



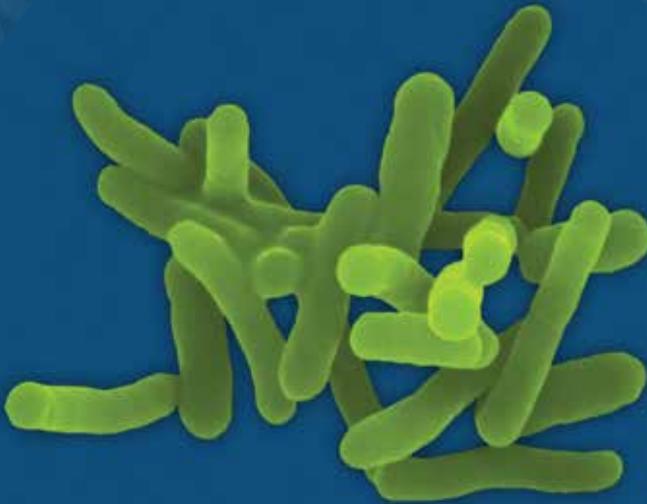
Food and Agriculture
Organization of the
United Nations



World Health
Organization

Microbial safety of lipid-based ready-to-use foods for management of moderate acute malnutrition and severe acute malnutrition

FIRST REPORT



28

MICROBIOLOGICAL RISK
ASSESSMENT SERIES

Microbial safety of lipid-based ready-to-use foods for management of moderate acute malnutrition and severe acute malnutrition

FIRST REPORT

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Abbreviations used in the text

a_w	Water activity
CCP	Critical Control Point
cfu	colony-forming unit
CoA	Certificate of Analysis
FAO	Food and Agriculture Organization of the United Nations
FUF	Follow-up formula
GHP	Good hygienic practice
GMP	Good manufacturing practice
HACCP	Hazard analysis critical and control point [system]
LNS	Lipid-based nutrient supplement
LRTI	Lower respiratory tract infection
MAM	Moderate acute malnutrition
MSF	Médecins Sans Frontières
OC	Operating characteristic
OPRP	Operational prerequisite programme
PIF	Powdered infant formula
PRP	Prerequisite programme
RUF	Ready-to-use food for the management of moderate acute malnutrition and severe acute malnutrition
RUSF	Ready-to-use supplementary food for the management of moderate acute malnutrition
RUTF	Ready-to-use therapeutic food for the management of severe acute malnutrition
SAM	Severe acute malnutrition
UNICEF	United Nations Children's Fund
UTI	Urinary tract infection
WFP	World Food Programme
WHO	World Health Organization

Executive summary

Lipid-based ready-to-use foods (RUF) for the nutritional management of moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) are low-moisture foods (LMF) provided to children from 6 months to 59 months of age within the context of emergency feeding programmes supervised by governments, the World Food Programme (WFP), the United Nations Children's Fund (UNICEF), Médecins Sans Frontières (MSF), and other non-governmental organizations. Microbiological purchase specifications for RUF include criteria for Enterobacteriaceae, *Salmonella*, *Cronobacter* species, mesophilic aerobic bacteria, coliforms, and *Listeria* species.

In 2012, UNICEF and WFP found *Cronobacter* species in certain lots of lipid-based RUF. At the request of WFP and UNICEF, FAO and WHO convened a technical meeting in FAO headquarters, Rome, Italy, from 11 to 14 December 2012, to help formulate a science-based response to this finding and to provide guidance on appropriate microbiological specifications to include among other purchase requirements to enhance the safety of lipid-based RUF. *Cronobacter* spp. are recognized foodborne pathogens of infants, and opportunistic pathogens of infants, young children, the elderly, and other health-compromised persons. *Cronobacter* spp. have been identified in a large range of foods and in the environment and an international *Cronobacter* spp. standard has been established, but only for powdered infant formula (PIF) based on the strength of evidence of a causal association between presence in reconstituted PIF and illness in infants. The meeting reviewed the information on RUF, the feeding programmes, and the characteristics of children served that was provided by WFP, UNICEF, and MSF, and the available published data on infections in the population of interest and the microbial contamination of LMF.

Lipid-based RUF provide necessary energy, fatty acids, protein and micronutrients tailored for the needs of children with MAM and SAM based on a mixture of a standardized set of ingredients and are produced by blending the water-soluble carbohydrates, vitamins and minerals into a lipid-rich paste. Thorough mixing of the dry ingredients into the viscous paste is important for product uniformity and to prevent separation during storage; applying heat during mixing facilitates the process but is limited by the risk of rancidification of the lipid components, and RUF do not achieve pasteurizing time and temperature conditions during manufacture. The low water activity (a_w) of the resulting nutrient- and energy-dense paste prevents microbial growth but does not kill contaminating pathogenic bacteria. Because no validated microbial kill step exists at present in the produc-

tion of lipid-based RUF, their microbiological safety is completely dependent on the microbial content of the ingredients and the implementation of procedures throughout manufacture to control ingress and growth of pathogens.

The safety of a food product is the responsibility of its manufacturer and is the sum of many factors, encompassing validated control measures throughout the entire chain of manufacturing. The adherence to and the effectiveness of these control measures can be verified by the application of appropriate sampling and testing relevant to the step of the process. The technical meeting expressed concern about the level of ingredient and process control demonstrated by producers of RUF, and provided guidance on sanitation and other prerequisite programs (PRPs), HACCP, and sampling strategies that would provide greater assurance of process control and product safety. The technical meeting recommended that manufacturers review their existing control programmes, including those aspects related to facility design, PRPs (e.g. environmental monitoring, GMPs, GHPs) and HACCP, with a specific focus on the hazards of concern to assure adequate process control and hygiene, specific to each facility and product.

After conducting a generalized risk assessment for RUF based on available information on RUF currently in use and the current manufacturing conditions, the microbiology and epidemiological history of other similar LMF, and the clinical and public health issues relevant to 6 month to 59 month old malnourished children, the technical meeting concluded that *Salmonella* was the pathogen of greatest concern for lipid-based RUF. The technical meeting considered it appropriate to establish a criterion for *Salmonella* in RUF but determined that there was not adequate justification at this point to establish such a criterion for other pathogens such as *Cronobacter*. The technical meeting considered that replacing the current microbiological product standard for *Cronobacter* with quantitative standards for Enterobacteriaceae would provide more information on the safety of RUF and the level of process control and overall hygienic conditions through trend analysis of quantitative Enterobacteriaceae data.

For the RUF currently on hold because of positive *Cronobacter* species tests, the technical meeting determined that retesting lots and sublots for *Cronobacter* was not necessary or helpful, and provided a rationale for 3 possible approaches the agencies might use for final disposition of lots that had been found to be contaminated with *Cronobacter* species.

For RUF to be produced in 2013-2014, the technical meeting considered it important to rapidly determine the current capacity of manufacturers to control their process relative to the presence of *Salmonella* and the levels of Enterobacteriaceae in end products, and to use the information to establish temporary mi-

microbiological criteria for lot acceptance while indicators of process control were being established and applied. Therefore, the technical meeting requested additional end-product microbiological data and, based on results obtained during the first 3 months of 2013, recommended interim end-product purchase specifications for Salmonella and for Enterobacteriaceae to be developed. The goal of the recommendations was to achieve sufficiently low levels of bacterial density in RUF to protect the intended consumers and to drive continuous improvement in production practices and conditions, but that also were generally achievable to assure the availability of an uninterrupted supply of lipid-based RUF. The technical meeting further recommended that WHO and FAO enlist another group of experts after two years to revisit the issue of the microbial safety of lipid-based RUF, conduct another risk assessment based on the accrued microbiological data on the product, newly published reports on immunology and infectious diseases in malnourished populations, and additional information on LMF, and revise the recommendations for purchase specifications.

The technical meeting also encouraged research on manufacturing processes, including those that could be validated to deliver an adequate reduction in pathogen numbers, allow development of programs of safety assurance based on verification of process control, and enable moving away from end product standards.



Background

Consistent with the need to provide safe food for young children, particularly during the complementary feeding period between 6 and 24 months and the period of rapid development to age 59 months, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened a technical meeting in FAO headquarters, Rome, Italy, from 11 to 14 December 2012 that addressed the microbial safety of ready-to-use foods (RUF) for the management of acute malnutrition. RUF may be lipid-based products packaged in sachets or pots and non-lipid-based products such as biscuits and bars. This consultation focused on the microbial safety of lipid-based RUF used to manage moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) in children aged 6 to 59 months. When greater specificity is required in this report, the lipid-based RUF used to manage MAM are referred to as RUSF and the lipid-based RUF used to manage SAM are referred to as RUTF.

