codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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RISK PROFILE OF *ENTEROBACTER SAKAZAKII* AND OTHER MICROORGANISMS IN POWDERED INFANT FORMULA

Prepared by the United States of America and Canada

INTRODUCTION

The United States and Canada request that an additional item be considered during the 36th Session of CCFH under the Agenda Item for Other Business and Future Work.

During the 24th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), the subject of pathogens in infant formulas was raised. It was recommended that CCNFSDU submit a request asking CCFH to revise the Recommended International Code of Hygienic Practice for Foods for Infants and Children (CAC/RCP) 21-1979). The requested revisions would address, among other things, concerns with pathogens in infant formula, including *Enterobacter sakazakii*. Delegates and Observers at the 24th Session of CCNFSDU overwhelmingly supported this recommendation (Alinorm 03/26A, paragraphs 132-134).

With regard to CCFH, the Committee at its 33rd session generally recognized the necessity of revising the Code for Egg and Egg Products and the Code for Foods for Infants and Children (Alinorm 01/13A, paragraph 150). Work has been initiated for the revision of the Code for Egg and Egg Products, however, no work has yet been initiated for the revision of the Code for Foods for Infant and Children.

At its 35th Session, CCFH requested that the United States, in consultation with Canada, update the risk profile described above. The purpose of the update is to include any new information related to *E. sakazakii*, since the initial writing of the risk profile. Additionally, the update is to expand the risk profile to address "other pathogens of concern that may be present in powdered infant formula, including *Clostridium botulinum*, *S. aureus*, and other types of *Enterobacter* ….."(17).

BACKGROUND

Enterobacter sakazakii has been associated with a variety of severe and life-threatening conditions including meningitis, bacteremia, and necrotizing enterocolitis, especially in neonates and infants. The organism is a gram-negative rod within the family Enterobacteriaceae, genus *Enterobacter.* It was called "yellow-pigmented *Enterobacter cloacae* until 1980 when it was renamed *Enterobacter sakazakii*. The first two known cases of meningitis were reported in 1961 by Urmenyi et al. (1). Subsequently, cases of meningitis, septicemia, and necrotizing enterocolitis due to *E. sakazakii* have been reported worldwide. While the overall frequency of *E. sakazakii* infections appears to be low, the consequences can be dire. Although most reported cases have involved infants, reports have described infections in children and adults as well (2). Overall, reported case-fatality rates have varied considerably with rates as high as 50 percent in some instances. Infections from *E. sakazakii* have occurred as both sporadic cases and as outbreaks. It is the latter that has lead to the link with powdered infant formula, especially in the context of neonatal intensive care settings (3-6).

SCOPE AND RATIONALE

A number of outbreaks that have resulted in serious adverse health consequences and death underscore the need to better manage the risk of *E. sakazakii* in powdered infant formula. While it is not clear if powdered infant formula is the only source of *E. sakazakii* which leads to infection in infants and neonates, it is one source that is well documented and in need of an appropriate risk management strategy. There are many gaps in our knowledge of *E. sakazakii* including a better understanding of the at-risk population, the exposure vehicle, the infectious dose, host factors contributing to susceptibility and the molecular pathogenesis of the organism itself. However, *E. sakazakii* is known to be present in a proportion of powdered infant formula, such formula has been epidemiologically linked with illness in neonates, and the disease may be life threatening. That alone is enough to seriously consider appropriate strategies to reduce this documented risk.

PATHOGEN-FOOD COMMODITY COMBINATION(S) OF CONCERN

Pathogen of concern: *Enterobacter sakazakii*

Description of the food or food product and/or condition of its use with which problems due to this pathogen have been associated:

Powdered infant formula is the food item that has been linked with *E. sakazakii* infections. In 1988 Muytjens et al (7) reported recovery of Enterobacteriaceae from >50% of 141 dry infant formula powder products from 35 countries. *E. sakazakii* was recovered from 20 (14%). All were in compliance with Codex Alimentarius since the concentration of the organisms did not exceed 1 colony-forming unit/gram dry powder. More recently, Nazarowec-White and Farber (8) studied the incidence of *E. sakazakii* in powdered infant formula obtained from five different manufacturers that sell at the retail level on the Canadian market. A total of 120 samples (cans) from different lots manufactured on different days were obtained and evaluated. *E. sakazakii* was cultured from 8 cans of product with levels in positive samples averaging 0.36 CFU/100g. There have been a number of outbreaks of neonatal *E. sakazakii* infection attributed to powdered infant formula in which identical organisms were isolated from ill neonates and previously unopened containers of formula (see below).

DESCRIPTION OF THE PATHOGEN AND PUBLIC HEALTH PROBLEM

THE PATHOGEN

Enterobacter sakazakii is a gram-negative rod within the family Enterobacteriaceae, genus *Enterobacter.* It was called "yellow-pigmented *Enterobacter cloacae* until 1980 when it was renamed *Enterobacter sakazakii*. Little is known about specific virulence mechanisms but the organism appears to have a propensity to infect the central nervous system to cause meningitis, cysts or brain abscess. Subsequent developmental delay and hydrocephalus is a well-recognized sequela (2). *Enterobacter sakazakii* has also

been associated with necrotizing enterocolitis in at least one outbreak in Europe (4). In relation to thermal tolerance, Nazarowec-White and Farber (9) reported ten Canadian *E. sakazakii* strains (5 clinical and 5 food isolates) in which they determined the heat resistance at 52, 54, 56, 58 and 60 °C in reconstituted dried-infant formula. D-values of 54.8, 23.7, 10.3, 4.2 and 2.5 min were obtained for each temperature, respectively. The overall calculated z-value was 5.82 °C. They concluded that in a comparison with D-values of several members of the Enterobacteriaceae in dairy products, *E. sakazakii* appeared to be one of the most thermotolerant organisms. A notion supported by at least one report of the isolation of *E. sakazakii* from thermal springs (10).

