CRONOBACTER SPP.

(FORMERLY ENTEROBACTER SAKAZAKII)

THE ORGANISM/TOXIN

Enterobacter sakazakii was recently reclassified into eight distinct taxa of a new genus *Cronobacter* (Iversen *et al.,* 2008). All have been linked retrospectively to clinical cases in adults and infants (FAO/WHO, 2008). To avoid confusion the organism will be referred to here as *Cronobacter* spp. (*E. sakazakii*). The bacterium is Gram-negative, motile, rod shaped, non-spore-forming and will grow in aerobic and anaerobic conditions (FAO/WHO, 2008). It is considered an opportunistic pathogen. Enterotoxin-like compounds are produced by some strains (FAO/WHO, 2004).

THE FOOD

Powdered formulae (PF) can be used to supplement or replace human breast milk. As a powder, it has advantages of cost and storage over the liquid form, however liquid (ready-to-use) infant formula is commercially sterile and is rarely implicated in human illness. PF includes all types of powdered formulae for infants and young children, including:

- Powdered infant formulae (PIF) and infant formulae for special medical purposes;
- Follow-up formulae (FUF);
- Human milk fortifiers used to supplement breast milk.
  (CAC, 2008)

In general, PF products have been identified as high-risk foods for the growth of *Cronobacter* spp. (*E. sakazakii*) although only PIF has been implicated in cases of *Cronobacter* spp. (*E. sakazakii*) infection.

PIF is intended for newborns to weaning infants. Its composition closely resembles human breast milk. It is subject to stringent hygiene controls and microbial criteria in its manufacture. Current international standards (CAC, 2008) require *Cronobacter* spp. (*E. sakazakii*) to be absent in 30 samples of 10 grams.

Follow up formula (FUF) is a liquid food (derived from milk and/or other constituents of animal/plant origin) that is suitable for weaning infants from their 6^{th} to 12^{th} month. FUF may contain a wider variety of dry-mix ingredients that diversify the diet, e.g. cocoa powder, fruit/vegetable powders or flakes and flavours. FUF generally has a higher protein, iron and mineral content and a higher renal solute load compared to PIF (MoH, 2008a).

International evidence suggests that FUF has been consumed by infants <6 months old, and occasionally <1 month old (FAO/WHO, 2008). A general consensus has been reached by the Codex Alimentarius Commission not to establish a microbial criterion for *Cronobacter* spp. (*E. sakazakii*) in FUF (CCFH, 2009). This is mostly due to a lack of evidence associating illness with FUF, but also because feeding FUF to infants <6 months old contradicts manufacturers' directions. Unintended use or misuse of FUF has led to calls for clearer labelling and education of caregivers and healthcare professionals regarding the appropriate preparation and use of PIF and FUF.

NZFSA and the Ministry of Health have produced the following advice regarding *Cronobacter* spp. (*E. sakazakii*) and formula preparation:
http://www.nzfsa.govt.nz/consumers/food-safety-topics/recalls-and-product-advice/infant-formula-sakazakii/index.htm

Further advice on formula preparation is available from the Ministry of Health (MoH, 2008b):
http://www.healthed.govt.nz/uploads/docs/HE1521.pdf

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GROWTH AND CONTROL

Growth

Temperature

- Range 5.5-45°C (Nazarowec-White, 1998).
- Optimum 39.4°C (Kandhai et al., 2006).

Generation time 5 h at 10°C, 40 min at 23°C (Lambert and Bidlas, 2007), 20 min at optimum. It has been shown to grow in breast milk and breast milk with fortifiers (calorie and/or nutrient supplements) at 23°C and 37°C. The addition of fortifiers slowed growth at both temperatures, the effect was especially pronounced at 10°C (Lenati et al., 2008).

pH

- Minimum 3.89 (Lambert and Bidlas, 2007).
- Optimum 5-9.
- No maximum value found in the literature.

Atmosphere

Grows in aerobic and anaerobic conditions.