The meeting was held at the request of the World Food Programme (WFP) of the United Nations, and the United Nations Children's Fund (UNICEF) to help them formulate a science-based response to the finding of *Cronobacter* spp. in lipid-based RUF and to provide guidance on appropriate microbiological specifications to include among other purchase requirements to enhance the safety of lipid-based RUF. *Cronobacter* spp. are recognized foodborne pathogens of infants, and opportunistic pathogens of infants, young children, the elderly, and other health-compromised persons, by routes of infection that are not always clear (FAO-WHO, 2004, 2006, 2008; CDC, 2012). *Cronobacter* spp. have been identified in a large range of foods and in the environment (Beuchat *et al.*, 2009; Iversen, Lane and

Forsythe, 2004). An international *Cronobacter* spp. standard has been established, but only for powdered infant formula (PIF) based on the strength of evidence of a causal association between presence in reconstituted PIF and illness in infants (CAC, 2009).

In this context the specific meeting objectives were:

- To determine the clinical significance of foodborne exposure of malnourished children between the ages of 6 months and 59 months to *Cronobacter* species in RUF at the levels of contamination identified,
- To provide guidance on the proper disposition of the lots of RUF that had been found to be contaminated, and
- To provide preliminary guidance on appropriate microbial purchase specifications used when obtaining RUF from producers.

In addressing these objectives the meeting reviewed the available information on RUF, the manner in which it is used by WFP, UNICEF and MSF in programmes for the management of MAM and SAM, and the characteristics of children served. Information on these aspects were primarily provided by WFP, UNICEF, and MSF. In addition, the available published data on infections in the population of interest and the microbial contamination of LMF were considered.

This report also documents some of the follow-up actions that were taken in response to the recommendations of the technical meeting, including an overview of the results of a snapshot study on the distribution of Salmonellae and Enterobacteriaceae in lipid-based ready-to-use products and the interim specifications recommended by FAO and WHO.”

This is the first FAO/WHO report on this issue. A follow-up second report will be published as volume 29 in this series.



Introduction

Globally, approximately 180 million children suffer from malnutrition (Black *et al.*, 2008). Rates are highest in Africa, where up to 50% of children may be affected. Of the 180 million malnourished children, an estimated 40 million children each year develop severe, acute malnutrition (SAM) (UNICEF, 2007). The diagnosis of SAM is determined by the level of oedema, the mid-upper arm circumference, and weight for height of the malnourished child. Within the category of acute malnutrition, infants and children are divided into those who are severely malnourished and have medical complications that require in-patient care and who are fed F-75¹ until well enough to receive F-100 or RUTF; those who are severely malnourished but are well enough to be treated with RUTF on an out-patient basis; and those who are moderately malnourished (MAM; children who are not as severely affected as those with SAM but with reduced weight-for-height and at risk of developing SAM) and whose locally available diets can be adequately supplemented with RUSF on an out-patient basis. While the most proximal determinants of malnutrition are diet, health, and care from nurturing adults, SAM results additionally from an array of social and environmental problems, including drought, famine, social unrest and war (Black *et al.*, 2008). Efforts to mitigate SAM, and to prevent MAM from developing into SAM, must therefore be multi-faceted (Black *et al.*, 2008; Bhutta *et al.*, 2008). It is important that caregivers for children with SAM and MAM properly engage and assist the children as they eat, after washing their own hands and the child's hands with soap (Bhutta *et al.*, 2008). To further prevent

¹ F-100 and F-75 (also known as Formula 100 and Formula 75) are therapeutic milk products designed to treat severe malnutrition. When reconstituted with 500 ml water, they provide 100 and 75 kcals/100 ml, respectively.

diarrhoea—a contributor to malnutrition and one of the leading causes of death in malnourished children—the children need an adequate supply of clean drinking water (Arnold and Colford, 2007). Additionally, for the best results, children need a safe place to live, protected from violence and the elements; appropriate health care; and social and cognitive stimulation (Black *et al.*, 2008; Walker *et al.*, 2011; UNICEF, 2007). Within this complex mix of factors critical to these children's survival and development, the FAO/WHO technical meeting was charged with addressing issues related to the microbial safety of lipid-based RUF.

Acutely malnourished children require not only optimal nutrients in the correct amounts, but these foods also should be free of pathogens and toxins, within the limits of feasibility. F-75 and F-100 are formulas based on skimmed milk powder, which are reconstituted with water before use, and fall into the category of formulas for special medical purposes, even if not restricted to use in infants. They are therefore addressed in the relevant Codex documents, principally CAC/RCP 66-2008 (CAC, 2009) and Codex Std 72-1981 (CAC, 2011), but also other related Codex documents. RUTF, like F-100, contains dried skimmed milk powder, and can be used interchangeably with F-100, depending on the malnourished child's medical condition and environment. However, RUTF is not a formula to be reconstituted with water before feeding and, therefore has specific food characteristics that require their own food safety criteria. At the same time, lipid-based RUTF and RUSF share similar ingredients; methods of production and use; and food characteristics. They therefore may share particular food safety criteria. It should be noted that for the purposes of the expert consultation, the most vulnerable consumers, those with SAM, were referred to as the most important group in the risk assessment that was conducted. Although products used for treatment and other products used for prevention of malnutrition were discussed during the meeting, the emphasis was given to lipid-based RUF used to manage acutely malnourished children, rather than products used to prevent malnutrition and the non-lipid-based products used to manage malnutrition. An overview of the range of products, their characteristic and proposed use is provided in Appendix 1.

Outcomes and sequelae of SAM can be grave. Children with SAM typically experience derangements across multiple organ systems, including nervous, gastrointestinal, immune, haematological, and integumentary. Mortality rates among children with untreated SAM are estimated to be 20–40%. Malnourished children who survive frequently fail to reach their educational, developmental and economic potentials (Black *et al.*, 2008). Children with MAM and SAM are at elevated risk of dying from diarrhoeal disease, pneumonia, malaria, measles and other diseases. In children under 5 years of age, under-nutrition is directly or indirectly responsible for an estimated 3.1 million deaths annually (Black *et al.*, 2013). A cyclical rela-

tionship exists between malnutrition, immune dysfunction, increased susceptibility to infection, and the metabolic response to infection that further alters nutritional status (Rodriguez, Cervantes and Ortiz, 2011; Golden, 1996; Schlaudecker, Steinhoff and Moore, 2011). Malnutrition increases the risk of infection and death, with acute diarrhoea and acute respiratory infections responsible for most deaths in children less than 5 years of age. Malnutrition increases the frequency and duration of diarrhoea. SAM is associated with an approximately 2-fold increase in shigellosis and in the number of days with diarrhoea (Khatuni *et al.*, 2011; Guerrant *et al.*, 1992), and there is a linear relationship between the number of days of diarrhoea in the previous 4 weeks and risk of subsequent pneumonia among malnourished, but not normal children (Schmidt *et al.*, 2009). The bacterial pathogens that cause most cases of severe acute diarrhoea include *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp. and *Escherichia coli* of various pathogenic types. Furthermore, members of the family Enterobacteriaceae, particularly *Klebsiella* spp., *E. coli* and *Salmonella* spp., are important bacterial causes of pneumonia in SAM, in addition to *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* (Rodriguez, Cervantes and Ortiz, 2011).

A recent study found that children who were randomized to a one-week course of amoxicillin or cefdinir upon enrolment in an outpatient therapeutic feeding programme for SAM in Malawi were less likely to die during several weeks of follow-up (Trehan *et al.*, 2013). While any reduction in mortality among children with SAM is welcome news, this study leaves many questions unanswered that should be addressed before adopting widespread use of antimicrobial agents in this highly vulnerable population. For example, the HIV status of children in the study was frequently unknown, and infection status could substantially confound the results of this study. Further, approximately half of the deaths in the antibiotic groups occurred more than 2 weeks after enrolment in the programme, raising the possibility of important ongoing infectious exposures through food, water or the environment. Another possible factor contributing to the late mortality in the intervention group in the Trehan study is that hosts became more vulnerable to infections in the wake of antimicrobial therapy. Rates of β -lactam antibiotic resistance among Enterobacteriaceae in Africa and worldwide are high (Kiiru *et al.*, 2012; Lu *et al.*, 2012). In fact, in a recent study in Niger, 17 (31%) of 55 children were found to harbour extended-spectrum β -lactamase-producing Enterobacteriaceae upon admission to a re-feeding centre for SAM, suggesting widespread community transmission of such resistance genes (Woerther *et al.*, 2011). When antibiotics are used in a host harbouring drug-resistant pathogens, this problem is compounded: antibiotics kill susceptible flora within a host, allowing proliferation of any drug-resistant isolates already present or introduced to the host shortly after the course of antibiotics. The host may then develop infections that are much

more difficult to treat, and may be more likely to transmit the problematic bacterial strains to others. This could influence the hazard analysis and necessary microbial safety specifications of lipid-based RUF.