THE PUBLIC HEALTH PROBLEM

E. sakazakii has been isolated from a variety of sterile sites including blood and cerebrospinal fluid in humans with clinical conditions consistent with Gram-negative infections. While *E. sakazakii* has caused disease in all age groups the majority are in infants less than 2 months old. There are approximately 50 cases reported in infants less than 60 days old. Data on these infants is incomplete but what is available indicates that approximately three quarters of them had a birth weight of <2500 g, and three quarters were premature being born at <37 weeks gestation. One of the key questions is the susceptibility of term infants. There have been cases reported in term infants, and while some have major congenital abnormalities (e.g. neural tube defects, Downs syndrome), others have no reported evidence of compromised host defence yet have been afflicted with *E. sakazakii* sepsis or meningitis (2).

Mortality rates from *E. sakazakii* infection have been reported to be >50% but this figure has declined to <20% in recent years. While the disease is usually responsive to antibiotic therapy, a number of authors have reported increasing antibiotic resistance that are commonly used for initial treatment of suspected *Enterobacter* infection. Reports have also been made of β-lactamases and cephalosporinase from *E. sakazakii* (11). Even in the context of sensitive organisms long-term neurological sequelae are well recognized (2, 12).

While the reservoir for *E. sakazakii* in many cases is unknown, a growing number of reports have suggested a role for powdered milk infant formula as a vehicle for infection (3-6). In several investigations of outbreaks of *E. sakazakii* infection that occurred among neonates in neonatal intensive care units, investigators were able to show that the strain of *E. sakazakii* recovered from the ill neonates was indistinguishable from the strain recovered from unopened cans of infant formula used to feed the neonates (4-6). These reports strongly suggest that the infant formula contaminated intrinsically with *E. sakazakii* served as the source of infection for the neonates that subsequently became ill.

In addition to laboratory evidence corroborating contaminated infant formula as a source of infections in outbreaks of illness, epidemiologic associations subsequently confirmed by the laboratory have been established as well. Three cases of neonatal infection caused by *Enterobacter sakazakii* are reported from Iceland (5, 12). These infections occurred during a 9-month period in 1986 and 1987. Two of the neonates, who were normal at birth, survived but were left with brain damage. The third, which had Down's syndrome and severe cardiac malformations, died. *E. sakazakii* was not isolated from any environmental sources in the neonatal wards or in the milk kitchen, but it was grown from several lots of the powdered-milk formula used in the hospital. The *E. sakazakii* strains isolated from the neonates were indistinguishable from 22 strains grown from the formula. A combination of typing methods (plasmid analysis, antibiograms, chromosomal restriction endonuclease analysis, ribotyping, and multilocus enzyme electrophoresis) were used to evaluate the isolates from each outbreak as to their relatedness. The typing results differed among outbreaks, but in each one, patient and formula isolates shared the same typing pattern. The only exceptions were disk antibiograms, which often varied among colonies selected from each of the isolates. Plasmid analysis, chromosomal restriction endonuclease analysis, ribotyping, and multilocus enzyme electrophoresis all were effective as epidemiological typing methods for *E. sakazakii*, especially when used in combination. By using this typing scheme, the authors confirmed that *E. sakazakii* from intrinsically contaminated dried infant formula was the source of neonatal infection (12).

Simmons et al (3) reported an outbreak of *E. sakazakii* infection and colonization in neonates related to an infant formula contaminated during the manufacturing process. The outbreak occurred in a 20-bed neonatal intensive care unit during a six-week period in 1988, and involved a total of four infants. Three infants had

sepsis and three had bloody diarrhea; all patients responded to intravenous antibiotics and recovered without complications. The *E. sakazakii* isolated from the formula had the same plasmid and multilocus enzyme profile as those isolated from patients.

Van Acker et al (4) described an outbreak of necrotizing enterocolitis that occurred in a neonatal intensive care unit. A total of 12 neonates developed NEC in June-July 1998. For two of them, twin brothers, the NEC turned out to be fatal. *E. sakazakii* was isolated from a stomach aspirate, anal swab, and/or blood sample for 6 of the 12 neonates. A review of feeding procedures revealed that 10 of the 12 patients were fed orally with the same brand of powdered milk formula. *E. sakazakii* was isolated from the implicated prepared formula milk as well as from several unopened cans of a single batch. Molecular typing by arbitrarily primed PCR (AP-PCR) confirmed, although partially, strain similarity between milk and patient isolates. The investigators described what turned out to be an inadvertent re-challenge test involving the formula implicated as the cause of the outbreak. This occurred after a decision was made to discontinue the use of that particular formula in the neonatal intensive care unit on 10 July 1998 immediately after the investigators suspected a possible link between that formula, *E. sakazakii*, and development of necrotizing enterocolitis. However, because their initial cultures demonstrated the presence of *E. sakazakii* only in prepared milk and not in original powder, the formula was released again on 20 July 1998. One patient given the released formula developed symptoms of necrotizing enterocolitis on 23 July 1998, and *E. sakazakii* was isolated from her stomach aspirate and anal swab. At the same time, further cultures demonstrated the intrinsic contamination of the powdered milk with *E. sakazakii*, including a matching molecular profile of the *E. sakazakii* recovered from this new case and from the powder obtained from an unopened can. From then on, feeding with that formula was suspended and no further cases of necrotizing enterocolitis occurred.

