

Overview of a Risk Assessment Model for *Enterobacter sakazakii* in Powdered Infant Formula

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For

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Foreword

The risk assessment model described in this document was initiated at an FAO/WHO expert meeting on *Enterobacter sakazakii* and other microorganisms in powdered infant formula, held in Geneva, Switzerland on 2 – 5 February 2004 (FAO/WHO, 2004). It was subsequently recommended by the Codex Committee on Food Hygiene that this risk assessment should be further elaborated by JEMRA (Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment) to address some of the more specific risk management questions from the committee related to revising the international code of hygienic practice for foods for infants and children. FAO and WHO commissioned two well recognized risk assessment consultants, Mr Greg Paoli and Dr Emma Hartnett, to undertake this work. This report provides an overview of the risk assessment model that was developed and selected key assumptions and data.

A web-based version of this model is currently under preparation and will be made publicly available via the Internet by FAO and WHO in 2007.

1 Introduction

This document presents a quantitative risk assessment model developed for *Enterobacter sakazakii* in powdered infant formula (PIF). The risk assessment addresses PIF that is intrinsically contaminated with *E. sakazakii*, therefore, the risks associated with the potential contamination of the powder from environmental sources after retail, for example in the environment in which the formula is prepared or the equipment used in preparation (e.g. blenders), are not considered in this assessment. This is an important consideration for users of this model.

This risk assessment considers the preparation, storage and feeding of PIF to infants. The model describes the effect that each of the preparation and storage stages have upon the intrinsic microbiological quality of the PIF in terms of *E. sakazakii*. It examines the impact of different preparation and handling strategies on *E. sakazakii* in PIF and describes the outputs in terms of the relative risk posed to infants.

The risk assessment model estimates the risk of *E. sakazakii* illness posed to infants from PIF that is contaminated. Experimental studies suggest that *E. sakazakii* contamination of powdered formula is at low levels, with reports in the literature suggesting contamination levels of less than 1 cfu/g (Muytjens, Roelofs-Willemsse and Jaspar, 1988). While low levels of PIF contamination are reported, *E. sakazakii* has an observed growth range between 5.5°C and 49°C (Nazarowec-White & Farber, 1997a). These growth characteristics provide the opportunity for growth of any contaminating populations during the preparation of infant formula, resulting in potentially high levels of *E. sakazakii* at feeding.

1.1 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 the software called Analytica®. The risk assessment has three main components:

- 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.

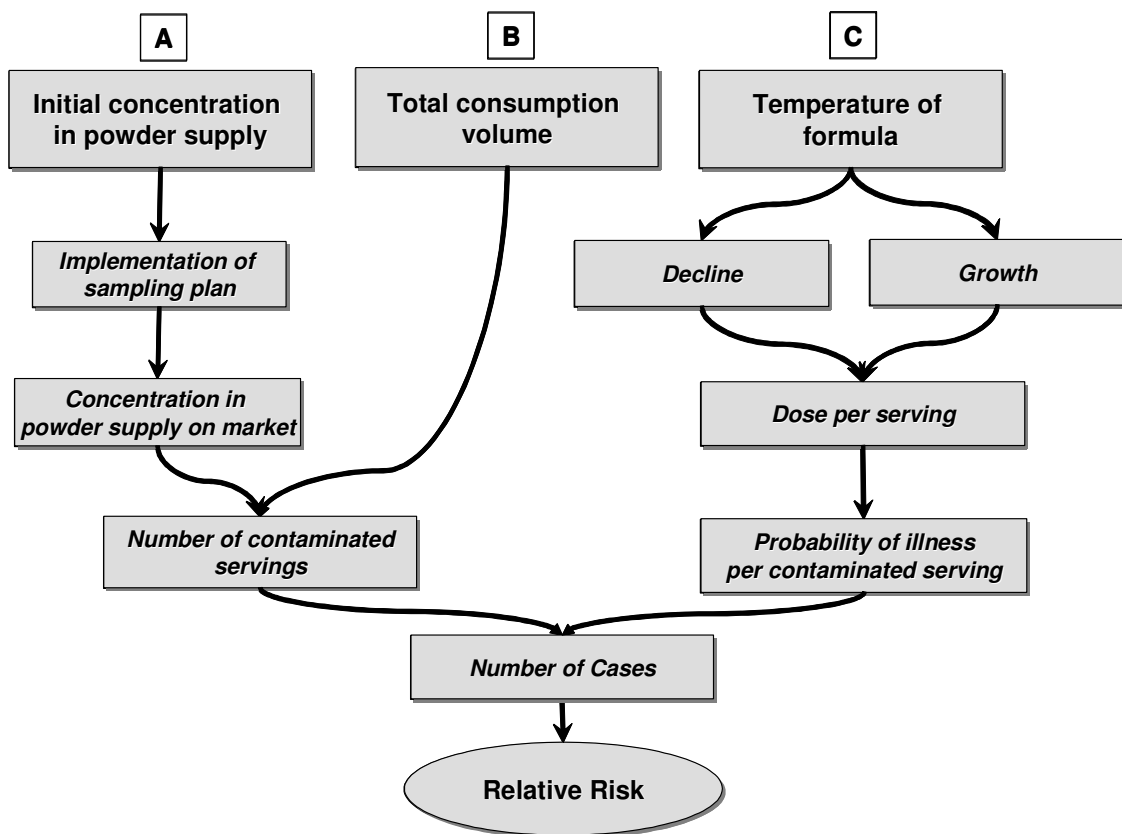


Figure 1: Schematic representation of the components of the risk assessment model

2 Hazard Characterization

The probability that illness results from the contamination of PIF with d_c cfu of *E. sakazakii* at the time of preparation is given by the exponential dose-response model, specifically $P_{ill} = 1 - \exp(-rd_c)$ 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 in the dry product. 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 are no data currently available to estimate the value of the dose-response parameter r which is likely to be specific for each of the infant groups considered in the model. Therefore, six options are presented in the model for some baseline value of r . The options available range from 1×10^{-5} to 1×10^{-10} . Once selected, multipliers of this baseline value of r can also be entered thus enabling this baseline value of r to be adapted to represent the relative susceptibility of each of the infant groups. As a default no pattern of susceptibility is assumed to apply across the infant groups. Therefore, values of 1 are implemented for the dose-response multiplier. Providing such options in the model enables a direct comparison of the impact of the assumptions regarding the value of r , and exploration of the relative susceptibility of the infant groups in terms of estimates of risk.