Water activity

Maximum salt concentration permitting growth: 9.1% (Lambert and Bidlas, 2007).

Survival

Favoured in PIF at low a_w and temperature.

In a long-term survival experiment the organism was inoculated into PIF to achieve a final reconstituted concentration of 10^6 cfu/ml and the PIF stored in screw-capped bottle at room temperature for 2 years. A final concentration of approximately 300 cfu/ml was measured in the reconstituted product (a 3.4 $\log_{10}$ reduction). Most of the reduction occurred in the first 5 months (Edelson-Mammel *et al.,* 2005).

Temperature

Survived 6 months of freezing in reconstituted PIF without a decrease in concentration.

Ten strains did not grow in reconstituted PIF stored at 4°C but could be detected by enrichment 72 h after preparation Gurtler and Beuchat, 2007a).

pH

Ability to survive moderate acid conditions is pH-dependent. Ten of twelve strains reduced by less than 1 $\log_{10}$ during a 5-hour challenge at pH 3.5 (at 36°C) (Edelson-Mammel *et al.,* 2006).

Water Activity

Survives in PIF (a_w = 0.2). Survived better in PIF at a_w 0.25-0.30 than in PIF at a_w 0.43-0.50 at both 21°C and 30°C (Beuchat et al., 2009).

Exponential-phase cells are more sensitive to drying than stationary-phase cells in low water activity environments (Pagotto *et al.,* 2007). Dried stationary phase cells survived 46 days at 25°C and 47°C, reducing by around 2 $\log_{10}$ CFU/ml in the first 20 days then remained stable (Breeuwer et al., 2003).

Inactivation

No synergistic interactions between inhibitory factors such as weak acids, pH, salt and temperature (Lambert and Bidlas, 2007).

Temperature

Substantial diversity in thermal resistance of strains with two distinct heat resistance phenotypes observed. At 58°C the D time varied by almost 20 fold between strains (Edelson-Mammel and Buchanan, 2004).

Reference temperature	D value in reconstituted PIF (minutes)	Reference temperature	D value in reconstituted PIF (minutes)
52°C	54.8 [1]	60°C	1.1 – 4.4 [3,4]
54°C	6.4 – 23.7 [1,2]	62°C	0.2 – 0.3 [4]
56°C	1.1 – 21.1 [2,3]	65°C	0.6 [3]
58°C	0.27 – 9.9 [2,3]	70°C	0.07 [3]

[1] Nazarowec-White and Farber 1997; z-value 5.8°C.
[2] Breeuwer *et al.,* 2003 (in phosphate buffer); z-value 3.1-3.6°C.
[3] Edelson-Mammel and Buchanan, 2004 (strain 607 described as most heat resistant in their study); z-value 5.6°C
[4] Iversen *et al.,* 2004; z-value 5.7-5.8°C.

A >4 $\log_{10}$ reduction of the pathogen in powdered milk and PIF when they are reconstituted with water at ≥70°C) has been demonstrated (Edelson-Mammel and Buchanan 2004; Osaili *et al*., 2009). However, manufacturers recommend using cooled boiled water. Advice varies regarding the temperature to be achieved but it is generally < 50°C before addition of powder to reduce (1) nutrient loss, particularly vitamin C, (2) clumping of powder and (3) potential for burns/scalds to infants or carer. Higher temperatures may also activate bacterial spores. The Ministry of Health (2008a, 2008b) and the NZFSA advocate cooling water and refrigerating for use on the same day if not used directly. At three months of age, tap water from a town supply can be used. An internet-based risk assessment model (JEMRA, 2007) considers the initial level of contamination, consumption patterns, preparation and handling (including water temperature). This enables comparisons between different preparation and feeding scenarios.

pH
Over a 5-hour challenge at pH 3.0, the decline in 12 strains was 4.9 to >6.3 $\log_{10}$. The rates of inactivation varied considerably between strains (Edelson-Mammel *et al.,* 2006).