The van Acker report (4) is also noteworthy because it demonstrated that relatively low levels of *E. sakazakii* were present in the samples of powdered milk formula that were implicated as the cause of the outbreak. For example, the manufacturer's microbiological quality control data for the batch of formula implicated in the outbreak showed that, of the five samples analyzed, one yielded 20 coliforms/g whereas in the other four samples less than 1 coliform/g was found. These results fulfilled the requirements of the Codex Alimentarius (a minimum of four of five control samples with <3 coliforms/g and a maximum of one of five control samples with >3 but $\leq$20 coliforms/g) (7) but not the requirements of Belgian law (i.e., < 1 coliform/g in all control samples) (4). After the incident, the product facility was upgraded, appropriate hygienic measures were taken, and more stringent release norms for dietetic specialties (<0.3 coliform/g, 0 *E. sakazakii* isolates/10 g) were applied by the manufacturer.

A more recent outbreak of colonization and infection with *E. sakazakii* occurred in a neonatal intensive unit in Tennessee in 2001 (6). In the Tennessee outbreak, the investigators demonstrated a statistically significant association between *E. sakazakii* colonization/infection and powdered milk formula ingestion. Specifically, in that study, the investigators showed that nine of the nine infants who were infected/colonized with *E. sakazakii* had been fed a specific formula product compared to 21 of 40 infants who were not infected/colonized with *E. sakazakii* (P =0.008).

The recovery of *E. sakazakii* from samples of powdered infant formula has been reported in at least one survey of commercially produced formulas. For example, in an examination of a total of 141 different powdered formulas obtained in 35 countries for the presence of members of the *Enterobacteriaceae*, Muytjens et al. (7) cultured *E. sakazakii* from 20 formulas (14% of the 141 formulas); the formulas from which *E. sakazakii* was recovered were available in 13 countries and made by a number of different manufacturers. It is important to note that all of the formula tested met the FAO (1977) recommendation for bacterial coliform count in powdered infant formula (less than 3 CFU/g). Interestingly, however, the investigators speculated that these powdered formulas had the potential to serve as a reservoir for future outbreaks. It has been suggested that the high thermal resistance of *Enterobacter* spp. in comparison to other members of the *Enterobacteriaceae* can possibly explain their high prevalence in powdered and prepared formula milk (9).

While the above examples have discussed intrinsic contamination of powdered infant formula with *E. sakazakii,* extrinsic contamination has also been associated with disease in infants. In one instance a blender used in rehydration and rehydrated formula were found to be contaminated with *E. sakazakii* (13). In another instance a cracked contaminated blender used to prepare formula from dry powder was implicated in

an outbreak that involved two infants. These included one case of sepsis and meningitis complicated by cerebral infarction, and one case of sepsis. In addition, three cases of intestinal colonization were identified (14, 15).

FOOD PRODUCTION, PROCESSING, DISTRIBUTION AND CONSUMPTION

Powdered infant formula, which is not a sterile product, is the food commodity of interest for this risk profile. *E. sakazakii* is considered to be an environmental organism, and as such is likely to be present in both manufacturing facilities as well as domestic situations. Molecular epidemiology has clearly demonstrated that *E. sakazakii* present in powdered formula has caused serious human illness. It is unclear at what stage in the manufacturing process the organisms get into powdered formula. In some instances however, the contamination appears to have arisen from equipment used to prepare the formula in milk kitchens.

Little is known about the growth rates of different *E. sakazakii* isolates in formula, but it is likely that they grow readily in formula held at room temperature for prolonged periods. Nazarowec-White and Farber (8) reported that minimum growth temperatures for *E. sakazakii* in culture media varied from 5.5°C to 8°C; however, no growth occurred at 4°C. These authors also reported that generation times for *E. sakazakii* at 10°C varied from 4.18 to 5.52 h. They concluded that due to its relatively short lag time and generation time, even low levels of *E. sakazakii* may pose a safety concern. Hence, improper storage of reconstituted powdered infant formula at ambient temperature e.g., on a bedside table for night feeding, may permit the growth of *E. sakazakii*. Lack of specific information related to infectious dose and rate of growth of the organism makes it difficult to determine the adverse health consequences of a specific level of contamination of powdered formula. Several approaches have been suggested to minimize the risk from using powdered infant formula. These include: preparing only a small amount of reconstituted formula for each feeding to reduce the quantity and time that formula is held at room temperature for consumption; Minimizing the holding time, whether at room temperature or while under refrigeration, before a reconstituted formula is fed and; Minimizing the "hang-time" (i.e., the amount of time a formula is at room temperature in the feeding bag and accompanying lines during enteral tube feeding). Some have suggested using heated water to reconstitute the formula, but this has raised questions regarding safety to the infant (or handler if boiling water is used), destruction of nutrients and unknowns regarding thermal tolerance of the organism.

There is a paucity of data regarding the microbial ecology of *E. sakazakii*. Muytjens and Kollee reported that they were not able to isolate the organism from surface water, soil, mud, rotting wood, grain, bird dung, rodents, domestic animals, cattle or raw cow's milk (16). So the precise environmental niche for *E. sakazakii* remains unclear.

OTHER RISK PROFILE ELEMENTS

Published reports of *E. sakazakii* infections are largely from developed nations, and even there a significant level of underreporting of infections is likely. The lack of reports from developing countries is likely due to lack of recognition of the problem rather than there being no illness. Arguably the problem may be even greater in developing countries where cleaning and maintaining equipment poses a greater problem both in manufacturing facilities as well as hospitals. Also the numbers of susceptible infants is likely to be greater in developing countries.