3 Exposure Assessment

The level of exposure, or dose, at the point of consumption, denoted d_c , is a result of the level of contamination in the PIF at preparation and the overall effect of the conditions of preparation of the formula and holding of the prepared formula between preparation and use upon the magnitude of any *E. sakazakii* populations contaminating the powder. *E. sakazakii* is a mesophilic organism, with an observed growth range

between 5.5°C and 49°C (Nazarowec-White & Farber, 1997a). These characteristics, 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 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 their 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 components of the model are:

- Level of powder contamination
- Sampling plan employed
- Method of preparation employed

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

The level of *E. sakazakii* in the PIF just before preparation is a result of the initial level of *E. sakazakii* in the product, the impact of any microbiological criteria (and associated sampling plans) 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.

3.1.1 E. sakazakii concentration in dry product

E. sakazakii has been reported to be present in powdered product at low levels (for example Muytjens, Roelofs-Willemse, and Jaspar, 1988 and others (see Table 20 in FAO/WHO, 2006)). However, studies reporting levels in powder usually involve testing of product on the market as opposed to product testing in the manufacturing environment prior to release for sale. One of the objectives of the risk assessment was to examine the effect of microbiological criteria and their associated sampling plans upon estimates of risk. In order 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. Such data were submitted to FAO/WHO as part of the *Call for Data*.

There is interest in considering Enterobacteriaceae as indicators of process hygiene. In developing the risk assessment consideration was also given as to whether it was possible to evaluate the impact of testing for Enterobacteriaceae on risk reduction of *E. sakazakii* illness in infants. Therefore, in addition to estimating the concentration of *E. sakazakii* in powdered product it is also necessary to estimate the level of Enterobacteriaceae in the product. Sample testing data for PIF in the manufacturing environment was provided as part of the FAO/WHO *Call for Data*. The data provided included the following:

- Sample size for Enterobacteriaceae samples (g)
- Number of samples tested for Enterobacteriaceae
- Number of samples positive for Enterobacteriaceae
- Sample size for *E. sakazakii* samples (g)
- Number of samples tested for *E. sakazakii*
- Number of samples positive for *E. sakazakii*

Assuming the organisms are distributed randomly following a Poisson distribution in the powder, the probability of obtaining at least one positive result can be calculated from $P > 0 = 1 - \exp(-C \times s)$ given a sample size of s grams and a concentration in the powder of C per gram. Using this, the concentration in the product can be estimated from $C = \frac{-\ln[1 - P > 0]}{s}$ where C is the concentration (per gram), $P > 0$ is the probability of recording a positive sample, and s is the samples size (grams).

This was applied to estimate the concentrations in the product of Enterobacteriaceae and *E. sakazakii*. In total 60 records were provided. Each reports the number of positive samples for Enterobacteriaceae and the number of positives for *E. sakazakii*. For Enterobacteriaceae, 35 samples yielded positive results. For *E. sakazakii*, 23 samples gave positive results. Note that only where the sample size was specified can a concentration be estimated. For ten records the sample size was not recorded. These samples were not included in the analysis. The estimated concentration for each of the positive results are provided in Table 1. These data are summarized in Table 2.

Table 1: Estimates of the concentration of the concentration of Enterobacteriaceae and *E. sakazakii* in PIF based upon samples taken of the product during manufacture

Data Point	Estimated log cfu/gram <i>E. sakazakii</i>	Data point	Estimated log cfu/gram Enterobacteriaceae
1	-3.44	1	-1.16
2	-3.21	2	1.38
3	-3.70	3	-1.14
4	-3.35	4	-1.44
5	-4.05	5	-0.51
6	-4.31	6	-0.49
7	-4.33	7	-0.29
8	-3.92	8	-0.35
9	-4.66	9	-0.27
10	-4.33	10	-0.22
11	-5.17	11	-0.22
12	-3.86	12	-0.18
13	-3.21	13	-0.12
14	-4.68	14	-1.25
15	-5.24	15	-2.08
16	-4.37	16	0.25
17	-3.66	17	0.99
18	-2.79	18	-0.49
19	-3.00	19	-1.76
20	-3.81	20	-2.44
21	-3.31	21	-2.36
22	-2.79	22	-2.40
23	-3.71	23	-2.23
		24	-2.29
		25	-0.56
		26	-1.03
		27	0.69
		28	-0.01
		29	-2.54
		30	0.004
		31	-0.42
		32	0.61
		33	-0.10
		34	-1.08
		35	-2.65

3.1.2 Exploring the relationship between Enterobacteriaceae and *E. sakazakii* contamination levels

In total, 22 of the records reported positive results for both Enterobacteriaceae and *E. sakazakii*. The estimates of concentration are given in Table 3. Note that for one record 0/50 positive samples was obtained for Enterobacteriaceae but 2/6 samples were positive for *E. sakazakii*. This record is removed from further analysis as it was only a 1 gram sample.

Table 2: Summary of the estimates of concentration of Enterobacteriaceae and *E. sakazakii* in PIF in the manufacturing environment.