2.1 LIPID-BASED READY-TO-USE FOODS FOR MANAGEMENT OF MODERATE ACUTE MALNUTRITION AND SEVERE ACUTE MALNUTRITION

Lipid-based RUF for the management of MAM and SAM are produced by mixing water-soluble carbohydrates, vitamins and minerals into a lipid-rich paste (Figure 1—Product flow figure for RUF) (Manary, 2006). Particle size and thorough mixing of dry ingredients into the viscous paste are important for product uniformity and to prevent separation during storage; applying heat during mixing facilitates the process but is limited by the risk of rancidification of the lipid components. The low water activity (a_w) of the resulting nutrient- and energy-dense paste minimizes opportunities for microbial growth, and nitrogen-flushed sachets have a two-year shelf-life.

Lipid-based RUSF include brands such as PlumpySup™; eeZeeRUSF™; and AchaMum™. They are used for community-based management of MAM, for preventive blanket feeding, and as a vehicle for micronutrient supplementation. Lipid-based RUSF are designed to provide between a quarter to half of a child's energy requirements and 100% of their micronutrient needs, but are not intended to be a sole-source food. Lipid-based RUTF include brands such as PlumpyNut™; eeZeePaste NUT™; MANA™ RUTF; NuttyButter™ and Instapaste™ in addition to a number of generic RUTF brands. These are used in a community-based setting to treat SAM in children without medical complications during the outpatient (rehabilitation) phase of treatment, and to provide 100% of the energy and micronutrient requirements of 6- to 59-month-old infants and children. RUTF is fed for a period of between 4 and 8 weeks. RUTF facilitates community management of SAM, and is part of a clinical protocol that includes regular health examinations, amoxicillin, anti-malarial drugs, vitamin A, anthelmintic drugs and measles vaccination.

While no studies have been conducted that demonstrate a pattern of unintended use, it is recognized that within-family sharing of RUF occurs. This practice is believed to increase as the health of the child improves, and is more common with RUSF than RUTF. Sometimes RUF are found for sale in local markets. More importantly with respect to foodborne infections, recommended hygiene during consumption may not be followed and frequently the access to a supply of safe water can be inadequate (Dorion *et al.*, 2012). Performance indicators demonstrate the

success of the approach used to treat SAM, with the 20–40% mortality in untreated SAM reduced to 5–10% inpatient mortality, which is further reduced to 2–4% in children being treated at home with RUTF. Management of MAM is also associated with a health centre, but the health status of recipients of RUSF is followed less intensively; nonetheless, minimal health evaluations are performed fortnightly or monthly when the RUSF sachets are dispensed. The programme reaches about 7 million malnourished children, effectively managing MAM and minimizing clinical progression to SAM. Children who recover from SAM are often also discharged to a MAM programme to prevent relapse.

Lipid-based RUF provide necessary energy, fatty acids, protein and micronutrients tailored for the needs and tastes of the children served in the emergency feeding programmes based on a mixture of a standardized set of ingredients. These include peanuts, chickpeas, soy flour, milk protein (dry skimmed milk and/or whey), sugar, vegetable oil, a vitamin and mineral premix, and small amounts of emulsifiers and

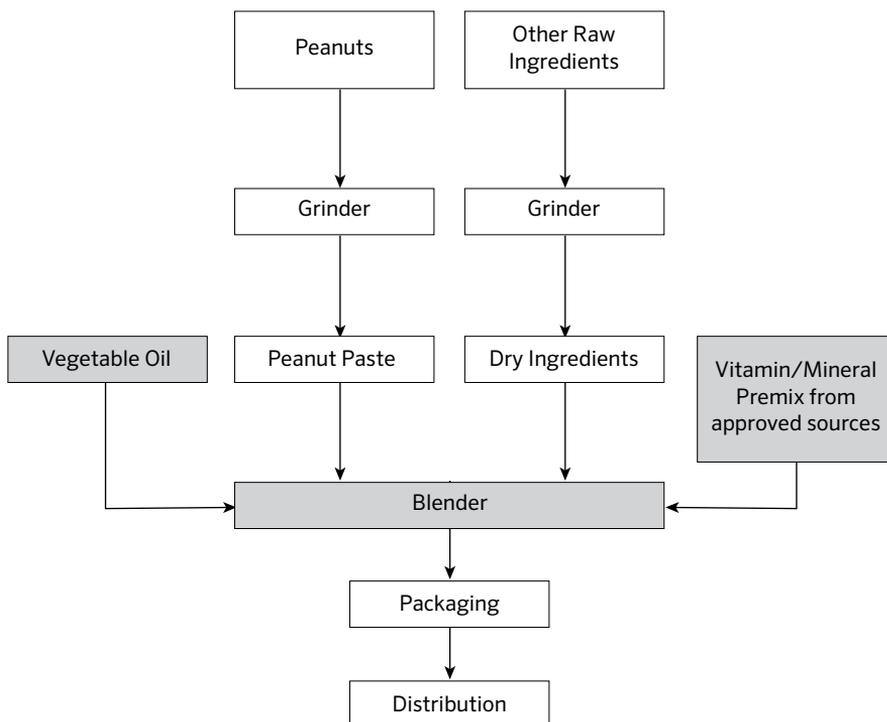


FIGURE 1: Generic schematic for the production of lipid-based RUF for management of MAM and SAM

stabilizers. The ingredients are relatively inexpensive commodity items that are broadly available. In addition, although the industry has developed considerably from its early days of using kitchen-style equipment, lipid-based RUF are relatively easy to manufacture without specialized or unique equipment, which makes possible their production in the country of use. At present, there are approximately 20 manufacturers of lipid-based RUF located in Africa, Asia, Europe and North America.

2.2 EXISTING SPECIFICATIONS FOR LIPID-BASED READY-TO-USE FOODS FOR MANAGEMENT OF MODERATE ACUTE MALNUTRITION AND SEVERE ACUTE MALNUTRITION

SAM management programmes have traditionally focused on provision of therapeutic foods, treatment of acute medical problems, and measles vaccination. Programmes initially required in-patient management of children with SAM, using fortified milk-based formulas, and this approach continues to be used in children whose SAM is complicated by other medical issues that require inpatient care, or who suffer anorexia, intractable vomiting, convulsions, lethargy, unconsciousness, lower respiratory tract infection (LRTI), high fever, severe dehydration, severe anaemia, hypoglycaemia or hypothermia in addition to SAM, and for children who reject RUTF or who reside in settings that do not support community-based treatment of SAM. Manufacturers follow relevant ISO, Codex and other food safety requirements (e.g. ISO 22000:2005 (ISO, 2009); CAC/RCP 1-1969 (CAC, 2003); CAC/RCP 66-2008 (CAC, 2009)). Pre-packaged milk-based formulas F-75 and F-100 are packed in sachets or pouches of 102.5 g that are to be opened and mixed with 500 ml of boiled water, to prepare approximately 600 ml of liquid diet. Microbiological specifications, which vary slightly among UNICEF, WFP and MSF, include criteria for Enterobacteriaceae, *Salmonella* spp., *Cronobacter* spp., mesophilic aerobic bacteria, coliforms, and *Listeria* spp. A Certificate of Analysis (CoA) must be issued and forwarded prior to each shipment for each lot (batch). This certificate must identify the laboratory methods employed and the target values for *Salmonella* spp., *Cronobacter* spp. and mesophilic aerobic bacteria.

SAM is treated in an in-patient setting based on two phases: first for children who are in the worst condition and who are prescribed a regimen of F-75 and medicines to treat their illnesses. The second phase is used for children who have recovered from the worst illnesses, and they begin to receive F100 until their malnutrition is resolved. F-75 is expected to be used for children who have medical complications until they can be transferred to F100 or RUTF. RUTF has the same composition as F100 in terms of ration of fat, protein and carbohydrate; both RUTF and F100 are made with dried skim milk powder and therefore have similar properties in

terms of digestibility. However, because F-75 and F-100 are to be reconstituted with water before use, their hazard profiles are more consistent with those of powdered formulae for infants and children (CAC, 2009).

When children 6 to 59 months of age with SAM no longer have medical complications, eat RUTF with good appetite, are active and alert, and reside in settings that support community-based management, they graduate to community-based treatment with RUTF. Since the inception of the community-based management of acute malnutrition, there has been less need for F-100 because children can be transferred from F-75 to RUTF. Children who do not have medical complications and receive RUTF directly without receiving in-patient treatment are generally considered in better condition, although, of course, their status must be monitored closely, since it can deteriorate quickly if illness occurs. RUTF was developed in 1999 as a peanut-based paste nutritionally equivalent to the milk-based formulas. Because this product does not require reconstitution and is shelf-stable and highly portable, it allowed programmes to transition from primarily inpatient to primarily out-patient management of SAM, from around 2006. Because out-patient programmes are less resource-intensive, coverage of malnourished populations greatly expanded. In Ethiopia, treatment programmes reached 50 times more children in 2009 than they had in 2003 (UNICEF, 2007). In 2009, UNICEF procured 14 million kg of RUTF, which indicates the broad reach of this type of product (UNICEF, 2007) and the need to ensure its safety.