RISK ASSESSMENT NEEDS AND QUESTIONS (UPDATED)

The key needs in relation to the risk posed by the presence of *E. sakazakii* in infant formula are as follows:

- Are there susceptible populations to *E. sakazakii* from powdered formula, and if so what are those populations?
- What is an acceptable level of *E. sakazakii* contamination of powdered infant formula? Does this vary depending on the age or immune status of the consumer?
- What are the appropriate risk management strategies to control *E. sakazakii* in manufacturing facilities, in the hospital or at home?

- What is the contribution of hospital practices to contamination (primary or additional) of the prepared product?
- What is the contribution to infectious risk of the feeding practices and delivery systems for prepared formula?
- What is the contribution to infectious risk of powdered human milk fortifier?

At the current time there is insufficient scientific knowledge to perform a quantitative risk assessment. However, this emerging food safety public health concern would benefit from a formal evaluation of the risks associated with this pathogen-product pair including a consideration of available control measures and their likely effectiveness for improving public health.

AVAILABLE INFORMATION AND MAJOR KNOWLEDGE GAPS

Information regarding *E. sakazakii* is limited to a relatively small number of case reports of sporadic cases and outbreaks. Currently there is no active surveillance for *E. sakazakii.* There has been no risk assessment undertaken for *E. sakazakii* in powdered infant formula. While there are many major knowledge gaps, some of which have already been mentioned, the following are some of the more important ones:

- What are the susceptible infant populations to *E. sakazakii* in infant formula?
- What are the differences in virulence, thermal tolerance, growth kinetics between different *E. sakazakii* isolates.
- What is the infectious dose of *E. sakazakii* and how does this vary between susceptible populations?

CONCLUSIONS

E. sakazakii is an emerging infection that has clearly been linked with the consumption of contaminated powdered infant formula. The illness caused by *E. sakazakii* is often severe and life threatening with significant long-term sequelae in those who recover, particularly if the infection involves the central nervous system. The risk of potentially fatal infections appears to be highest for neonates in hospital settings, especially if low birth weight and, or immunocompromised. While the risk may diminish for older infants, reports in the literature indicate that there is still some degree of risk to this older population from consumption of powder formula containing *E. sakazakii.* Other than patient susceptibility, factors about which little is known that may contribute to the risk include the level of contamination in the formula, thermal stability, rate of bacterial growth, infectious dose and virulence of the pathogen. Powdered formula is not a sterile product and risk management strategies have to be developed in order address the presence of *E. sakazakii* in this product.

RECOMMENDED RISK MANAGEMENT ACTIONS

Considering the current state of knowledge related to this emerging foodborne pathogen, it is recommended that the Codex Committee on Food Hygiene undertake the following risk management activities.

- CCFH designate the Code for Foods for Infants and Children as the next code of practice that should be updated and request permission from the Codex Alimentarius Commission to initiate this new work. CCFH establish a working group to draft the revised code of practice for consideration at the next CCFH meeting. Revision of the Code should take into full account the issue of *E. sakazakii* in dried infant formula.
- CCFH should request that FAO/WHO undertake an expert consultation to articulate the current state of scientific knowledge related to *E. sakazakii* and identify and evaluate potential risk reduction strategies. To the extent possible, the consultation should address impact of this emerging pathogen within a risk analysis framework, including the areas identified in the sections on Risk Assessment Needs and Available Information; however a detailed quantitative microbial risk assessment is not mandatory at the current time.

- CCFH should encourage member nations and international health agencies to increase both their surveillance and research activities related to this microorganism.

UPDATE OF THE RISK PROFILE OF *E. SAKAZAKII* IN POWDERED INFANT FORMULA (FOLLOWING THE 35TH SESSION OF THE CCFH)

At its 35th Session, 27 January-1February 2003, CCFH requested that the United States, in consultation with Canada, update the risk profile described above. The purpose of the update is to include any new information related to *E. sakazakii*, since the initial writing of the risk profile. Additionally, the update is to expand the risk profile to address "other pathogens of concern that may be present in powdered infant formula, including *Clostridium botulinum*, *S. aureus*, and other types of *Enterobacter* ….."(17).

The United States Food and Drug Administration Food Advisory Committee *Meeting on Enterobacter sakazakii Contamination in Powdered Infant Formula* was held 18-19 March 2003. The public meeting included experts with backgrounds in pediatrics, epidemiology, gastroenterology, nutrition, and microbiology, as well as representatives of industry and consumer groups. Complete transcripts of the meeting and slide presentations are available on the FDA web site (www.fda.gov/ohrms/dockets/ac/cfsan03.html). A copy of the Summary Minutes (Annex 1) and the Table, Line-List of *Enterobacter sakazakii* Infection in Infants Reported in the Peer-Reviewed English Literature (Annex 2) are attached.

The Advisory Committee concluded:

> "*Yes, there is a risk [of E. sakzakii illness due to the consumption of contaminated powdered infant formula]. Populations at risk are preterm infants born at less then 36 weeks gestational age up to a post-term age of 4 to 6 weeks, immunocompromised infants at any age, and term infants hospitalized in level 2 and level 3 neonatal intensive care units (NICUs). Every effort should be made to avoid feeding powdered infant formula to these at-risk infants. Use of powdered products for these at-risk infants should be considered only when no appropriate liquid product is available.*
>
> *There is probably a low, but as yet unquantified, risk in healthy term infants, which cannot be described with data available at this time."*

The Committee went further to suggest intervention strategies in the manufacturing process, including prerequisite programs to assure the microbial quality of raw material, hygienic design and maintenance of equipment, and enhanced HACCP programs with verification. It also encouraged the development of a microbial testing program and the preparation of educational documents to be attached to formula products and targeted to health care providers for at-risk infants. It stated, "Available information is insufficient to permit specification of an allowable lower level of microbial detection of *E. sakazakii* in powdered infant formula." The Committee concluded by identifying a list of knowledge gaps and research priorities needed to better address the public health problem in the US.