Statistic	Enterobacteriaceae (log cfu/gram)	<i>E. sakazakii</i> (log cfu/gram)
Mean	-0.77	-3.91
Standard Deviation	1.07	0.67

Table 3: Data pairs of the estimates of Enterobacteriaceae and *E. sakazakii* in samples of PIF taken in the manufacturing environment based upon data submitted to FAO/WHO.

Estimated log cfu/gram Enterobacteriaceae	Estimated log cfu/gram <i>E. sakazakii</i>
-1.44	-3.44
-0.51	-3.21
-0.49	-3.70
-0.29	-3.35
-0.35	-4.05
-0.27	-4.31
-0.22	-4.33
-0.22	-3.92
-0.18	-4.66
-0.12	-4.33
-2.08	-5.17
0.99	-3.86
-1.76	-3.21
-2.44	-4.68
-2.23	-5.24
-2.29	-4.37
-0.56	-3.66
0.69	-3.00
0.00	-3.81
0.61	-3.31
-1.08	-2.79
-2.65	-3.71

These data were analyzed to explore any possible relations between the concentration levels of the two groups of organisms. A plot of the concentrations is given in Figure 2. It can be seen that there is no clear trend although a slight positive relationship is indicated, specifically as the estimated concentration for Enterobacteriaceae increases so does the estimated concentration for *E. sakazakii*.

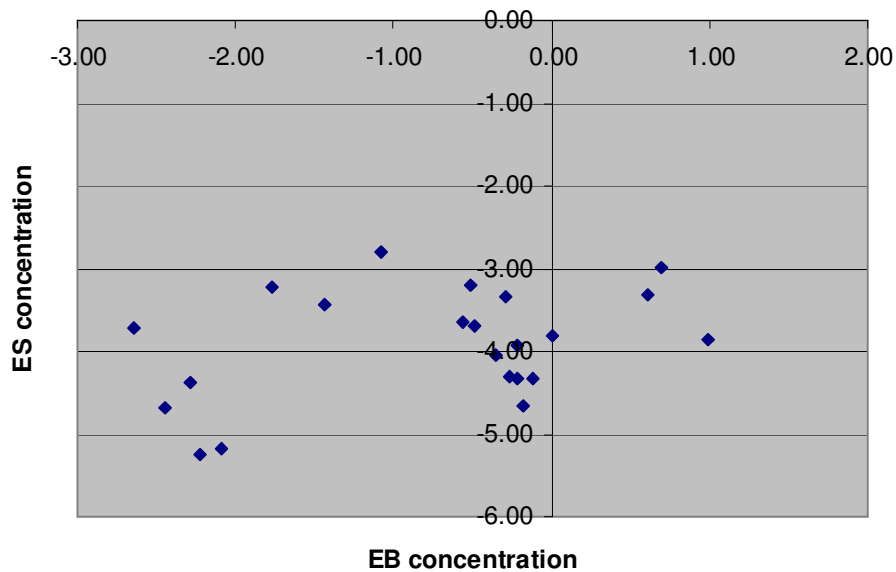


Figure 2: Scatter plot of the estimated Enterobacteriaceae (EB) and *E. sakazakii* (ES) concentrations as predicted by from the data submitted to FAO/WHO

To explore the relationship further, two statistics were calculated to describe the relationship. These are the Spearman's rank correlation coefficient and the Pearson product moment correlation coefficient.

Spearman's rank correlation coefficient is a non-parametric measure that assesses how well an arbitrary monotonic function could describe the relationship between two variables, without making any assumptions about the frequency distribution of the variables. Unlike the Pearson product-moment correlation coefficient, it does not require the assumption that the relationship between the variables is linear. Using Spearman's

rank the correlation coefficient, ρ , is given by $\rho = 1 - \left(\frac{6\sum(\Delta R)^2}{n(n^2 - 1)} \right)$ where ΔR is the difference in rank of the data in a data pair and n is the number of data pairs. For a given estimate of ρ the level of significance of the estimate of the coefficient given the number of data pairs the estimate is based upon is given in Table 4.

Table 4: Table of critical values for significance levels of $p=0.05$, $p=0.02$ and $p=0.01$ for the Spearman's rank co-efficient

Number of pairs	Critical value of coefficient		
	<i>P 0.05</i>	<i>P 0.02</i>	<i>P 0.01</i>
5	1	1	
6	0.886	0.943	1
7	0.786	0.893	0.929
8	0.738	0.833	0.881
9	0.683	0.783	0.833
10	0.648	0.746	0.794
12	0.591	0.712	0.777
14	0.544	0.645	0.715
16	0.506	0.601	0.665
18	0.475	0.564	0.625
20	0.45	0.534	0.591
22	0.428	0.508	0.562
24	0.409	0.485	0.537
26	0.392	0.465	0.515
28	0.377	0.448	0.496
30	0.364	0.432	0.478

The estimate of ρ from the 22 data pairs shown in Table is 0.2. From the table above the critical value for a significance of $p= 0.01$ given 22 data pairs is 0.562, therefore these data indicate that a strong positive relationship cannot be inferred between the concentration of Enterobacteriaceae and *E. sakazakii*.

The Pearson product moment correlation coefficient is a dimensionless index that reflects the extent of a linear relationship between two data sets. The value ranges from -1.0 to 1.0. A value of 1 shows that a linear equation fully describes the relationship, with all data points lying on the same line and with Y increasing with X . A score of -1 shows that all data points lie on a single line but that Y increases as X decreases. A value of 0

shows that a linear model is inappropriate and that there is no linear relationship between the variables. This assumes that the data follow an underlying Normal distribution. The estimate of the Pearson product moment correlation coefficient using the data presented in Table 4 is 0.37. These data therefore indicate that the relationship is unlikely to be adequately described by a linear model.