Pressure
High hydrostatic pressure as a non-thermal pasteurisation treatment has been reported. Under experimental conditions, a 7-$\log_{10}$ cycle reduction was achieved at 350-400 MPa for 10-15 minutes at ambient 25°C (Pérez *et al*., 2007).

Disinfectants / Sanitisers
Not applicable.

CLINICAL PICTURE

Incubation: Little information available.
Symptoms: Gastrointestinal symptoms such as diarrhoea. Neurological sequelae include brain liquefaction, seizures, high fever. A full review of symptoms with references is given by Gurtler *et al*., 2005.
Condition: Meningitis, septicaemia, necrotising enterocolitis.
Dose: A dose-response curve is not available because of a lack of data. Multiplication prior to consumption is required to cause illness. Approximately 1000 cells may be sufficient to cause an infection (Iversen and Forsythe, 2003). Current knowledge suggests that <3 cfu/100g in PIF followed by multiplication after reconstitution can lead to infection (FAO/WHO, 2004).
At Risk Groups: Causes disease in all age groups. However, those at greatest risk are neonates and infants <2 months old, particularly premature, low-birth weight or immuno-compromised infants (FAO/WHO, 2008). Worldwide, 9 cases of adult infection have been documented. Groups with low gastric acid secretion and absence of natural gut flora may be at more risk as the pathogen may survive stomach passage into the intestine.
Long Term Effects: Because of underreporting in most countries, the long-term effects of infection are not clear. Mental retardation and quadriplegia have been reported. Mortality rates vary, historically from 10% to 80% (since 1958), but declining to under 20% in recent years (FAO/WHO, 2004).
Treatment: Antibiotics. There is reported increased resistance to broad-spectrum penicillins and cephalosporins (Lai, 2001).

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SOURCES

Human: Has been recovered from clinical specimens of cerebrospinal fluid, blood, sputum, throat, nose, stool, gut, wounds, bone marrow, eye, ear, stomach aspirates, anal swabs and breast abscess.
Animal: Flies and rodents are reported as source and vector, although a low prevalence (0.2%) in stable flies has been reported (Mramba *et al*., 2006).
Food: First documented case linked to PIF was reported by Clark *et al.* (1990). The pasteurisation for IF manufactured using a wet-mix process must achieve a minimum 10 log unit reduction for vegetative bacteria but theoretically achieves a 50-80 log unit reduction (FAO/WHO, 2008). This suggests that contamination occurs post-pasteurisation. Current standards do not require PIF to be sterile, but liquid ready-to-feed infant formula is commercially sterile. In 50-80% of cases, PIF is both the vehicle and source (direct or indirect) of illness (FAO/WHO, 2004). The ability to monitor formula has improved in recent years. Cheese products, dried herbs, spices, rice seeds, lettuce, minced beef, sausage meat and vegetables, fermented cassava, mung bean and alfalfa sprouts, and crab meat reported to contain the organism but not linked to cases.
Environment: Isolated from water, dust, soil, plant materials, mud and vacuum cleaners (Pagotto *et al.,* 2007).
Transmission Routes: Two different routes for *Cronobacter* spp. (*E. sakazakii*) to enter PIF; (1) contaminated ingredients added after drying and before packaging (intrinsic contamination) or (2) through reconstitution and handling (external contamination). No evidence of infant-to-infant transmission. Not all infants with infections have been exposed to PIF, suggesting another environmental source. A case documented in Brazil appears to be the first of mother-to-infant transmission via breast milk (Barreira *et al.,* 2003).

OUTBREAKS AND INCIDENTS

NZ Incidence: Became notifiable in 2005 following the death of a premature infant in a Waikato neonatal unit (ESR, 2007). One invasive disease case was notified in 2005; an elderly male with peritonitis who was on a renal ward (ESR, 2007). The Ministry of Health has reported that newborn twins contracted *Cronobacter* spp. (*E. sakazakii*) meningitis in the neonatal intensive care unit at National Women's Hospital in 1991. Both babies survived but one suffered brain damage and spastic quadriplegia (MoH, 2005).
Overseas incidence: A 2002 USA survey estimated the rate of infection among infants as 1/100,000 and the rate among low birth weight neonates as 8.7/100,000 (WHO 2004, citing pers. comm.). Since the first case was documented in 1958 there have been around 120 documented cases worldwide, and at least 27 deaths (to July 2008) (FAO/WHO, 2008).