The same microbiological specifications were applied in 2005 to RUTF as are used for therapeutic milk formulas. However, there are important differences between F-100 and RUTF that influence the appropriateness of microbial targets for specifications and concentrations of concern. For example, F-100 is a milk-based powder that is reconstituted with water before feeding, just as is the case for PIF. Like PIF, if F-100 contains *Cronobacter* spp. and is held at warm ambient temperature after it is reconstituted, the small numbers of *Cronobacter* spp. that are in the dry powder may grow to the larger numbers that are assumed to be necessary to produce serious invasive disease. Because of *Cronobacter*'s short generation time in reconstituted formula at favourably warm temperatures, numbers of bacterial cells can increase a thousand-fold in just 3 hours. In contrast, RUTF is distributed as a thick paste with intrinsic low a_w and is not reconstituted with water. It is packaged in single-serving sachets, which are to be opened and consumed in one sitting. Product characteristics provide minimal opportunities for pathogen growth even when consumption stretches over a few hours; furthermore, while consumers of RUTF are certainly at greater risk of infections with serious complications than are well-fed children of similar age, they fall outside the age group known to be at highest risk for the most serious complication of *Cronobacter* spp. infection, i.e. meningitis (Bowen and Braden, 2006). Therefore, the microbiological standard of no detected counts of *Cronobacter* spp. in

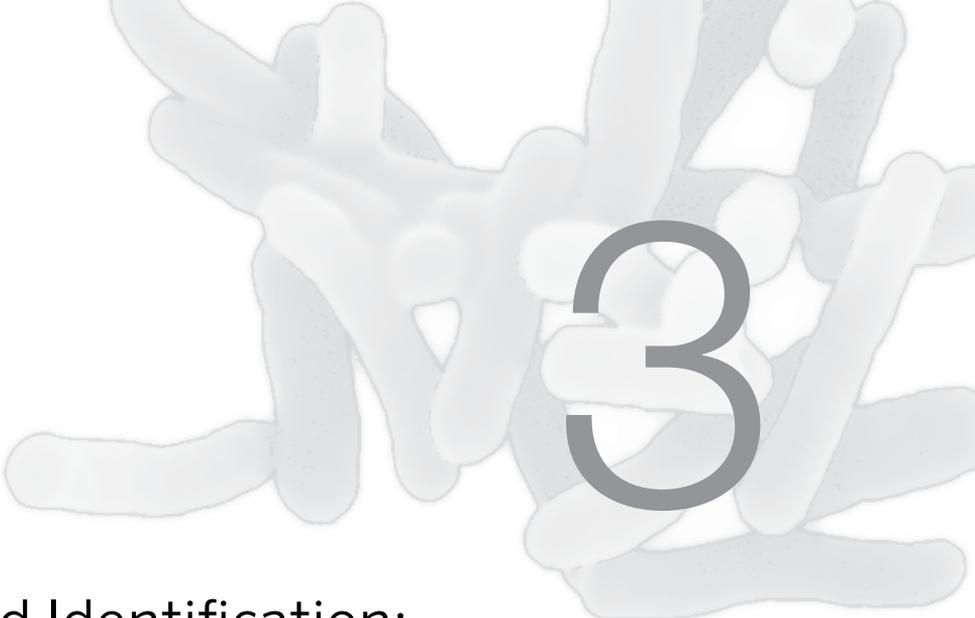
30 samples of 10 g each of product, as would be applied to PIF and other formulas for special medical purposes intended for infants, may not be the most appropriate indicator of safety risks associated with lipid-based RUF.

In addition to looking at Codex PIF standards for guidance on appropriate microbiological standards for lipid-based RUF, the food safety and quality assurance teams from UNICEF, WFP and MSF also reviewed and appropriated other Codex and EU food standards for additional microbiological specifications that appeared relevant to lipid-based RUF. UNICEF, WFP and MSF purchase approximately 95% of the supply of RUF, and their food safety and quality assurance teams collaborate on audits of manufacturers, based on Codex and ISO 22000 standards, to assure uniformity across all emergency feeding programmes that combat malnutrition. However, each organization keeps its own independent quality system and decision tree for the validation of products and manufacturers.

2.3 MICROBIOLOGICAL HAZARDS ASSOCIATED WITH LOW-MOISTURE FOODS

The water activity (a_w) of foods is a measurement of the concentration of water available for biological reactions. Pathogenic and spoilage micro-organisms are not capable of growing in low-moisture foods with $a_w \leq 0.60$. Lipid-based RUF, with a maximum a_w of 0.60, fall in the low-moisture food category. The low availability of water provided by low-moisture foods, however, does not always kill micro-organisms and, indeed, may maintain them in a metabolically dormant state. Microbial contaminants may gain access to low-moisture foods through ingredients or from the processing environment because of poor management, including failure to apply good manufacturing practices (GMPs). Peanuts, chickpeas and soybeans—the main raw commodities used in lipid-based RUF formulations—contain a wide range of naturally occurring bacteria and fungi, some capable of causing human diseases. Therefore, even low-moisture foods with sufficiently low a_w to prevent the growth of bacteria can be vehicles of pathogens in outbreaks of foodborne disease (Beuchat *et al.*, 2013).

Mild heat treatment that can eliminate foodborne pathogens in high-moisture foods may not be sufficient to eliminate the same pathogens in low-moisture foods. The increased heat resistance of bacteria afforded by low-moisture environments can be affected by many factors, including cell form (vegetative or spore), type of solute present in the food, pH, and physiological differences among genera of micro-organisms. It is imperative that validation of processes used to manufacture specific lipid-based RUF formulations be done using equipment at sites where they are produced. Otherwise, a false sense of process control may result.



3

Hazard Identification: Micro-organisms of concern in lipid-based ready-to-use foods for management of moderate acute malnutrition and severe acute malnutrition

The potential range of contaminating micro-organisms is very wide and includes one large important family, the Enterobacteriaceae. Members of the Enterobacteriaceae family include Gram-negative, facultatively anaerobic, rod-shaped bacteria of worldwide distribution that are found in soil, water, plants and animals from insects to humans. While not all genera and species in the group are known to infect and cause disease in humans, some do either as opportunistic pathogens (those that require a special “opportunity” to enter a human host and cause disease) or as pathogenic bacteria with attributes that make them more or less dangerous to everyone. Two examples of members of the Enterobacteriaceae family that are important to this discussion of lipid-based RUF are *Cronobacter* spp. at the opportunistic end of the pathogenic spectrum and *Salmonella* spp. at the more universally pathogenic end, but many other bacteria in the Enterobacteriaceae family can and do cause human illness. In infants, *Cronobacter* spp. infections primarily present as meningitis and septicaemia; other presentations include urinary tract infections (UTI), diarrhoea, and perhaps necrotizing enterocolitis. This bacterium can be cultured from foods with a low a_w , including milk powder, PIF, follow-up formula (FUF), and in all manner of meat, fish and plant-based foods and ingredients (Iversen, Lane and Forsythe, 2004). Therefore, their presence in ingredients

included in the manufacture of lipid-based RUF is likely. Because no temperatures currently achieved during processing and packaging have been validated to reliably kill *Cronobacter* spp. that are introduced through the ingredients or from the processing environment, their presence in finished products is not unexpected (it is important that presumed isolates of *Cronobacter* spp. isolated from lipid-based RUF be carefully distinguished from closely-related *Enterobacter* spp.; in addition, subtyping of confirmed *Cronobacter* spp. is desirable). *Cronobacter* spp. can persist in low moisture foods for at least 2 years (Caubilla-Barron and Forsythe, 2007). Although most people appear to ingest *Cronobacter* spp. in a variety of foods on a continual basis, clear attribution of disease to specific foods is restricted to the special case of high-risk infants consuming reconstituted PIF that has been held at temperatures for a period adequate for microbial growth to occur, suggesting that even susceptible hosts require a large exposure dose. However, it is somewhat more difficult to identify *Cronobacter* spp. than *Salmonella* spp. in stools, and etiologic diagnosis rarely is attempted for uncomplicated diarrhoeal illness, especially in resource-poor areas. Therefore, the health impact of small numbers of *Cronobacter* spp. in low-moisture foods, while undocumented, is uncertain.

Salmonella also survives well in foods with low a_w . However, in contrast to *Cronobacter* spp., *Salmonella* has been involved in many outbreaks and food recalls associated both with low-moisture and high-moisture foods, often causing disease at low exposure doses (Beuchat *et al.*, 2013) (see Table 1). Peanuts, peanut products and powdered milk, which are ingredients in lipid-based RUF, have been implicated as sources of *salmonellae* that have caused several outbreaks of salmonellosis. The potential presence of pathogenic strains of *Escherichia coli*, *Shigella* spp., *Klebsiella* spp. and *Citrobacter* spp.—all members of the Enterobacteriaceae family—in lipid-based RUF also pose some level of safety risk to intended consumers. The relative likelihood of the presence of one or more pathogenic members of the family can be inferred in lipid-based RUF while quantitatively monitoring these products for Enterobacteriaceae as an indicator of general hygiene.