The greatest risk reductions are likely to be obtained by removing the product with concentrations in the upper tail of the distribution. Since sampling plans are aimed at this purpose, a high measurement of the Spearman's rank correlation coefficient may be an important indication of relatedness with respect to the ability to reduce risk (i.e., a highly ranked Enterobacteriaceae concentration is likely to be associated with a highly ranked *E. sakazakii* concentration; there is not necessarily any particular additional benefit to the relationship being linear). In the presence of a high level of rank correlation, a sampling plan aimed at rejecting lots with high levels of Enterobacteriaceae will be predisposed to also reject lots with higher levels of *E. sakazakii*. This aspect requires more analysis, and ideally an improved dataset specifically aimed at uncovering the nature of this relationship.

3.1.3 Exploring the impact of microbiological criteria upon the level of contamination in powdered product

For the purposes of the calculations and discussion that follows, the following general assumptions are employed regarding the manufacturing and sampling of powdered infant formula (PIF):

- PIF is assumed to be produced in discrete lots.
- Microbiological sampling is applied on a lot-by-lot basis, with results of sampling applying to the disposition of the individual lot.
- Sampling results apply only to decisions regarding the sampled lot and do not impact the microbiological quality of subsequent lots (e.g., by triggering changes or any other process control adjustment).
- Product lots of PIF that are rejected by the sampling criteria never enter the market.

- Product lots are of equal size.

The following technical assumptions regarding the distribution of contamination between and within lots of PIF are employed in the calculations:

- There is variation in the level of contamination between lots of PIF.
- There is variation in the level of contamination within lots of PIF.
- The level of contamination between lots is assumed to be log-normally distributed, such that the arithmetic mean concentration of the organisms across lots would follow a log-normal distribution (or equally, would be normally distributed when transformed to the log-scale).
- The level of contamination within lots is also log-normally distributed, such that measurements of the local concentration of organisms, taken randomly, within the lot would follow a log-normal distribution (or equally, would be normally distributed when transformed to the log-scale).

The following technical assumptions regarding the sampling process are employed in the calculations:

- When a number of samples is specified, samples are assumed to be taken randomly from within the mass of PIF in a single lot.
- While the distribution of local concentrations within the lot is assumed to be log-normally distributed, the distribution of the number of organisms within a small mass (e.g., like the mass of one sample) is assumed to be locally homogeneous.
- As a result of local homogeneity, the number of organisms that will be captured in a sample follows a Poisson process, with the intensity given by the random log-normal concentration where the sample is taken. Thus, the number of organisms (and any other statistics of the sampling) are derived from the Poisson Lognormal distribution (PLN).

Two-Class Plans

Using standard terminology, a two-class plan employs a threshold concentration (m , usually referred to as 'little- m ' to distinguish it from M , or 'big- M ' which is additionally

employed in three-class plans). The threshold concentration is a concentration above which a sample is considered defective. The number of samples taken (n) is specified. Generally, the size of the sample (s) in terms of the mass or volume of product is also specified. The number of defective samples (labelled, c) that will be tolerated while still accepting the lot is also specified.

Three-Class Plans

In a 3-class plan, sampling results are assumed to fall into three distinct categories. A sample falling below m is considered acceptable. A sample result exceeding the concentration m but not exceeding M is considered marginally acceptable, such that the lot is accepted only if the number of such samples does not exceed c . A sample result exceeding M is unacceptable and the product lot is rejected. Therefore, there are three scenarios in which a lot may be rejected in a 3-class plan: a) where more than c samples fall between m and M , or b) where any sample exceeds M , or c) where both a) and b) apply.

Co-existence of Two- and Three-Class Plans

Two-class plans and three-class plans may be instituted in parallel, with either of the criteria resulting in the decision to reject a lot. In such cases, the two-class plan may be applied to pathogens, while the three-class plan is applied to indicator organisms. The two-class plan may be instituted with qualitative sampling whereby the test on the sample only provides presence/absence of the pathogen in the sample, rather than a concentration. In other situations, multiple plans (three-class followed by two-class plan, or a sequence of two-class plans) may be applied in series such that the sampling for the second plan (related to a pathogen) is conditional on the results of the first plan (applied to indicator organisms).

Calculation of Risk Reduction via Sampling Plans

It is possible to calculate the risk reduction that would be achieved as a result of the implementation of microbiological criteria in isolation of the other components of the risk assessment model. The calculation of the risk reduction that is achieved by decisions based on microbiological criteria requires assumptions regarding the relationship between the distribution of pathogens in the accepted product and the ultimate risk. This risk assessment employs the following assumption with respect to *E. sakazakii* in PIF:

- Given manufacturing conditions and subsequent die-off of the organisms during storage, we assume that concentrations are sufficiently low such that when the product is eventually separated into serving-sized units, there will be only one colony-forming unit in a serving.
- While the potential for subsequent growth of the organisms is a critically important factor in overall risk generation, each contaminated serving that originated in PIF (as opposed to the preparation environment) stems from a single cfu.

The impact of this assumption is that each organism in the raw material acts as a source of risk independently of any other organisms. As such, the risk (from manufactured powder, as opposed to other sources of contamination) is proportional to the number of organisms in the manufactured powder. A further impact is that the number of contaminated servings is equal to the number of organisms, since there is a one-to-one relationship between organisms and contaminated servings. As a result, for calculation of risk reduction associated with the supply of PIF, it is sufficient to calculate the change in the total number of organisms that are in the supply. For example, if the decisions resulting from a sampling plan or any other control measure reduce the number of organisms by a factor of 10, then the risk is reduced by a factor of 10. As a further implication, it is important to note that the arithmetic mean of the concentration (and not the geometric mean, or log-mean) is proportional to risk, since it is, in turn, proportionate to the total number of organisms.