Since January 2003, the US Centers for Disease Control and Prevention provided consultation for the investigation of three sporadic *E. sakazakii* infections in infants. Although powdered infant formula was not confirmed as the vehicle of transmission for these cases, neither could it be ruled out. An opened container of infant formula associated with one case yielded at least four *E. sakazakii* strains as determined by distinct pulse-field gel electrophoresis patterns of selected colonies. Two cases were unusual in that both infants were living at home, and one 8-month-old was a previously healthy term infant (C. Braden, CDC, personal communication). The Centers for Disease Control and Prevention is establishing a collection of *E. sakazakii* clinical isolates and a database of associated pulsed field gel electrophoresis patterns. This collection and database is shared with the FDA laboratory responsible for culture and pulse-field gel electrophoresis of *E. sakazakii* from infant formula.

The United States Food and Drug Administration also presented data at the Advisory Committee meeting from a field survey of powdered infant formula producers. Twenty-two finished product samples were taken, and five (22.7%) were positive at the lowest limit of quantitation (0.3 CFU/100 g). In addition to the finished product samples, ingredients were also cultured, and one of 38 carbohydrate samples (2.6%) and one

of 31 protein samples (3.2%) were positive. There was no correlation of a positive result with the method of manufacture, i.e., wet mixing-spray drying versus dry blending, or with the product type, soy versus milk.

A recently published risk profile of *E. sakazakii* as "an emergent pathogen associated with infant milk formula", presents a good review of the literature and generally agrees with this updated document and with most of the conclusions of the FDA Advisory Committee. This "*Viewpoint"* article, however, goes beyond the available data in discussing infectious dose and suggested sampling plans, and does not address pathogens other than *E. sakazakii* (17a).

OTHER MICROBIOLOGICAL CONCERNS

CONTAMINATION DURING MANUFACTURING

Current Codex microbiological specifications for powdered infant formula allow certain limits for coliforms which includes *E. sakazakii* (n=5, c=1, m<3, M=20) (18). These specifications apply to all members of the family *Enterobacteriaceae* capable of fermenting lactose and resistant to bile salts, which, like *E. sakazakii*, have been considered non pathogens or "opportunistic pathogens" in the past. However, there are an increasing number of reports demonstrating the ability of *E. agglomerans*, *Hafnia alvia*, *Klebsiella pneumoniae*, *Citrobacter koseri (diversus),* and *C. freundii*, to cause invasive disease in neonates and young infants. Most of this literature consists of case reports describing clinical symptoms and some characteristics of the microorganism but does not address the potential for foodborne disease. One outbreak of *C. freundii* infections in a neonatal intensive care unit did identify infant formula as the vehicle of transmission within the nursery. However, it is unclear how the food became contaminated (19). As with *E. sakazakii*, there is at least one report of an invasive *C. koseri (diversus)* infection in a previously healthy term infant, who had been at home for approximately 4 weeks (20).

Although the current Codex specifications allow limits for coliforms and mesophilic aerobic bacteria (n=5, c=2, m=1,000, M=10,000), there are no recognized safe levels for pathogens such as *Salmonella* (n=60, c=0, m=0). Historically, *Salmonella*-contaminated dried milk products caused outbreaks of illness as early as the 1950's in the United Kingdom and Bulgaria (21). A 1966 multi-state outbreak of *Salmonella* Newbrunswick infections in the United States, primarily in infants, occurred over a ten month period and was eventually linked to consumption of instant nonfat milk (22). The outbreak was recognized through a retrospective analysis of *Salmonella* surveillance data that showed a startling increase in the number of reported salmonellosis cases due to this serotype that had been rarely isolated from human specimens. Twenty-five primary cases were identified in 17 states across the United States from April 1965 through January 1966. Twelve patients were under one year of age, while other cases were distributed throughout all age groups. The epidemiology suggested the product was distributed nationwide, was consumed largely by infants, had a long shelf life (or was continuously contaminated), and that, due to the relatively small number of cases, there was a low level or sporadic contamination of the product. The outbreak investigation ultimately linked the illnesses with consumption of dried milk predominately from one manufacturer. Subsequently, *S.* Newbrunswick was isolated from unopened containers, the manufacturing plant environment, and other milk products on the premises. Investigators concluded that the spray drier and instantizing system presented several cleaning problems, an issue raised during the FDA Foods Advisory Committee Meeting in connection with *E. sakazakii* contamination of powdered infant formula.

In a situation similar to the *S.* Newbrunswick outbreak, a large increase in the number of reported *S.* Ealing infections was recognized during routine surveillance for salmonellosis in the United Kingdom in 1985 (23). The proportion of infants infected was substantial and cases were geographically widespread. A subsequent case-control study implicated one brand of infant formula. Of a total 76 people infected with *S.* Ealing during 1985, 48 were infants, 14 were siblings or adult contacts of infants, 2 adults had consumed other products from the implicated factory, and 12 had no known source. Independent engineers experienced in dried product preparation, reported that the manufacturing plant was "functioning correctly." However, a follow up investigation revealed *S.* Ealing in waste powder as well as several pinpoint holes, weld cracks, and a larger hole measuring 1x3 cm, in the inner lining of the spray drier. Powder recovered from the larger hole was positive for *S.* Ealing. Intensive bacteriological sampling by 33 Public Health Laboratories examined 4554 samples of 658 batches found *S.* Ealing in only 4 of 267 sealed packets. The most likely number of pathogens was 1.6 organisms per 450 g. Again, this outbreak was detected only because there

was an existing salmonellosis surveillance system which included a central laboratory, serotyping the *Salmonella* isolates, and infrequency of isolation of this serotype from humans. The other significant feature of this investigation is the very low number of salmonellae estimated to be present in the powder; this level of contamination would be difficult to detect in quality control sampling practiced at that time (50g). The article describing this outbreak also referenced to an outbreak of *S.* Bredeney infections (rare serotype) in infants in Australia, where infant formula had become contaminated through "tiny cracks in the stainless steel wall" of the spray drier, where insulation was also contaminated (24).