In addition to the Gram-negative Enterobacteriaceae, the potential presence of Gram-positive foodborne bacterial pathogens in lipid-based RUF for management of MAM and SAM should be recognized. *Bacillus cereus*, and rarely other *Bacillus* spp., can produce a heat-stable emetic toxin in foods and diarrhoeagenic toxin in the gastrointestinal tract. *Clostridium botulinum*, *C. perfringens* and rare strains of other clostridia are known to cause foodborne intoxications. Infant botulism has been associated, in at least one case, with consumption of reconstituted PIF (Brett *et al.*, 2005). Intestinal toxæmia botulism in adults has been associated with consumption of peanut butter containing *C. botulinum* (Sheppard *et al.*, 2012). Spores of *Bacillus* spp. and *Clostridium* spp. have high heat resistance, and are likely to survive thermal processes used to manufacture lipid-based RUF. Listeriosis associated with consumption of low-moisture foods has not been documented. However,

TABLE 1. Identification of potential hazards associated with lipid-based RUF for management of MAM and SAM

Hazard	Potentially in ingredients	Potentially in processing environment	Potentially will survive processing	Potentially pathogenic at low dose	Potential severity
Mycotoxins*					
Non-typhoidal <i>Salmonella</i> serovars	+	+	+	+	Serious
Other Enterobacteriaceae (includes <i>Escherichia coli</i> , <i>Klebsiella</i> , <i>Shigella</i> , <i>Enterobacter</i> , <i>Cronobacter</i> , <i>Citrobacter</i> and <i>Proteus</i>)	+	+	+	+	Variable
<i>Clostridium botulinum</i>	+	+	+	+ **	Severe
<i>Listeria monocytogenes</i>	+	+	+	± ***	Serious
<i>Bacillus cereus</i>	+	+	+	--	Moderate
Enterotoxigenic <i>Staphylococcus aureus</i>	+	+	+	--	Moderate
<i>Clostridium perfringens</i>	+	+	+	--	Moderate

* Mycotoxins are included on the hazard identification list to acknowledge their importance; however, they were not considered in this consultation. Likewise, it is possible that various parasites and viruses known to cause enteric disease among populations receiving lipid-based RUF should be included in the hazard identification for lipid-based RUF, but insufficient information was available during the consultation.

** The concern is for intestinal colonization botulism syndrome in which spores germinate and produce toxin in the intestinal tract because of decreased gut motility, reduced diversity of gut microflora, and impaired immunity. In some circumstances 10–100 *C. botulinum* spores per serving may constitute a small but present risk.

*** For foods that do not support growth of *L. monocytogenes*, ≤100/g is generally considered safe. This may need review, specifically for PlumpyMum™ because of the influence of pregnancy on the risk of listeriosis.

Listeria monocytogenes can survive in foods with low a_w ; an initial population of 4.4 log cfu/g of peanut butter (a_w 0.33) stored at 20°C for 24 weeks decreased by only 0.6 cfu/g (Kenney and Beuchat, 2004). While the presence of Gram-positive pathogens in lipid-based RUF has not yet been reported, the potential for contamination exists and they should not be dismissed as having no safety significance.



4

Hazard characterization

Not every foodborne or other type of exposure to pathogens results in illness, and many host factors can contribute to the likelihood of acquiring a foodborne infection and becoming seriously ill from that infection. Commonly involved host factors that increase the risk of infections and illnesses include being at the extremes of age (e.g. premature babies, neonates, people more than 60 or 70 years of age) and being immune suppressed because of illness or therapy. Another factor that increases the risk of infection and illness that is particularly relevant to this discussion of lipid-based RUF is malnutrition. Malnutrition deranges all of the body systems of a child and, compared with children without malnutrition, children with MAM have about a 3-fold higher all-cause mortality rate and those with SAM have a greater than 9-fold increase in all-cause mortality rate. The underlying physiological reasons for this are many, but the changes caused by malnutrition that may have the most impact on foodborne infections that could be due to contaminated lipid-based RUF include atrophy of lymphoid tissues (e.g. tonsils, thymus and gut-associated lymphoid tissue); decreased antibody production, including of the antibody type (IgA) that is secreted into the digestive system; decreased cell-mediated immunity; poorly functioning complement and cytokine systems; decreased production of gastric acid (the first major barrier to ingested foodborne pathogens); decreased intestinal motility and barrier function; decreased production of intestinal mucus; and hepatic dysfunction, including decreased bile production. In addition, children served by emergency feeding programmes are administered oral antimicrobial agents for a variety of reasons, and this can alter the gut microflora in negative ways that persist long after administration of the antimicrobial agents has ended.

The epidemiological characterization of *Cronobacter* spp. infections is based on sparse data drawn largely from informal or passive surveillance, published case series and case reports, and a small number of epidemiological studies in a few countries around the world. These data suggest that the risk of serious invasive disease (meningitis and bacteraemia) following exposure to *Cronobacter* spp. is greatest for premature and very low birth weight infants, and otherwise typical infants during the first month or two of life (CDC, unpublished data), and this age-associated elevation in susceptibility to infection with serious consequences is supported by a neonatal mouse model that is being developed to understand the pathophysiology of *Cronobacter* infections (Richardson, Lambert and Smith, 2009; Richardson *et al.*, 2010, 2012). In 1.5-, 3.5-, 5.5- and 9.5-day-old neonatal mice challenged orally with *C. sakazakii* in reconstituted powdered infant formula, mortality in mice treated at 1.5 days old was much higher than in mice at 3.5 days old (13% vs 1%) and by one week after challenge no mortality was seen in mice treated at 5.5 or 9.5 days old. Isolation of *Cronobacter* spp. from brain tissues showed a similar age-dependent pattern, with isolation from 26%, 7% and 3% of mice challenged at 1.5, 3.5 and 5.5 days old, respectively, but no *Cronobacter* spp. could be isolated from any neonate treated at 9.5 days old (Richardson *et al.*, 2012). This is consistent with an age-dependent susceptibility to *C. sakazakii* invasion of tissues, and resulting mortality.

The risk for meningitis in humans is closely tied to age-related development of an effective blood-brain barrier, and the risk of meningitis appears to drop off substantially beyond the second month of life in the normal host. In the neonatal mouse model described above, the only age group that demonstrated a dose-responsiveness to *C. sakazakii* was the youngest tested, 1.5-day-old neonates, with a statistical increase in invasion of brain tissues at 10^7 and 10^{10} cfu *C. sakazakii*. For mortality, the only dose showing a statistical increase compared to control was 1010 cfu *C. sakazakii* in 1.5 day old neonates (Richardson *et al.*, 2012). The risk of bacteraemia is also reduced with increasing age beyond the neonatal period in humans, especially beyond the first year of life, and then the risk of bacteraemia again climbs in the elderly. The epidemiology of *Cronobacter* spp. in neonates suggests that infection requires a high dose (i.e. the low concentration of *Cronobacter* spp. in dry PIF appears to require a period for growth after rehydration before consumption for infections to occur), but large data gaps constrain the acceptance of this suggestion with confidence. The results of infection also are strongly influenced by the species and subtype of *Cronobacter* spp. (Joseph *et al.*, 2012a, b). Almost nothing is known about infectious dose and the role of food as a vehicle for infection for age groups beyond the neonatal period; the infectious dose is likewise unclear for other routes of exposure and other clinical forms of infection (e.g. urinary tract infections (UTI)).

A recent active surveillance study in North America found 0.66 cases of *Cronobacter*-associated disease per 100 000 population of all ages per year, with the highest attack rates in persons 70 or more years of age, followed by infants and children less than 1 year of age (Anna Bowen, pers. comm.). Besides reconstituted PIF consumed by neonates, sources of infection generally are unclear and, while the infectious dose is presumed to be high, that is also poorly documented. In infants, meningitis and septicaemia are the best recognized and most severe syndromes; other presentations include UTI, diarrhoea, and perhaps necrotizing enterocolitis. Outside the neonatal period, the most common manifestation is UTI, followed by wound infections; septicaemia is the most common serious manifestation of infection outside the neonatal period, and meningitis rarely occurs.

Surveillance and disease reporting are poor among children with MAM and SAM, and how well the data above and the inferences drawn from them relate to the general conditions of susceptibility to infection and serious disease in malnourished children has not been well studied. While it would be expected that the general increase in susceptibility to infection caused by malnutrition would also increase the susceptibility of malnourished children to infection with *Cronobacter* spp., these infections do not appear to have been documented in various studies involving malnourished children, with the possible exception of one study of bacteraemia in children in rural Kenya, not all of whom were malnourished and below the age of 6 years. In this study, 22 of 1132 blood isolates were Enterobacter spp. and some of these 22 isolates could have been later re-classified as *Cronobacter* spp. (Berkley *et al.*, 2005). Of the remaining 1110 blood isolates, 166 were non-typhoidal *Salmonella* spp., 121 were *Escherichia coli*, 36 were *Klebsiella* spp., and other Gram+ and Gram- bacteria made up the balance.