Since the microbiological criteria are assumed to apply on a lot-by-lot basis, the most important variable in describing the lots is the arithmetic mean concentration of pathogens. When a lot of powder is rejected, the number of pathogens that are removed from the powder supply is proportionate to the arithmetic mean concentration of the lot. It is then possible to calculate the risk reduction factor associated with implementation of microbiological criteria as follows:

$$\text{Sampling Risk Reduction Factor} = E[C_{\text{pre-sampling}}] / E[C_{\text{accepted}}],$$

where $E[C_{\text{pre-sampling}}]$ is the mean concentration in the powder supply before lot acceptance decisions are made, and $E[C_{\text{accepted}}]$ is the mean concentration in the

accepted powder supply. Thus, if the *accepted* powder has, on average, 5 times fewer pathogens than the available powder before sampling, then the sampling risk reduction factor will be 5.

In order to calculate the sampling risk reduction factor, the risk assessment simulates the lot-by-lot implementation of decisions based on microbiological criteria. Then the average concentration of *accepted* lots is calculated and compared to the average concentration of the *pre-sampling* powder supply using the equation above to calculate the net risk reduction effect of the sampling program.

The following is a brief overview of the simulation process for determining the risk reduction factor associated with a two-class sampling plan where the sample results are qualitative (sample indicates presence or absence) and the lot is rejected if any samples are positive:

- 1) Choose a number of lots (L) to simulate.
- 2) Choose the distribution for the between-lot variation in average concentration (BC) of *E. sakazakii* in PIF. The mean of this distribution is MBC .
- 3) Choose the distribution shape and standard deviation ($WC|BC$) for within-lot variation in the concentration of *E. sakazakii* in PIF. The mean of this distribution is given by the random samples from the between-lot distribution (BC) specified in 2.
- 4) Randomly choose an arithmetic mean concentration from BC .
- 5) Given BC , determine the distribution for WC , such that the mean of WC equals BC .
- 6) Randomly simulate n samples of size s of PIF with the concentration of powder in each sample drawn randomly from WC .
- 7) Calculate the probability (P_{reject}) that at least one of the n samples are positive for this lot. Calculate $P_{accept} = 1 - P_{reject}$
- 8) Accept the lot, or reject the lot with probability P_{accept} or P_{reject} respectively.
- 9) Repeat 4-8 until L lots have been simulated.
- 10) Calculate the expected value of the concentration of accepted lots (MAC).
- 11) Calculate the risk reduction ratio $RR_{sampling} = MBC/MAC$.

The following sequence of calculations is provided to illustrate the process for the simulation of 4 lots (L=4) and 5 samples per lot (n=5) with samples of 10 grams.

- 1) $\log_{10} BC$ is normally distributed with mean -3 and standard deviation 1.
- 2) Within-lot concentration is assumed to be log-normally distributed. Assume $\log_{10}(WC)$ is normally distributed with standard deviation 1
- 3) For the 4 lots, the random samples from BC are: [0.0009, 0.0008, 0.0016, 0.0767]. Mean arithmetic concentration between-lots (MBC) is 0.02 cfu/g.
- 4) For the four lots, randomly drawn within-lot concentrations at each sampling point are given by the following table:

	<i>Sample</i>				
LOT	1	2	3	4	5
1	4.78E-04	4.88E-04	3.74E-03	5.95E-04	6.46E-05
2	5.67E-05	1.10E-05	1.10E-04	9.58E-05	3.09E-04
3	2.21E-05	4.62E-05	1.86E-04	5.10E-03	6.03E-06
4	6.07E-02	4.04E-02	3.21E-02	1.51E-02	1.13E-01

- 5) The probabilities that at least one of the samples are positive (P_{reject}) and that none of the samples are positive (P_{accept}) are given by the table below.

<i>LOT</i>	<i>Lot Mean Conc.</i>	<i>P_{reject}</i>	<i>P_{accept}</i>
1	0.0009	0.052	0.948
2	0.0008	0.0058	0.9942
3	0.0016	0.052	0.948
4	0.0767	0.927	0.073

- 6) Calculate the expected concentration in accepted lots. This is equal to the weighted average of the mean concentrations of each lot, weighted by the probability of their being accepted. Note that the mean of P_{reject} is approximately 0.25 indicating that on average 1 of the 4 lots will be rejected (usually, lot 4).
- 7) Calculate the ratio of the original between-lot concentration from the 4 lots simulated (MBC) and the expected concentration of accepted lots (MAC).

Description	Variable	Value
Mean Between-Lot Concentration before Sampling	MBC	0.02
Mean Between-Lot Concentration in Accepted Powder	MAC	0.0029
Risk Reduction Factor associated with application of microbiological criteria	RR = MBC/MAC	6.6
Proportion of Lots Rejected	Mean(P_{reject})	26%

This small simulation demonstrates a number of concrete elements of the way that the sampling scheme reduces risk. Lots 1, 2 and 3 are relatively uncontaminated, while Lot 4 is relatively highly contaminated. Lots 1, 2 and 3 have a low, but non-zero probability of being rejected. Lot 4 is very likely to be rejected. This is the primary reason for the risk reduction. The risk reduction is determined by the fact that the expected concentration in the accepted powder (*MAC*) is significantly reduced when compared to the original powder supply (*MBC*) primarily due to the small probability that the accepted powder supply will include Lot 4.

Stability of Estimates

The estimates above are based on only 4 lots and therefore do not represent a stable estimate of the impact of the risk reduction associated with sampling. Below are the final result tables for simulations with 50,000 and 100,000 lots respectively. These larger simulations provide a more robust estimate of the risk reduction that would be expected 'in the long run.'