There continue to be published reports of *Salmonella* illnesses associated with the consumption of powdered infant formula in the 1990's. A report from CDC in 1993 describes infections with a lactose-fermenting strain of *S*. Tennessee in the United States and Canada. The atypical organism was eventually isolated from the product and the manufacturing plant (25). A published report from the Health Ministry in Spain described 48 *S.* Virchow infections in children mostly under 7 months, occurring in 14 of the 17 regions of Spain; the unique lactose-fermenting strain was also found in powdered infant formula implicated through case-control studies (26). Authors of the article concluded that detection and intervention of the outbreak would not have been possible without the National Epidemiological *Salmonella* Surveillance Network and the Spanish National Reference Laboratory for *Salmonella* and *Shigella*. Because both of these outbreaks were caused by lactose fermenting stains of *Salmonella* and that biochemical characteristic is often a key factor in identification, the reported cases are probably much lower than the actual number. In the Spanish outbreak, the powdered formula was sold only in Spain, and the lack of illnesses reported to "Salm-Net" (European surveillance system) appeared to confirm that exposure pattern.

In a 1998 publication, Threlfall, at al. report an outbreak of *S.* Anatum illnesses, involving 15 infants and 2 relatives in the UK and 2 infants in France during 1996/ early 1997 (27). As previously reported, the outbreak could only be detected through laboratory-based surveillance and targeted epidemiological investigations (through case-control studies). Additionally, this outbreak employed molecular subtyping to more specifically identify the epidemic strain and rapid communication/collaboration through the Salm-Net network to identify an international outbreak.

It is clear from these reports published in the peer-reviewed literature, that the problem of *Salmonella* contamination of powdered infant formula is extremely difficult to detect in all respects:

(a) Clinically, there were hospitalizations and even deaths, but the majority of patients had a diarrheal illness, which might go uncultured or unreported, even in a "sophisticated children's hospital (28),

(b) Epidemiologically, the reported cases were small in number and probably would have gone unnoticed within the background of salmonellosis cases if the *Salmonella* strains had not been unique in some way,

(c) Also epidemiologically, the cases were geographically dispersed and would not have been identified without established *Salmonella* surveillance networks with laboratories capable of serotyping isolates,

(d) Food microbiological samples taken for routine monitoring processes would probably not have been adequate to detect the low numbers of sporadic contamination which appears to occur in the spray drier. {The international survey of powdered infant formula by Muytjens, et al. did not detect any *Salmonella* spp. in 141 samples of powdered infant formula from 35 countries (7)},

(e) Food microbiology laboratories could not have cultured an adequate sample of products without the targeted strategy, which requires epidemiological investigations such as case-control studies and

(f) Environmentally, it was not possible to culture the epidemic strain from the manufacturing plant until, on at least one occasion, investigators isolated the strain from waste material and then worked their way back to the spray drier source (23).

In addition to *Enterobacteriaceae*, other microorganisms have been linked with infant diarrhea and may be hazards in infant formula. The pasteurization temperatures used by most manufacturing processes would not eliminate clostridial spores introduced through contaminated ingredients. In general, this has not caused a documented problem either as a hazard in food or risk in infants with one possible exception. There is one

report of infant botulism linked to contaminated infant formula in June 2001 in the UK. The microorganism was isolated from the infant and the powdered product; the report stated that molecular "fingerprinting" of the isolates was underway (29). There are two other published reports suggesting a link between *Clostridium difficile* and *C. perfringens* infections (associated with sudden infant death syndrome and neonatal necrotizing enterocolitis) and consumption of infant formula; however, the reports do not clearly show causality or describe the type of formula or the possible route of infection (30).

Staphylococcal food poisoning is generally a self-limited gastroenteritis and outbreaks were historically associated with dried milk and dried whey products in the 1950's. Contamination was attributed to raw ingredients and abusive holding temperatures. The preformed staphylococcal enterotoxins were then able to survive the drying process (31). Based on a current search of the English language, scientific literature, *S. aureus* does not appear to be a public health problem associated with powdered infant formula.

Although potential problems with clostridia and staphylococci (and/or their toxins) are far less defined than those for *Enterobacteriaceae*, more information and monitoring of ingredients may be warranted.

POST MANUFACTURING CONTAMINATION

Microbial contamination can occur after the manufactured product is distributed. This can occur in the preparation kitchen of a nursery, as described above, or it can occur during the procedure of enteral feeding (32). The cause of contamination can be food handlers, contaminated water used to reconstitute the product, or the indwelling nature of the enteral tube used for feeding. All of these events have been documented and contribute to the international concerns surrounding weaning foods in general (33).

CONCLUSIONS

Since the Risk Profile of *Enterobacter sakazakii* in Powdered Infant Formula was presented at the 35th Session of CCFH, some additional information has become available and a national body of experts assembled in the US. However, the risk profile, including the Conclusions and Recommended Risk Management Actions, remain essentially the same. There is still a pressing need to obtain additional information on what public health impact *E. sakazakii* has in developing nations. This may require targeted epidemiological and microbiological studies in country or sentinel site (s).

