Exposure assessment to *Cronobacter sakazakii* in powder infant formula in Ireland

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**Background**

*Cronobacter sakazakii* represents a significant risk to the health of neonates. This bacterium is an emerging opportunistic pathogen that is associated with rare but life-threatening cases of meningitis, necrotizing enterocolitis, and sepsis in premature and full-term infants. Although, the organism nature has not been fully understood, molecular typing methods have identified PIF as a source and vehicle of neonatal infection. The recovery of *C. sakazakii* from numerous locations in powdered milk production facilities (Kandhali et al., 2004a,b), suggests that contamination of the final product occurs via the environment of the processing facility (Drudy et al., 2006a; Mullane et al., 2006).

Although studies have epidemiologically linked contaminated PIF to neonatal infection, no report exists to conclusively link *C. sakazakii* recovered in the manufacturing environment to the final PIF product (Mullane et al., 2007).

**Introduction**

The introduction of legislative microbiological criteria for foods (Official J L338 (2005) 1) has increased the exposure of enforcement and industrial laboratories to the need to test multiple food samples for organisms, such as *C. sakazakii* in PIF. For this organism the attributes sampling scheme is adopted, where the sample results are qualitative (sample indicates presence or absence) and the lot is rejected if any samples are positive.

**Objectives**

The objective of this study is to consider some statistical aspects relating to the probability of accepting and rejecting a lot, considering two surveys carried out in Ireland to detect the contamination of *C. sakazakii* in PIF.

**Materials and methods**

The assumptions used to calculate the probability of accepting or rejecting a lot are the ones adopted by the WHO risk assessment model for Enterobacter sakazakii in powdered infant formula (www.miramodels.es/SAKRAModelWizard.aspx) and fully described by Paoli & Hartnett (2006).

The level of contamination between and within lots is assumed to be log-normally distributed, while the distribution of the organism in the 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) is derived from the Poisson Lognormal distribution (PLN).

For the calculation of the rejection rates, we considered:

1) The log-normal distribution of *C. sakazakii* across all lots of PIF (cfu/g);
2) The between-lot standard deviation (σ) across all PIF lots; and
3) The within-lot standard deviation (σw) for individual PIF lots.

Two recent studies on monitoring of *C. sakazakii* were conducted in Ireland and reported 7 positives in 276 samples and zero positives in 719 samples (Mullane et al., 2007; Ireland National Microbiological Survey, 2006). For each of these two studies, true prevalence was estimated (mean: 2.9 and 0.14%) from apparent prevalence using the Bayesian approach based on beta (1, 1) and assuming the microbiological analyses was without error, thus considering sensitivity and specificity equal to 1. The concentration of *C. sakazakii* in the product is estimated from C = ln[1– P rej]/σ / S where C is the concentration (per gram), P > 0 is the distribution of prevalence estimated, and σ is the samples size (grams). From the log-normal distribution of the lot variability, 100 samples were randomly collected and a second order model was performed (Fig 1).

Next, lots were simulated and tested against the microbiological criteria established in the EC 2073/2005 (absence in 10 g, 30 samples per unit). The probability of accepting each sample was calculated,

$$P_{\text{accept}} = e^{-d_{i} / k_{i}}$$

where $d_{i}$ is the expected prevalence of contamination and $k_{i}$ is the sample size. Finally the probability distribution of rejecting a lot was obtained:

$$P_{\text{reject}} = 1 - \prod_{i=1}^{n} (1 - P_{\text{accept, } i})$$

**Results and Discussion**

A visual analysis of the results suggests that the total uncertainty is more affected by variability rather than by uncertainty around prevalence data (Fig. 1,2,3).

Graphically are presented several distributions describing the probability of rejecting a lot assuming 10 different values for the within (σ) and between lot variability (σw) (Fig. 3 & 4). Simulating for different between-lot variability values (0.2 to 2), we obtained probabilities of rejecting a lot ranging from 0.09 to 0.33 and 0.01 to 0.14 for the first and second survey, respectively. It is apparent from the results of the simulations (Fig. 2), that increasing the within lot variability for the survey with the higher positive proportion of 2.9%, the probability of rejecting the lot decreases because more chances are that some positive samples are not picked. When the within lot variability decreases, the CFU’s among units are more uniform, therefore the probability of rejecting the lot increases. In contrast (Fig. 3), decreasing the between lot variability, the probability of rejecting the lot also decreases. As shown in Figure 4, rejection rates also are more sensitive by varying the between-lot variability instead of the within-lot variability.

**Conclusions**

Although uncertainty around prevalence data was analysed, two different isolation methods were used to recover *C. sakazakii* in this two surveys, therefore, as part of future work, also sensitivity and specificity of the method will be taken into account.

Based on available survey data, which indicate presence or absence of the organism in PIF, we were not able to estimate the within and between-lot variability, but we could only assume a certain range of values. As it is clearly shown by the results, changing these two values during simulation, gives a great range of results in terms of probability of rejecting a lot. The challenge for further work is to quantify the within and between-lot variability, to allow processors estimate with confidence the lot rejection rate arising from CFU with EU legislation.

**References**