With L = 50,000

Description	Variable	Value
Mean Between-Lot Concentration before Sampling	MBC	0.014
Mean Between-Lot Concentration in Accepted Powder	MAC	0.00454
Risk Reduction Factor associated with application of microbiological criteria	RR = MBC/MAC	3.11
Proportion of Lots Rejected	Mean(P_{reject})	11.5%

With $L = 100,000$

Description	Variable	Value
Mean Between-Lot Concentration before Sampling	MBC	0.014
Mean Between-Lot Concentration in Accepted Powder	MAC	0.00454
Risk Reduction Factor associated with application of microbiological criteria	RR = MBC/MAC	3.01
Proportion of Lots Rejected	Mean(P_{reject})	11.5%

It can be seen that the larger simulations converge to a fairly stable estimate of the risk reduction factor (around 3) and the proportion of lots that will be rejected (approximately 11.5%).

Lot Rejection Rate

At the same time as calculating the risk reduction associated with microbiological criteria, it is important to keep track of the proportion of the lots of powder that are rejected by the sampling scheme. While large risk reductions may be possible through sampling plans, they may achieve this by requiring disposal of significant proportions of powder. Efficiency measures can be calculated, such as risk reduction per lot rejected. As such, a plan that reduces risk indiscriminately will have a relatively low efficiency measure. A plan that reduces risk by selectively rejecting highly contaminated lots will yield a higher efficiency score. An appropriate sampling plan would strike a balance between maximizing risk reduction and minimizing powder lot rejection, presumably by being most selective for highly contaminated lots of powder.

3.1.4 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). Results indicated that during storage, an initial decline of 0.014 log units per day for the first 153 days of storage followed by a period decline at a slower rate of 0.001 log units per day (measured up to 687 days after the start of the experiment). Within the risk assessment, options are provided for different storage durations of 0, 30, 100 and 365 days. Following discussions at the expert meeting (16 – 20 January 2006) of the experimental

studies considering survival of *E. sakazakii* in powder it was concluded that it was most appropriate to use the reduction of 0.014 log units per day for all storage durations..

3.2 Estimating impact of preparation and holding

During preparation, holding and feeding of the reconstituted formula, the formula will be subject to temperatures that provide the opportunity for both increase and decline in the concentration of contaminating *E. sakazakii*. The model provides the option to define specific preparations scenarios. Each of these methods is described in terms of 4 main stages, specifically

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

For each of the scenarios, these four stages are defined in terms of the duration, the ambient temperature, and the rate at which the formula is heated or cooled. It is assumed that regardless of the scenario specified that the formula is cooled/warmed to a specified feeding temperature and that this process takes 30 minutes. Below are four possible scenarios to illustrate the types of scenarios that can be described:

- Premixing of PIF in 1 litre container, cooled briefly and then poured into servings with an extended time to consumption
- Mixing of PIF occurs in the feeding bottle, followed by refrigeration with a short time to consumption
- Mixing of PIF occurs in the feeding bottle but there is no refrigeration of the product, and there is an extended time to consumption
- Mixing of PIF occurs in the feeding bottle but there is no refrigeration of the product, and there is an extended time to consumption at a very warm room temperature

The specifications for the six scenarios are presented in Table 5 and the values assigned to these specifications are presented in Table 6. Using the information for the scenarios that are specified by the user, 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. This profile is then used to estimate the extent of growth or temperature-related inactivation that may occur in any

contaminating *E. sakazakii* populations. The accumulation of the growth and decline of the population over the time from preparation to the end of the feeding period provides an estimate of the level of *E. sakazakii* ingested.

Table 5: Assignment of variables to describe the six preparation scenarios specified in the risk assessment model

Example Preparation Scenario	Stage duration (hours)			Stage temperature (°C)			Cooling rate (h ⁻¹)		
	Prep	Cool	Feed	Prep	Cool	Feed	Prep	Cool	Feed
Premixing of PIF in 1l container, cooled briefly and then poured into servings with an extended time to consumption	0.25	1	6	RT	RRT	WRT	SAC	SAB	SAB
Mixing of PIF occurs in the feeding bottle, followed by refrigeration with a short time to consumption	0.25	6	2	RT	RRT	WRT	SAB	SAB	SAB
Mixing of PIF occurs in the feeding bottle but there is no refrigeration of the product, and there is an extended time to consumption	0.25	1	6	RT	RRT	WRT	SAB	SAB	SAB
Mixing of PIF occurs in the feeding bottle but there is no refrigeration of the product, and there is an extended time to consumption at a very warm room temperature	0.25	1	6	VRT	VRT	VRT	SAB	SAB	SAB

Key: RT – Room Temp, WRT – Warm room temp, VRT – Very warm room temp, RRT - Refrigeration temp, SAC – Still air container, SAB – Still air bottle,

A description of all the different preparation, holding and feeding scenarios that have been evaluated to date are provided in the report of the FAO/WHO expert meeting implemented in early 2006 (FAO/WHO, 2006).

Table 6: Example of values assigned to the variables presented in Table5 in the risk assessment model

Variable	Description	Value	Source
RT	Room temperature	20°C	Assumption
WRT	Warm room temperature	27°C	Assumption
VRT	Very warm room temperature	35°C	Assumption
RRT	Refrigeration temperature	7°C	Assumption
SAC	Cooling rate - still air, formula in a 1 litre can	100u per second	Zwietering, pers. comm.
SAB	Cooling rate - still air, formula in a bottle	200u per second	Zwietering, pers comm.

Considering holding as a time-sequenced event and assuming that the stages preparation, cooling, warming and feeding occur consecutively, the temperature time profile for the PIF is determined and the growth and/or decline of the population predicted. The process is divided into discrete time steps (for example 0.01 hour). 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. If the temperature is greater than the maximum permissible growth temperature then cell death occurs and population decline is predicted. Calculations are conducted in \log_{10} space, facilitating the development of an additive model to describe a complex process.