Overseas outbreaks
Belgium, 1998, necrotising enterocolitis, 12 infants, 2 fatalities. All cases fed PIF (van Acker *et al.,* 2001).
Knoxville, USA, 2001, 49 infants in intensive care (Weir, 2002).
France, 2004, 9 infants, 2 fatalities (Coignard et al., 2006).

REFERENCES

Barreira ER, Costa de Souza D, de Freitas Gois P, Fernandes JC (2003) Meningite por *Enterobacter sakazakii* em recem-nascido:relato de caso. Pediatria (São Paulo); 25: 65-70.

Beuchat LR, Kim H, Gurtler JB, Lin L-C, Ryu J-H, Richards GM (2009) Cronobacter skazakii in foods and factors affecting its survival, growth, and inactivation. International Journal of Food Microbiology; 136: 204-213.

Breeuwer P, Lardeau A, Peterz M, Joosten HM (2003) Desiccation and heat tolerance of *Enterobacter sakazakii*. Journal of Applied Microbiology; 95: 967-973.

CAC, Codex Alimentarius Commission (2008) Code of hygienic practice for powdered formulae for infants and young children. CAC/RCP 66-2008. Available at: http://www.codexalimentarius.net/web/standard_list.jsp. Accessed 22 July 2010.

CCFH, Codex Committee on Food Hygiene (2009) Alinorm 09/32/13. Agenda item 4. Codex Alimentarius Commission. Available at: http://www.codexalimentarius.net/web/archives.jsp?year=09. Accessed 22 July 2010.

Clark NC, Hill BC, O'Hara CM, Steingrimsson O, Cooksey RC (1990) Epidemiologic typing of *Enterobacter sakazakii* in two neonatal nosocomical out breaks. Diagnostic Microbiology and Infectious Disease; 13: 467-472.

Coignard B, Vaillant V, Vincent JP, Leflèche A, Mariani-Kurkddijan P, Bernet C, L'Hériteau F, Sénéchal H, Grimont P, Bingen E, Desenclos JC (2006) Infections sévères à Enterobacter sakazakii chez des nouveau-nés ayant consommé une préparation en poudre pour nourrissons, France, octobre-décembre 2004. Bulletin Épidémiologique Hebdomadaire; 2-3: 10-13.

Edelson-Mammel SG, Buchanan RL (2004) Thermal inactivation of *Enterobacter sakazakii* in rehydrated infant formula. Journal of Food Protection; 67: 60-63.

Edelson-Mammel SG, Porteous MK, Buchanan RL (2005) Survival of *Enterobacter sakazakii* in a dehydrated powdered infant formula. Journal of Food Protection; 68: 1900-1902.

Edelson-Mammel S, Porteous MK, Buchanan RL (2006) Acid resistance of twelve strains of *Enterobacter sakazakii*, and the impact of habituating the cells to an acidic environment. Journal of Food Science; 71: M201-M207.

ESR (2007) Notifiable and other diseases in New Zealand. Annual Report 2006. Client Report FW0717 for the Ministry of Health. Institute of Environmental Science and Research Ltd, Porirua.

FAO/WHO (2004) *Enterobacter sakazakii* and other microorganisms in powdered infant formula. Microbiological Risk Assessment Series No. 6. Food and Agriculture Organization of the United Nations, World Health Organisation. Available at: http://www.who.int/foodsafety/publications/micro/mra6/en/. Accessed 22 July 2010.

FAO/WHO (2008) *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formulae. Microbiological risk assessment series No. 15. Food and Agriculture Organization of the United Nations, World Health Organisation. Available at: http://www.who.int/foodsafety/publications/micro/mra_followup/en/index.html. Accessed 22 July 2010.