The small number of reported cases and limited data on dose-response for *Cronobacter* spp. stand in sharp contrast to the large number of documented cases of non-typhoidal salmonellosis and many well-studied foodborne disease outbreaks of non-typhoidal salmonellosis, in which the health risk posed by very low doses in some foods (e.g. foods with high lipid content) has been well established. Each year in the United States of America, for every 100 000 infants approximately 1.8 are reported with *Cronobacter* spp. infections, compared with 100 reported cases of infant non-typhoidal salmonellosis (CDC, 2011); most of the salmonellosis cases are enteric infections in infants in the United States of America, and few cases of invasive disease are reported. Each year in Africa, in contrast, there are approximately 200 to 400 cases of invasive non-typhoidal salmonellosis per 100 000 population (Feasey *et al.*, 2012). The relatively large burden of severe, life-threatening invasive non-typhoidal salmonellosis in Africa is due in part to the impact of malnutrition, with additional contributions by HIV/AIDS, malaria and other factors.

Public health surveillance—the ongoing, systematic collection, interpretation and dissemination of data for public health action (Porta, 2008)—is vital to detect outbreaks and other public health problems, as well as to define the scope of such problems, monitor changes in agents of disease, and evaluate interventions (Thacker, Qualters and Lee, 2012). In the absence of formal surveillance, it is extremely difficult to detect outbreaks until the number of cases is very large, unless the syndrome caused by the disease typically is both very rare and severe (e.g. liver failure with jaundice rapidly progressing to death in acute aflatoxicosis). While it may be that the importance of salmonellosis in children receiving lipid-based RUF compared with the lack of documented *Cronobacter* spp. infections is due to frequent exposures to *Salmonella* spp. from food, water and the environment, and a relatively low frequency of exposures to *Cronobacter* spp. within these geographical areas, these data do not exist. Closing data gaps will be important to understanding the health impact of *Cronobacter* spp. in lipid-based RUF. While infections with *Cronobacter* spp. in malnourished children do not appear to be common and *Cronobacter* meningitis does not appear to have been documented in this group, septicaemia and death are not uncommon among children with SAM and MAM, and they rarely receive culture-based diagnostics when ill.



5

Control of microbiological hazards in low-moisture foods

The safety of a food product is the responsibility of its manufacturer. It is the sum of many factors, encompassing control measures throughout the entire chain of manufacturing. That is, safety is achieved through careful selection and handling of raw materials and the application of GMPs and Good hygienic practices (GHPs) in production areas and lines during their processing, as well as the identification of specific control measures and their continual monitoring according to hazard analysis and critical control point (HACCP) principles, which allows for rapid and adequate correction in case of deviations. The adherence to and the effectiveness of these control measures can be verified by the application of appropriate sampling and testing programmes relevant to the step of the process.

Process control by all manufacturers and for each product must be validated before the products can be used by any of the programmes. The manufacturers must have a food safety policy based on Codex and ISO 22000 standards, and process controls in place, including pre-requisite programmes (PRP) and HACCP plans. Manufacturers must establish specifications for all ingredients and packaging materials used and must require a CoA from their ingredient suppliers covering microbiological parameters of ingredients, and they all must conduct end-product analyses to release each lot. Use of common specifications, similar food safety procedures and requirements, and collaboration among the major purchasers of RUF effectively promotes uniformity among the programmes but, unfortunately, GMP violations observed during audits and inspections have been found across the manufacturers.

A hazard assessment, conducted as part of a HACCP study (CAC, 2003), will determine which of the known microbiological hazards are not sufficiently important and can therefore be adequately managed through a combination of PRP and GHPs, versus those that are sufficiently important to require specific control measures. Pathogens that normally produce mild illness when ingested, such as *Bacillus cereus* and *Staphylococcus aureus*, are usually ranked as less important, whereas presence of *Salmonella* spp. is frequently ranked as very important in low-moisture products. However, ranking cannot be generalized, and a definitive conclusion on the significance of individual microbiological hazards must be determined for each product. This is done by systematically assessing the likelihood of the occurrence of important hazards and the severity of the health impact in the likely and intended consumers (see Hazard Identification – Table 1). This assessment needs to be carried out for all the steps, from the raw materials, through the different processing steps, including filling and packaging, as well as for intermediate storage and distribution, preparation and use by the final consumer.

Significant hazards will require the implementation, in addition to PRP and GHPs, of specific control measures. Heat treatments, for example, that will effectively and reliably eliminate significant microbial hazards or reduce their concentration to acceptable levels represent such specific control measures. These treatments must be validated to demonstrate their effectiveness in achieving the desired target under the conditions of the process and, once implemented, managed as critical control points (CCP). Adequate management of CCPs requires appropriate monitoring procedures for the relevant parameters, e.g. time (flow rate) and temperature. Actions need to be defined in case of deviations from the established limits at the CCP, such as the diversion of product to the drain or re-processing to achieve the desired level of pathogen reduction to ensure that no insufficiently processed product is further handled. Verification through appropriate procedures is an additional element to consider, and is an integral element of a HACCP programme. Microbiological testing of finished product can be used to verify the effectiveness of control measures.

When considering the microbiological quality and safety of the finished products, the criteria against which the acceptability of the product is judged are normally defined by taking into account microbial hygiene or process indicators relevant to the type of product under consideration, and the typical processing steps applied. In addition, one or several epidemiologically relevant pathogens are generally also included in criteria. Microbiological criteria should be designed by applying the latest version of the general principles published by Codex Alimentarius (CAC, 2013).

Microbiological criteria for the finished products are used to derive criteria for raw materials used during their manufacture. Criteria for raw materials need to take into account whether they are used with or without a microbial kill step that will destroy relevant microbiological hazards. In the absence of any kill step during processing, criteria for raw materials need to be aligned with those of the finished products. Adherence to appropriate GMP and GHP to control contamination during processing is verified through the establishment of appropriate criteria for samples taken from processing lines (product contact surfaces) as well as from the processing environment itself.

5.1. CHALLENGES TO ADDRESSING THE SAFETY OF LIPID-BASED READY-TO-USE FOODS FOR MANAGEMENT OF MODERATE ACUTE MALNUTRITION AND SEVERE ACUTE MALNUTRITION

In answering the multifaceted question of the safety of lipid-based RUF, specifically for *Cronobacter* spp. and for foodborne pathogens in general, for their intended use by their intended consumers (malnourished 6- to 59-month-old infants and children), the technical meeting found that it had inadequate data regarding each of the facets on which to base firm conclusions:

- No evidence exists that any foodborne illnesses attributable to lipid-based RUF have occurred; however, specific surveillance for foodborne diseases and investigations to identify sources of infection have not been done. While some pathogens that can be transmitted by the foodborne route have been isolated from clinical samples from patients and study subjects from relevant populations in relevant geographical locations, very limited inferences can be drawn on the role of food in general in those infections, and no inferences are possible on the role of lipid-based RUF.
- The limited amount of historical end-product testing that has been done and the microbial contamination snapshot that was conducted in first quarter 2013 suggest that a relatively low level of intermittent product contamination with *Cronobacter* spp. and *Salmonella* occurs; however,
 - under the best of circumstances, negative end-product test results only imply that contamination exists below some pre-determined level based largely on the total sample volume tested, and one cannot infer on the basis of end-product testing that contamination is not present;
 - the sampling strategy used prior to the expert technical meeting meeting is insensitive statistically because of small sample volume (i.e. the number of samples tested and the amount of product per sample); and
 - while the statistical strength of the snapshot survey was greater than that derived from historical end-product testing, one cannot assume that

the results of this time-limited study conducted under possibly atypical conditions are representative of the daily output of manufacturers across all of the variability that arises from seasonal differences in ingredient supply, performance of equipment and personnel, and other factors that can influence safety.