While relatively rare, there is clear evidence, from established surveillance activities, that low level contamination of dried infant formula with various members of the *Enterobacteriaceae* (including the well known pathogen *Salmonella*) has led to cases of disease in neonates. While control of these organisms in this non-sterile food represents unique challenges to both the product manufacturer and the end user, these diseases should be largely preventable.

It is probable that risk management actions targeted for *E. sakazakii* would also address potential risks from other members of the *Enterobacteriaceae*. However, for well known pathogens such as *Salmonella* spp., the susceptible host population would likely be broader.

The use of infant formula can prevent the documented transmission of HIV from mothers who have AIDS to infants through breast feeding. It will be important to consider the risk of powdered infant formula for this potentially susceptible population as well as the risk of using sterile liquid formula, which could be vulnerable to contamination once opened. It will also be important to obtain related exposure and disease data in this setting.

Considering the difficulties in detecting dried infant formula associated microbiological problems in the developed country setting, it is extremely unlikely that it could be detected in developing countries without the resources and infrastructure to participate in laboratory-based surveillance programs.

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ANNEX 1

Contaminant and Natural Toxicants Subcommittee of the Food Advisory Committee
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
March 18-19, 2003

The Contaminants and Natural Toxicants Subcommittee ("Subcommittee") of the Food Advisory Committee convened a meeting on March 18-19, 2003 to consider *Enterobacter sakzakii* and powdered infant formula

Review of Charge and Questions, Discussion, and Responses to Questions

Charge 1: Characterize the infants at risk.

Question 1: Given available information on *E. sakazakii* and powdered infant formula, is there a risk? If so, identify the populations of infants at risk: identify infants at risk including consideration of factors, such as the extent to which immune status, age and/or general health status, etc., may impact on the susceptibility of infants to *E. sakazakii* infections.

For Charge 1, Question 1, the Subcommittee was asked to come to consensus. The committee voted unanimously to the following answer to question 1:

Yes, there is a risk. Populations at risk are preterm infants born at less than 36 weeks gestational age up to a post-term age of 4 to 6 weeks, immunocompromised infants at any age, and term infants hospitalized in level 2 and level 3 neonatal intensive care units (NICUs). Every effort should be made to avoid feeding powdered infant formula to these at-risk infants. Use of powdered products for these at-risk infants should be considered only when no appropriate liquid product is available.

There is probably a low, but as yet unquantified, risk in healthy, term infants, which cannot be described with data available at this time.

Charge 2: If there is a meaningful risk, how can this risk be addressed?

Question 1: What intervention strategies can be used in infant formula manufacturing processes and plants?

The Subcommittee developed a four-part recommendation in response to this question.

1. Intervention strategies which reduce bacterial presence in powdered infant formula should be used in manufacturing processes and plants. These include, but are not limited to, prerequisite programs to assure the microbial quality of raw materials, hygienic design and maintenance of equipment, hygienic zoning in plant design, and continuous use and improvement of HACCP programs and their verification.
2. The Subcommittee encourages the development of a microbiological sampling and testing program through joint efforts by industry and the FDA, the purpose of which is to assure greater clinical safety of this product. It would be highly desirable to formally assess the contribution of such a microbiologic testing program when added to the intervention measures described above.

3. The Subcommittee recognizes that with the currently available processing technologies for powdered infant formula, the risk for illness due to *E. sakazakii* cannot be completely eliminated for the at-risk populations specified above. Whether the additional interventional strategies described above can achieve this result is not clear.

4. Recognizing the important clinical purposes for which powdered infant formula is used among the populations most at-risk for *E. sakazakii* infection, the Subcommittee strongly encourages and enjoins the powdered infant formula manufacturers to develop product formulations which combine the

attributes of maximal infant growth promotion and microbiologic safety for use in the at-risk populations described above.

Question 2: Are there other intervention strategies? Include consideration of product labeling options for powdered infant formula (e.g., directions for preparation and use), and consider handling practices for the settings (hospitalized and non-hospitalized) in which powdered infant formula is prepared and consumed?

The Subcommittee answered Question 2 as follows:

FDA, with input from industry, should prepare educational documents to be attached to appropriate infant formula materials targeted to at-risk infants. These educational materials should alert all health-care users that powdered infant formulas are not sterile and the need for special handling, if used. The educational materials should be updated to reflect any new information that becomes available. They would be distributed through FDA outreach efforts.

Question 3: Is it possible, based on available information, to specify allowable lower levels of microbial detection of *E. sakazakii* in powdered infant formula, and do allowable levels vary by risk characteristics of the infant?

The Subcommittee answered Question 3 as follows:

Available information is insufficient to permit specification of an allowable lower level of microbial detection of *E. sakazakii* in powdered infant formula. Without knowledge for such specifications, it is not possible to answer the second part of the question.

Question 4: What are the critical knowledge gaps and research priorities relative to the need to address issues about the presence of *E. sakazakii* in powdered infant formula?

The Subcommittee compiled a list of gaps in knowledge and research needs, including the following:

1. Consider methods for post-drying inactivation of *E. sakazakii* in powdered infant formula and continued development of methods to detect E. sak
2. Continue to document occurrence of *E. sakazakii* in powdered infant formulas
3. Develop over time means, if possible, for sterilizing powdered infant formulas
4. Consider developing sterile liquid products for use with at-risk populations
5. Identify pathogenic factors, host-susceptibility factors and spectrum of disease
6. Population-based surveillance, perhaps through FoodNet, to provide denominators for incidence of *E. sakazakii* infections in infant populations
7. Assure that clinical laboratory procedures are able to isolate and identify *E. sakazakii*
8. Optimal therapy for infected infants

