2.21	+1.695

Vitamin (units/100 cal) Label declaration Reconstitution procedure		Products			
		Similac with Iron	Enfamil ProSobee Soy	CVS Infant Formula with Iron	Carnation Good Start with Iron
Sample no.:		147984	209219	209220	209221
"Use by" date:		1 Nov. 2005	1 Sept. 2005	28 Oct. 2004	Oct. 2004
Vitamin B_{12} (µg/100 cal)	Label	0.25	0.30	0.30	0.22
Per label		0.90±0.01	0.72±0.01	0.397±0.01	0.605±0.01
Boiling water		0.86±0.10	0.72±0.00	0.41±0.01	0.617±0.02
Change (%)		-4.4	0	+3.27	+1.98
Niacin (µg/100 cal)	Label	1 050	1 000	750	750
Per label		1 503±92	1 418±9	1 242±32	1 176±34.7
Boiling water		1 521±49	1 456±23	1 236±27	1 282±8.72
Change (%)		+1.2	+2.7	-0.48	+9.01
Folic acid (µg/100cal)	Label	15	16	7.5	15
Per label		36.0±0.95	32.3±0.75	26.9±0.35	43.9±0.40
Boiling water		36.1±1.27	33.3±0.29	28.3±0.25	45.2±1.44
Change (%)		+1.0	+3.1	+5.2	+2.96
Pantothenic acid (µg/100 cal)	Label	450	500	450	15
Per label		1010±26	793±19.7	633±15.3	77.±40.3
Boiling water		1060±42	803±21.0	649±1.53	755±34.8
Change (%)		+4.95	+1.3	+2.53	+1.948
Biotin (µg/100 cal)	Label	4.4	3	5.5	2.2
Per label		9.9±1.39	4.6±0.47	6.59±0.48	4.25±0.06
Boiling water		10.4±0.55	4.23±0.25	6.73±0.32	4.28±0.08
Change (%)		+5.1	-8.0	+2.12	+0.706
Vitamin C (mg/100 cal)	Label	9	12	8.3	8
Per label		16.6±0.29	26.2±0.55	10.7±0.06	18.3±0.85
Boiling water		11.5±0.81	9.0±0.61	10.1±0.06	15.0±0.85
Change (%)		-30.7	-65.6	-5.61	-18.03
Iron (mg/100 cal) as ferrous sulphate	Label	1.8	1.8	1.8	1.5

Notes:
Label = Value declared on product label.
Values represent means ± standard deviations of three independent determinations for each reconstitution procedure.
Mean change % calculated as follows: ([boiling water]/[per label]-1) × 100.
Reconstitution:
Method 1 "Per label": Generally, labels on powdered formula will instruct the user to bring tap water to boil in a separate container, cool to 40°C, and add the desired volume to the bottle. The appropriate amount of powdered formula (i.e. the number of scoops per volume) is then added, the bottle capped and the formula shaken until the powder is completely dissolved. For this study, distilled water was boiled in a separate container, cooled to 40°C and the desired volume was added to a suitable container. The appropriate amount of powdered formula was then added and the formula shaken until the powder was dissolved.
Method 2 "Boiling water": Distilled water was boiled in a separate container, and then the desired volume was immediately added to a suitable container. The appropriate amount of powdered formula was then added and the formula shaken until the powder was dissolved.
Three independent preparations by each method were made for each formula.

Appendix E

LIST OF CASES OF INVASIVE (STERILE-SITE) *ENTEROBACTER SAKAZAKII* INFECTIONS ANALYSED[1]

Year of report	Location	No. cases	Reference
1961	UK	2	Urmenyi and Franklin, 1961
1965	Denmark	1	Joker, Norholm and Siboni, 1965
1979	Georgia, USA	1	Monroe and Tift, 1979
1981	Indiana, USA	1	Kleiman *et al*., 1981
	Oklahoma, USA	1	Adamson and Rogers, 1981
1983	Netherlands	8	Muytjens *et al*., 1983
1985	Missouri, USA	1	Naqvi, Maxwell and Dunkle, 1985
1988	USA	2	Willis and Robinson, 1988
1989	Portugal	1	Lecour, Seara and Miranda, 1989
	Iceland	3	Biering *et al*., 1989
	Tennessee, USA	3	Simmons *et al*., 1989
1990	Maryland, USA	1	Noriega *et al*., 1990
1991	Ohio, USA	1	Gallagher and Ball, 1991
2000	North Carolina, USA	1	Burdette and Santos, 2000
2001	Israel	2	Bar-Oz *et al*., 2001; Block *et al*., 2002
	Belgium	1	Van Acker *et al*., 2001
2002[a]	Israel	2	Block *et al*., 2002
	Tennessee, USA	1	CDC, 2002
	Wisconsin, USA	1	CDC (unpublished data)
2003	USA	6	CDC (unpublished data)
2004	France	2	InVS, 2006
	USA	2	CDC (unpublished data)
2005	USA	2	CDC (unpublished data)

[a] The two cases referred to here occurred in 1993 and 1998.

[1] This table lists sterile-site infections only. Some of the references listed above may report more cases of *E. sakazakii*-related illnesses than are listed here; however, only those cases reported as sterile-site infection were included in this analysis. This list of cases was compiled and analysed in advance of the expert meeting by Dr Anna Bowen, CDC, United States of America. At that time, 46 sterile-site cases were identified. Of these, 1 was a urinary tract infection, and 45 either bloodstream or central nervous system infections. These 45 were further analysed, since there were enough cases to support subgroup analysis and draw conclusions. It is recognized that while this analysis included the majority of cases, it may not include all cases of invasive illness in infants caused by *E. sakazakii*.

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