To predict the temperature as a result of cooling, at each time step the temperature T_i at time step i is given by

$$T_i = T_f + (T_{i-1} - T_f) \exp^{-\beta t_i}$$

Using the above equation for each time step, T_f is the surrounding temperature associated with the stage, T_{i-1} is the starting temp of PIF at each time step given by the temperature at the end of the previous time interval $i-1$, β is the cooling rate associated with the particular stage and t_i is the length of time in the preparation stage (for example 0.01 hour). An example of a temperature profile for the preparation, cooling, warming and feeding stages is shown in Figure 3.

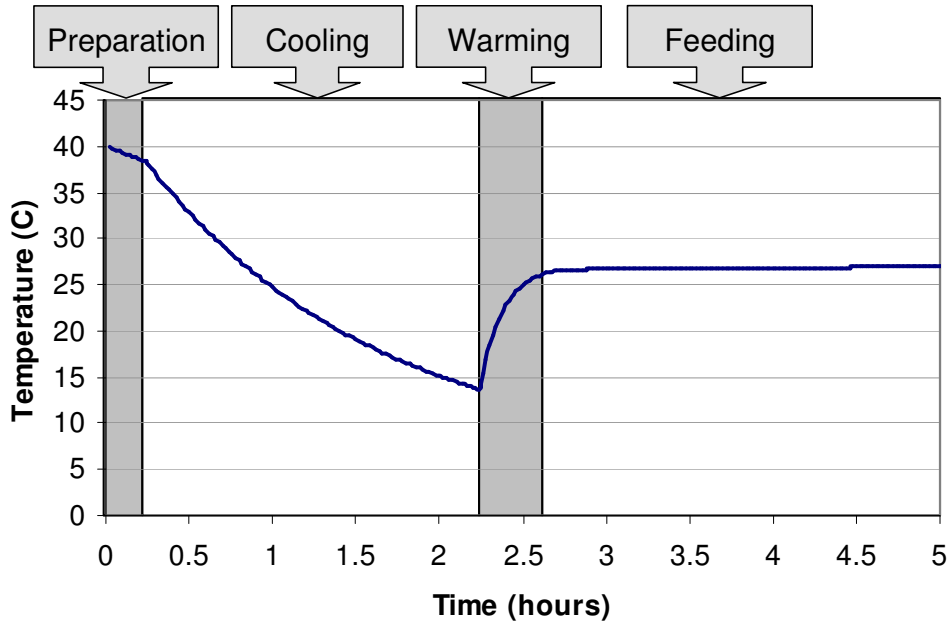


Figure 3: Example of a temperature-time profile generated by the risk assessment model for the stages of preparation, cooling, warming and feeding.

3.2.1 Estimating the change in contaminating population during preparation stages

3.2.2 Predicting growth

The change in the population size that may occur as a result of growth is described by the specific growth rate k . Using the Square Root Model for the full biokinetic temperature range, the value k (ln/hour) can be determined from $\sqrt{k} = b_G (T - T_{\min}) \{1 - \exp(c_G (T - T_{\max}))\}$ (McMeekin et al., 1993). Here T is the temperature of the PIF, T_{\min} and T_{\max} are the lower and upper temperatures at which the growth rate curve crosses zero (from the model fit), and b_G and c_G are parameters derived from the fit of the model. The growth model is parameterized using experimental data received as part of the *FAO/WHO Call for Data*. These data are used to estimate T_{\min} , T_{\max} , b_G and c_G . The estimates of the model parameters are given in Table . The resulting estimates of T_{\min} are consistent with published data (for example using

data presented by Nazarowec-White & Farber, (1997a) and Iversen, Lane and Forsythe, (2004), values of 2.5 and 2.1 respectively are obtained for T_{\min} . The dependency of the lag phase upon temperature is described by a logarithmic model using $\text{Log}_{10}(\lambda) = c_L \ln(T) + b_L$ where λ is the lag phase in hours, and b_L and c_L are parameters derived from the fit of the model. The resulting values for b_L and c_L are 4.309 and -1.141 respectively. At each time-step the temperature of the formula is determined and the lag phase estimated. The percentage of the lag phase that has passed is estimated from $\% \lambda = \sum_i \frac{t_i}{\lambda_i} \times 100$. Once the percentage of the lag phase that

has passed reaches 100% the magnitude of the growth, G_i , is given by $\frac{k_i}{\ln(10)} t_i$.

3.2.3 Predicting decline

The decline in the population size that may occur for any individual time interval, R_i , is given by

$$R_i = \frac{t_i}{10^{D_{ref} \left(\frac{T_{ref} - T}{z_{ES}} \right)}} \quad i = 0, 0.01, 0.02, \dots, d$$

Here T is the temperature of the PIF for a given time step, in the particular preparation stage as a result of cooling, T_{ref} and D_{ref} are a reference temperature and associated D-value for *E. sakazakii* respectively, t_i represents the incremental time steps through the duration of the preparation stage until time of completion, d ; and z is the z-value for *E. sakazakii*. The overall change in the contamination level, C , in the formula considering the effect of preparation, holding and feeding is given by $C = \sum_i G_i + R_i$.

This results in a profile of the change in the magnitude of the contaminating population of *E. sakazakii* as shown in Figure .

The model is parameterized with data describing the characteristics of *E. sakazakii* strain 607. Strain 607 is considered the most thermotolerant of the strains studied in the

literature (Edelson-Mammel & Buchanan, 2004) and therefore parameterising the model based upon the characteristics of this strain poses a worst-case scenario in terms of thermotolerance. At this stage there are insufficient data available for all aspects of the model to explicitly include other strains. The z-value reported for strain 607 is 5.6 (Edelson-Mammel & Buchanan, 2004) this is consistent with other studies, for example Nazarowec-White & Faber (1997b) report a z-value of 5.82 as the mean for a mix of strains, Iversen & Forsythe, (2004) report z-value of 5.7 for 2 strains. The D-value for strain 607 at 58°C is reported to be 0.16 hours (9.6 mins) (Edelson-Mammel & Buchanan, 2004). Other reports in the literature for other strains range from 1.3 to 3.8 mins at 58°C (Iversen & Forsythe, 2004) and 0.4 to 0.6 mins (Breeuwer *et al.*, 2001). The parameter values are summarized in Table 7.