ANNEX 2

LINE LIST ENTEROBACTER SAKAZAKII PUBLICATIONS

Table. Line-List of Cases of *Enterobacter sakazakii* Infection in Infants Reported in the Peer-Reviewed English Literature
(A case was defined as an infant meeting at least one of the following 3 parameters:
1] *E. sakazakii* recovered from one or more of the following normally sterile specimens: blood, CSF, brain tissue;
2] Infant involved in outbreak of necrotizing enterocolitis and *E. sakazakii* recovered from blood, stool, or stomach aspirate in >1 infant; or
3] Bloody diarrhea and *E. sakazakii* recovered from stool in pure culture)

Reference	Gender	Weight at birth	Gestation (wk)	Powdered Formula	Age at onset of illness	Illness	Outcome	Country
Lancet 1961	Male	6 lb 11 oz	38	?	11 d	Meningitis	Died	England
	Female	4 lb 7 oz	32	?	5 d	Meningitis	Died	
Dan Med Bull 1965	Female	3250 g	?	?	4 d	Meningitis	Recovered*	Denmark
J Clin Microbiol 1979	Male	2600 g	Term	Yes	7 d	Bacteremia	Recovered	U.S.A.
J Clin Microbiol 1981	Female	?	Term	?	5 wk	Meningitis	Recovered*	U.S.A.
Clin Microbiol Newsl 1981	Male	"Normal pregnancy and delivery"		?	5 wk	Meningitis/Sepsis	Recovered	U.S.A.
Tijdschr Kindergeneeskd 1982	Male	1900 g	32	?	4 d	Meningoencephalitis	Died	Netherlands
	Female	1670 g	32	?	3 d	Meningoencephalits	Died	
J Clin Microbiol 1983	Male	2830 g	36	?	5 d	Meningitis	Recovered*	Netherlands
	Female	2400 g	Term	?	3 d	Meningitis	Died	
	Female	1670 g	32	?	3 d	Meningitis	Died	
	Male	1900 g	32	?	4 d	Meningitis	Died	
	Female	2690 g	Term	Yes	5 d	Meningitis	Died	
	Male	2085 g	38	?	5 d	Meningitis/NEC	Died	
	Female	1370 g	"Premature"	?	5 d	Meningitis/NEC	Died	
Female	850 g	?	?	?	9 d	Meningitis	Recovered*	
Pediatr Infect	Female	?	?	?	21 d	Meningitis	Recovered*	U.S.A.

Dis 1985								
Pediatr Infect Dis J 1988	Male	?	Term	?	4 wk	Meningitis	Recovered*	U.S.A.
	Male	2040 g	37	?	8 d	Meningitis	Recovered*	
J Clin Microbiol 1989	Male	3144 g	36	Yes	5 d	Meningitis	Recovered*	Iceland
	Male	2508 g (Down's)	Term	Yes	5 d	Meningitis	Died	
	Male	3308 g	38	Yes	5 d	Meningitis	Recovered*	
*Infect Control Hosp Epidemio*l 1989	?	780 g	28	Yes	28 d	Sepsis	Recovered	U.S.A.
	?	950 g	29.5	Yes	57 d	Sepsis	Recovered	
	?	850 g	27.5	Yes	52 d	Sepsis	Recovered	
	?	1270 g	34.5	Yes	13 d	Bloody diarrhea	Recovered	
Infection 1989	?	?	?	?	?	Meningitis	Died	Portugal
Pediatr Infect Dis J 1990	Female	?	?	Yes	6 months	Bacteremia	Recovered	U.S.A.
Pediatr Radiol 1991	Male	2520 g	35	?	2 d	Meningitis	Recovered*	U.S.A.
Klin Padiatr 1994	Male	1420 g	31	?	?	Meningitis	Recovered*	U.S.A.
Pediatr Radiol 2000	Female	3000 g	35	?	6 d	Meningitis	Recovered	U.S.A.
J Clin Microbiol 2001	Male	850 g	27	Yes	55 d	Necrotizing enterocolitis	Recovered	Belgium
	Female	1930 g	31	Yes	16 d	Necrotizing enterocolitis	Recovered	
	Male	995 g	27	Yes	40 d	Necrotizing enterocolitis	Died	
	Male	965 g	27	Yes	33 d	Necrotizing enterocolitis	Died	
	Female	815 g	29	Yes	41d	Necrotizing enterocolitis	Recovered	
	Female	1,200 g	28	Yes	22 d	Necrotizing enterocolitis	Recovered	
	Male	1,100 g	28	Yes	9 d	Necrotizing	Recovered	

						enterocolitis		
	Female	590 g	27	Yes	39 d	Necrotizing enterocolitis	Recovered	
	Female	1,350 g	31	Yes	17 d	Necrotizing enterocolitis	Recovered	
J Clin Microbiol 2001	Female	1,490 g	32	Yes	9 d	Necrotizing enterocolitis	Recovered	
	Male	1,290 g	32	Yes	7 d	Necrotizing enterocolitis	Recovered	
	Male	1,550 g	30	Yes	4 d	Necrotizing enterocolitis	Recovered	
Acta Paediatr 2001	Female**	2,155 g	36	Yes	4 d	Bacteremia, meningitis	Recovered (with VP shunts	Israel
	Female**	620 g	27	Yes	9 d	Sepsis	Recovered	
Eur J Clin Microbiol Infect Dis 2002	Female	2,720 g	Term	?	6 d	Meningitis	?	Israel
	Female	"Neonatal bacteremia in a formula-fed full-term neonate"				Bacteremia	?	
MMWR 2002	Male	1,270 g	33.5	Yes	11 d	Meningitis	Died	U.S.A.

* Indicates presence of neurologic and/or developmental-delay sequelae following infection

** These two cases also presented and discussed in publication below (Eur J Clin Microbiol Infect Dis 2002)

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