- The experts believe, on the basis of a *priori* knowledge of food microbiology, that the local food supply in the geographical regions in which emergency food aid is administered is almost certainly contaminated at some frequency with *Cronobacter* spp. and *Salmonella* spp., and this will complicate attribution of infections that occur in children consuming lipid-based RUF. However, food-specific risk will need to be determined to assess the additive effect of contaminated lipid-based RUF on the burden of *Cronobacter* spp. and *Salmonella* spp. infections that become documented among consumers of RUF. These data are currently lacking, and, therefore, if infections are identified among consumers of lipid-based RUF, they must be assumed to be related to RUF, especially if they are phenotypically or genetically similar to isolates from lipid-based RUF, without being able to assess the possible role of other sources.
- The geographical area affected by malnutrition overlaps with the geographical area included in the meningitis belt in Africa and, when possible, the medical staff that cares for the malnourished infants and children confirms the aetiology of meningitis when it occurs. The published literature on this topic reviewed by the technical meeting does not include reference to *Cronobacter* spp. as a cause of meningitis in the population of interest, but it is not certain that the age group that consumes lipid-based RUF is at increased risk of developing *Cronobacter* meningitis.
 - Infants within the first couple of months of life are at greatest risk of developing *Cronobacter* meningitis because of their poorly developed blood-brain barrier, in addition to the immaturity of their innate and adaptive immune defence systems.
 - While malnutrition has an effect on all organ systems, its effects on the development of the blood-brain barrier are poorly studied, and there are no data to suggest that malnourished children at 6 months of age and older have an impaired blood-brain barrier. However, clarifying this is an important research need. It may be possible to design a study using protein- and calorie-deprived neonatal mice or some other animal model of infection with *Cronobacter* spp., and this should be discussed with experts in the field, but the data derived from animal studies may be difficult to extrapolate to the risk for intended consumers of RUF.

- The generalized all-cause increased susceptibility to infection and serious complications that exists among malnourished children has been well documented by numerous studies; however, for reasons that are not clear, these studies have not identified *Cronobacter* spp. by the foodborne or other routes as an important cause of illness and death among malnourished infants and children.
- Contamination of lipid-based RUF with *Cronobacter* spp. and by *Salmonella* spp. have been documented by historical testing data and by the microbial snapshot study, but the frequency, range of contamination levels, variability of contamination and important contributors to contamination cannot be inferred from existing data. *A priori* knowledge of food microbiology and the epidemiology of foodborne salmonellosis attributed to similar low-moisture foods are useful in estimating likely occurrence of *Salmonella* spp. contamination of lipid-based RUF, so appropriate sampling strategies could be designed, but, based on currently available data, large uncertainties exist regarding contamination of lipid-based RUF by pathogens.
- While other potential foodborne pathogens have been documented in similar low-moisture foods and probably exist at some level in lipid-based RUF, the risk they pose to malnourished infants and children cannot be quantified at present. The experts believe the risk of salmonellosis is much greater than that posed by all other potential foodborne pathogens; however, data that will allow better quantification of relative risk are needed and, at present, there is little evidence to support assumptions.

5.2 CONTROL OF SALMONELLA SPP. AND OTHER PATHOGENS IN LIPID-BASED READY-TO-USE FOODS FOR MANAGEMENT OF MODERATE ACUTE MALNUTRITION AND SEVERE ACUTE MALNUTRITION

The first step in designing a system to assure that process control is adequate to manage the hazards potentially present in a food is to list the potential hazards, based on the likelihood that sufficient levels will be present in the end-product to cause illness in the intended consumer. This is the manufacturer's responsibility, but requires strict review by purchasers and may require expert advice. The hazards are then ranked on the basis of likelihood and severity of disease (see Table 1). Hazards with relatively low probability of being present at levels that will cause illness, or hazards that produce minor illnesses in the population of interest, may only require assurance that the pre-requisite programmes of cleaning and sanitation and the general application of GMPs are in place. However, as the significance of the hazard increases, greater assurance is needed that more stringent food safety practices are being performed properly.

The basis for effective control of microbiological hazards during the manufacture of lipid-based RUF is achieved through the implementation of PRPs as outlined in various sections of the General Principles of Food Hygiene (CAC, 2003). While an essential element of any microbiological food safety management system, PRPs alone are not sufficient to control all microbiological hazards. PRPs include the zoning of processing areas and lines, hygienic design of premises and equipment, cleaning procedures, and control of movement of personnel and goods, and are key elements in controlling the risk of re-contamination with pathogens of human health importance from the processing environment or food contact surfaces after effective pathogen control has been achieved during processing. Depending on the microbiological requirements of the finished product, certain aspects of PRPs may need to be managed more specifically, such as Operational Pre-requisite Programmes (OPRP), a requirement that is determined during the HACCP study.

The assurance that only raw ingredients that are adequately controlled for the presence of important foodborne pathogens are used to manufacture lipid-based RUF is an important part of any system focused on microbiological safety and process control. Successfully achieving this goal recognizes factors and conditions that influence the survival of pathogens in low-moisture foods and food ingredients, as well as in environments in which they are stored, processed and packaged. For example, *Salmonella* spp. contamination of powdered milk and foods that contain powdered milk, such as powdered infant formula, has been recognized as a major health hazard for consumers for decades (Cahill *et al.*, 2008). Lessons learned during years of clinical, epidemiological and food microbiological research on powdered milk and powdered milk products, and outbreaks of disease associated with them, include that product contamination can result from in-coming ingredients and from chronically contaminated foci (harbourage sites) within the food manufacturing facility, and that contamination can be low level, sporadic and unevenly dispersed in the product, and require extraordinary efforts to document its presence in the product, and still cause very large outbreaks of illness among consumers. Efforts to control this problem in powdered infant formulas have required strict application of specific preventive measures, along with extensive microbiological product surveillance, and yet occasional public health issues continue to arise.

It well known that *Cronobacter* spp., *Salmonella* spp. and other bacterial pathogens of concern in lipid-based RUF can survive for several months in low-moisture foods. *Cronobacter* spp. can survive for at least 18 months in PIF stored at 20–22°C (Edelson-Mammel, Porteous and Buchanan, 2005) and in dried liquid infant formula for at least 30 months at 20–25°C (Caubilla-Barron and Forsythe, 2007). *Salmonella* spp. can survive for at least 26 weeks on peanuts (Proaña Peralta *et*

al., 2011) and 24 weeks in peanut butter (Burnett *et al.*, 2000) stored at 21–23°C. Survival of these and other foodborne pathogens is enhanced at refrigeration and freezing temperatures compared with ambient temperature, and holding raw ingredients or finished products for some time period does not ensure the elimination of pathogen(s) of concern.

As has been documented for powdered milk products (Cahill *et al.*, 2008), persistence of *Cronobacter* spp., *Salmonella* spp. and other foodborne pathogens in foci within facilities that manufacture lipid-based RUF is an important concern. If GMPs are not followed, pathogens may grow on food contact surfaces, floors and other areas where water and organic residues from ingredients have accumulated, which increases the risk of contaminating the finished products. Mullane *et al.* (2007) monitored a PIF processing facility for *Cronobacter* spp. for 1 year. Frequencies of isolation up to 31% were found in some locations. Sub-typing of 200 isolates revealed that 70% were clonally identical, demonstrating the persistence of resident strains. *Cronobacter* spp. was detected in 18 (12%) of 152 environmental samples (scrapings from dust, vacuum cleaner bags, and spilled product near equipment) taken from three powdered milk factories (Kandhai *et al.*, 2004). Thirty-two percent of 298 environmental samples from five powdered-milk factories were found to contain *Cronobacter* spp. (Craven *et al.*, 2010); PFGE analysis showed that most clones were unique to each factory and 7 of 49 were isolated from both milk powder and other areas in the same factory, including tanker bays, evaporator rooms, an employee's shoes, and external roofs. Several *Salmonella* serotypes were recovered from equipment in receiving, manufacturing and storage areas in an oilmeal plant (Morita *et al.*, 2006). The presence and persistence of *Cronobacter* spp. and *Salmonella* spp. in these factories suggest the possibility of similar situations in lipid-based RUF factories if GMPs are not firmly adhered to. The presence of pathogens in ingredients purchased from factories in which they have been found emphasizes the importance of measures that reduce the likelihood that ingredients are contaminated with pathogens.

Heat treatments are the most frequently applied measures to control significant microbial hazards. However, combinations of individual measures that achieve the same reduction are also possible, and then each individual measure needs to be managed according to the same principles. If the production process does not include a microbial kill-step, as has been generally the case for lipid-based RUF, but is limited to a mixing or blending of individual ingredients, then the control measures described need also to be applied by the ingredient suppliers. This is needed to ensure that the purchased raw materials comply with quality and safety criteria that are, in principle, the same as those of the finished product. Validated processes such as sterilization or roasting applied during the manufacture of these

raw materials can be considered as effective microbial kill steps, but validation of the ingredient process and verification of its application must be documented. This holds true as well as for the effectiveness of PRPs designed to prevent post-process contamination, and this can be demonstrated through environmental monitoring. More lenient criteria can be applied for raw materials submitted to a microbial kill step, and stricter criteria may apply for ingredients used in processes conducive to microbial growth.

Just as WFP, UNICEF and MSF need a mechanism to assess the safety of lipid-based RUF prior to purchase, the manufacturers of RUF must ensure that the ingredients they use are handled and prepared in compliance with effective control measures, and will therefore comply with established criteria. To use resources in the most effective way it is important to determine which ingredients represent the highest risk for presence of the significant pathogen, such as *Salmonella* spp. The likelihood of occurrence of *Salmonella* spp. can be established based on information from the literature (e.g. epidemiology, surveys and surveillance programmes) as well as from historical data on the ingredient and its suppliers. Based on such data, milk powder, whey powder, soya proteins, peanuts and chickpeas are considered as being potentially high risk ingredients, but each supplier of every ingredient will require ongoing evaluation.