Gurtler JB, Kornacki JL, Beuchat LR (2005) *Enterobacter sakazakii*: A coliform of increased concern to infant health. International Journal of Food Microbiology; 104: 1-34.

Gurtler JB, Beuchat LR (2007a) Growth of *Enterobacter sakazaskii* in reconstituted infant formula as affected by composition and temperature. Journal of Food Protection; 70: 2095-2103.

Gurtler JB, Beuchat LR (2007b) Survival of *Enterobacter sakazakii* in powdered infant formula as affected by composition, water activity, and temperature. Journal of Food Protection; 70: 1579-1586.

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

Iversen C, Lane M, Forsythe SJ (2004) The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. Letters in Applied Microbiology; 38: 378-382.

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

JEMRA, Joint FAO/WHO Expert Meetings on Microbiological Risk Assessments (2007). Risk Assessment for *Cronobacter sakazakii* in powdered infant formula. Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment. Available at: http://www.mramodels.org/ESAK/default.aspx. Accessed 27 July 2010.

Kandhai MC, Reij MW, Grognou C, van Schothorst M, Gorris LGM, Zwietering MH (2006) Effects of pre-culturing conditions on lag time and specific growth rate of *Enterobacter sakazakii* in reconstituted infant formula. Applied and Environmental Microbiology; 72: 2721-2729.

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

Lenati RF, O'Connor DL, Hébert KC, Farber JM, Pagotto FJ (2008) Growth and survival of *Enterobacter sakazakii* in human breast milk with and without fortifiers as compared to powdered infant formula. International Journal of Food Microbiology; 122: 171-179.

Lambert JW, Bidlas E (2007) A study of the gamma hypothesis: predictive modelling of the growth and inhibition of the *Enterobacter sakazakii.* International Journal of food Microbiology; 115:204-213.

Mramba F, Broce A, Zurek L (2006) Isolation of *Enterobacter sakazakii* from stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae). Journal of Food Protection; 69: 671-673.

MoH, Ministry of Health (2008a) Food and Nutrition Guidelines for Healthy Infants and Toddlers (aged 0-2). A background paper. Available at: http://www.moh.govt.nz/moh.nsf/indexmh/0-2-food-and-nutrition-guidelines-may2008. Accessed 22 July 2010.

MoH, Ministry of Health (2008b) Eating for Healthy Babies and Toddlers: From birth to 2 years old. Available at: http://www.healthed.govt.nz/uploads/docs/HE1521.pdf. Accessed 22 July 2010.

MoH, Ministry of Health (2005) Recommendation to make *E. sakazakii* meningitis notifiable reinforced by historical cases. Press release 7 April 2005, Ministry of Health, Wellington.

Nazarowec-White M, Farber JM (1997) Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. Letters in Applied Microbiology; 24: 9-13.

Nazarowec-White M (1998) Biological characterisation of *Enterobacter sakazakii.* PhD thesis, University of Ottawa. Available at: http://www.collectionscanada.gc.ca/obj/s4/f2/dsk2/ftp03/NQ36785.pdf. Accessed 22 July 2010.

Osaili TM, Shaker RR, Al-Haddaq MS, Al-Nabulsi AA, Holley RA (2009) Heat resistance of *Cronobacter* species (*Enterobacter sakazakii*) in milk and special feeding formula. Journal of Applied Microbiology; 107: 928-935.

Pagotto FJ, Lenati RF, Farber JM (2007) *Enterobacter sakazakii*. In: Doyle MP and Beuchat LR (eds.) Food Microbiology Fundamentals and frontiers. Third edition. ASM Press, Washington, USA.

Pérez PMC, Aliaga RD, Reyes SD, Lopez MA (2007) Pressure inactivation kinetics of *Enterobacter sakazakii* in infant formula milk. Journal of Food Protection; 70: 2281-2289.

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

Weir E (2002) Powdered infant formula and fatal infection with *Enterobacter sakazakii.* Canadian Medical Association Journal; 166: 1570.