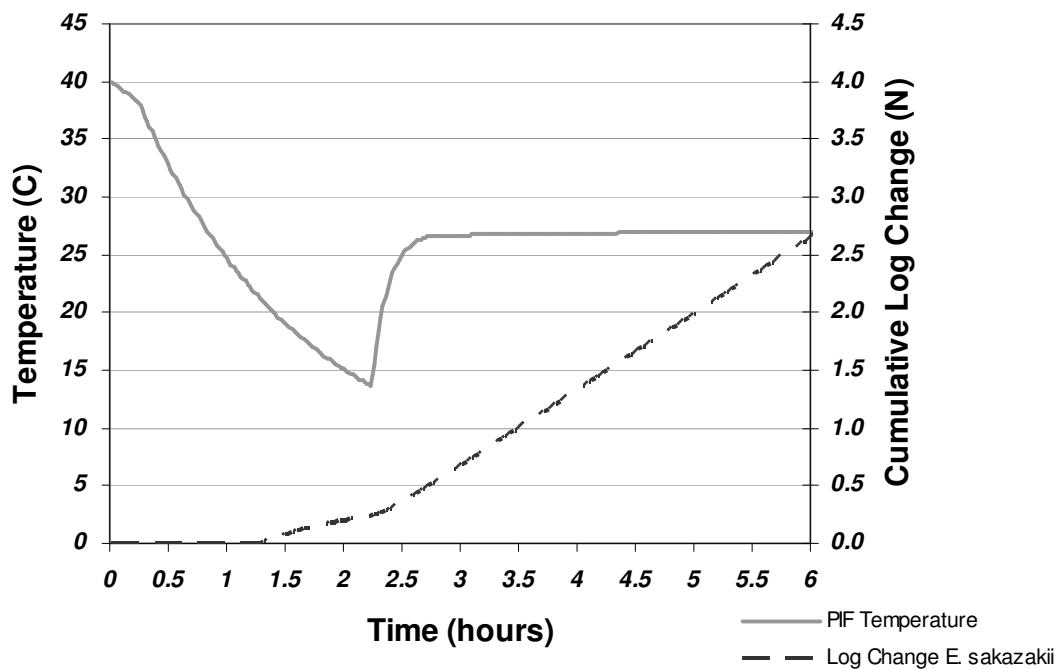


Figure 4: Example of the temperature profile and associated log change in *E. sakazakii* during the preparation, cooling, warming and feeding of PIF.

Table 7: Parameter values used in the risk assessment model to estimate the growth and decline of *E. sakazakii* in PIF.

Parameter	Description	Value	Reference
T_{opt}	Optimum temperature for growth	37°C	Iversen, Lane and Forsythe, 2004
T_{min}	Growth model parameter	2.5°C	FAO/WHO call for data, Kandhai et al., 2006
T_{max}	Growth model parameter	49°C	FAO/WHO call for data, Kandhai et al., 2006
b_G	Growth model parameter	0.053	FAO/WHO call for data
c_G	Growth model parameter	0.139	FAO/WHO call for data
b_L	Lag model parameter	4.309	FAO/WHO call for data
c_L	Lag model parameter	-1.141	FAO/WHO call for data
z	Z-value for <i>E. sakazakii</i>	5.6°C	Edelson-Mammel & Buchanan 2004
D_{ref}	D-value at reference temperature (hours)	0.16	Edelson-Mammel & Buchanan 2004
T_{ref}	Reference temperature used to determine D values	58°C	N/A
t_i	Length of time step	0.02 hr	N/A

4 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 PIF. The underlying assumption is that 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 powder consumption per 1 million infant-days, defined as N_{Es} , is estimated from

$$N_{Es} = \Theta \cdot C_m \cdot P_{ill}$$

Here, Θ is the concentration of *E. sakazakii* in the dry product at the point of preparation of the powdered formula (thus taking into account the impact of any sampling strategies

in place and any decline during storage); P_{ill} is the probability that illness results from the dry powder given an initial contamination level of 1 cfu of *E. sakazakii* in the 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 infant provided as options in the risk assessment, specified according to either the birth weight or age of the infant in any infant group. For each of these classes a recommended daily intake of formula is specified in the model. The daily powder consumption rate is given by converting the recommended ml/Kg per day associated with body weight to million Kg (MKg) per day per infant. The daily powder consumption rate per 1 million infants (C_m) is given by converting the recommended ml/kg associated with body weight per day to MKg 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 are defined by body weight and daily intake of PIF and are given in Table 8.

To simulate the model, an initial concentration of *E. sakazakii* is sampled and the concentration in finished powder estimated. The model iterates over the time from beginning preparation of the formula to completion of feeding predicting the change of any contaminating *E. sakazakii* population over time 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 calculated. The number of illnesses per million infant days is then calculated and converted to a relative estimate of risk across the scenarios considered. Estimates of risk can then be readily compared across infant groups and preparation scenarios to determine which scenarios present both desirable and achievable levels of risk mitigation for the infant group(s) of interest.

Table 8: Infant group definitions presented as options in the risk assessment model

Infant group	Definition	Weight (g)	Daily intake (MI/kg/day)
Extremely low birth weight	Birth weight <1000g	800	150
Very low birth weight	Birth weight <1500g	1250	200
Low birth weight	Birth weight <2500g	2000	200
Premature neonate	Prior to 37 completed weeks	2250	150
Term non-LBW Neonate	0 to 28 days of age	3600	150
Young Infant	29 days to 6 months of age	5000	150
Older Infant	6 to 12 months of age	9000	55.55

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