When processing steps that will effectively and reliably eliminate significant hazards or reduce their concentration to acceptable levels can be validated and implemented, a written plan for verifying their correct operation and the response to marginal performance and operating failures can be established and put into place. This provides the highest level of food safety, and when the steps that are critical to the safety of the end product can be identified and implemented, a properly designed and executed HACCP plan should be a purchase requirement. Whatever the process, the same principles would apply throughout the different steps in the food chain, from primary agricultural production of ingredients to consumption of the finished product, because the target is to ensure the safety of the product as consumed. Every step is linked in a chain of events that determines the safety of the end product.

5.3 ROLE OF SAMPLING IN ASSURING FOOD SAFETY

Microbiological testing is best used as part of the validation and verification procedures in HACCP and for assessing adherence to GMPs and GHPs, as well as the suitability of a food or ingredient for its intended purpose. Through microbial testing one can identify sources of contamination and potential pathogen har-

bourage sites (e.g. hot spots for Enterobacteriaceae), and verify effective processing and control of post-processing re-contamination. Microbial testing during primary agricultural production may be indicated when agronomic conditions have a major influence on the microbiological safety of the end product through ingress of pathogens in ingredients, and may include samples of irrigation water, fertilizer and other samples. Microbiological testing of ingredients is most useful in cases in which suppliers have a poor (or no) history of control, and when no effective kill step is included in the manufacturing process. In-process testing is used to verify a control or microbial kill step and to demonstrate the potential for re-contamination, generally by using indicator organisms like Enterobacteriaceae with quantitative results to test intermediate product, line residues, tailings, wash water and other types of samples. Testing the processing environment can identify harbourage sites and verify that the environment is under appropriate hygienic control, generally by using swabs and sponges to collect samples, followed by appropriate testing. Rapid testing methods such as ATP bioluminescence may be applied as a complement. End-product testing is used to demonstrate successful application of control measures and to assess the microbiological status of a lot when no other information exists to assess its status, but sampling probability issues reduce its value for assuring end-product safety. The safety of foods is principally achieved by controls focused at the source, product and manufacturing facility design, the process and its control, and all are based upon the consistent application of GHPs, GMPs, and HACCP principles during production. This preventive approach offers more control than microbiological end-product testing for lot release because the effectiveness of microbiological testing to assess the safety of foods is limited. The relative importance of end-product testing is lower than that for in-process and environmental testing because of the value of in-process and environmental testing in determining process control and their ability to provide early information on potential problems before those problems materialize.

'Variables' sampling plans² use a full range of numerical data that describe microbial loads on food being sampled, i.e. the hazard level is fully quantified, in contrast to 'attributes' schemes in which presence or absence of the attribute is all that is considered. Variables sampling plans and their interpretation are based on the known mean and standard deviation of log-transformed values of the bacterial counts of the product being tested. Consequently, variables sampling plans can only be used when these properties of the food are already known from previous, ongoing

² A sampling plan by variables is a method for evaluating the quality of a lot, and consists of measuring for each item the value of a variable characterizing the inspected commodity (CAC, 2004).

testing. Attributes plans³ can be used when we have no prior knowledge of the usual level of safety of the product. They are frequently used to establish whether a lot or batch of product is acceptable based on the assessment of a single attribute (e.g. *Salmonella* not detected per 25 g). Attributes sampling plans are defined⁴ by the number of samples to be analysed (n), the level that separates acceptable quality from marginally acceptable or unacceptable quality (m), the maximum number of sample units with unsatisfactory test results that are permissible (c), and sometimes a limit higher than m that can never be exceeded in an acceptable lot (M). For example, a 3-class attributes sampling plan specified by n = 10, c = 2, m = 100/g and M = 1000/g would require 10 samples of 1 g each, only 2 of which could contain 100 and none of which could contain more than 1000 of the specified micro-organism. The probability of detecting an unacceptable lot depends on:

- the actual proportion of units in the lot that are unacceptable;
- the number of samples tested;
- the total sample volume; and
- the distribution of contaminants, or the variability of contamination, within the lot.

The statistics governing the reliability of attributes sampling plans are well established and relatively easily calculated using computer software. Plotting operating characteristic (OC) curves for different sampling plans allows one to predict the probability of accepting a lot that contains an unacceptable level of defects. Producers may be motivated to design sampling plans that minimize the probability of rejecting acceptable lots, while the programmes purchasing lipid-based RUF will want sampling plans that minimize the probability of accepting lots that are contaminated above the specified limits. Understanding the performance of the sampling plan is important to avoid using more resources for product sampling than necessary and to preclude distributing defective RUSF and RUTF to malnourished children.

The probability of incorrectly accepting a batch that should be rejected is often set at 5%, i.e. we want to be 95% confident that we are detecting lots that should be rejected. However, if 20% of a lot is contaminated at >1 cell per 10 g, and the attributes plan is n = 10, c = 0, m = 0 per 10 g, the lot will incorrectly be accepted

³ A sampling plan for inspection by attributes is a method for evaluating the quality of a lot, and operates by classifying each increment of the sample as a conforming or non-conforming characteristic or attribute, depending on whether the specification is complied with or not. The number of increments having the non-conforming attribute are then counted, and if the acceptance number set by the plan is not exceeded the lot is accepted; otherwise, it is refused (CAC, 2004).

⁴ n = number of samples that must conform to the criterion; c = the maximum allowable number of defective sample units in a 2-class plan; and m = a microbiological limit which, in a 2-class plan, separates good quality from defective quality.

11% of the time. If only 0.1% of the lot is contaminated, testing 10 samples has only a 1% probability of detecting a contaminant, i.e. unacceptable lots would be accepted 99% of the time with the specified sampling scheme. Most of the time for food safety assurance, one wants to correctly reject a lot contaminated at the level of 0.001% or less, which requires hundreds of thousands of samples in order to be confident that a defective lot would be incorrectly accepted less than 5% of the time (ICMSF, 2002, 2011; van Schothorst *et al.*, 2009).

Microbiological specifications are based on the susceptibilities of the anticipated consumers and these specifications should, therefore, be the same for all similar products to be consumed by the same consumer groups. However, because the sampling frequency will be determined by the level of confidence the purchasing programme has that the supplier can consistently meet the microbiological specifications, sampling frequency may be different for the same product from different sources. One needs a reasonable level of sampling to achieve a reasonable level of confidence, and how much confidence one needs is determined by the impact of being wrong: one would need to be more confident of being correct when dealing with a pathogen that causes severe disease at a low dose and hence that, in turn, increases the number of samples that must be tested. Similarly, if contamination is not uniformly distributed within the batch, a larger number of samples is required to achieve the same level of confidence of the acceptability of the batch.

Microbiological criteria apply usually for individual lots or batches of products and are normally not dependent on the size of the lot. It is, however, important to determine what is a lot or batch of the product in question. The term lot has been defined in the Codex Alimentarius Standard “General Standard for the labelling of pre-packaged foods” as a definitive quantity of a commodity produced essentially under the same conditions (CAC, 2010). While the definition is not extremely precise, this implies the use of the same lots of raw materials, with continuous processing conditions and flow that assume no major interruptions or interventions due to stoppage or cleaning, storage of intermediate products for later usage and further processing, or use of re-work. Consideration needs also to be given to potential build up during production due to prolonged run times – in particular if growth is possible at certain steps of the process. While the manufacturer is certainly best placed to judge the suitability of a lot size with respect to their process, the assessment performed by the purchaser is a good opportunity to achieve a common understanding of what is acceptable and what is not. If a positive result for a pathogen is found in a sample drawn from a defined lot, the whole lot is considered as positive. In such a situation, no sub-lotting and re-testing is allowed, except for investigative purposes to determine the extent of the contamination, and results of re-testing would not be used for modifying the disposition decision.

Assuring the safety of lipid-based RUF requires moving away from a dependence on end-product testing for lot release, and toward a comprehensive system that will establish and document acceptable process control. Microbiological testing that is part of that documentation will indicate at some required level of confidence that the desired degree of control has or has not been achieved, and will include end-product testing as verification of process control, but will not make the incorrect assumption that safety can be assured by end-product testing alone.



6

Conclusions and recommendations

6.1 PATHOGENS OF GREATEST CONCERN

The technical meeting considered the range of potential microbiological hazards associated with lipid-based RUF for management of MAM and SAM and categorized them in terms of their ability to be present in the product ingredients, persist in the processing environment and in the end product, and their potential to cause illness in the consuming population (Table 1).

Salmonella spp. was identified as the highest priority infectious hazard and its control in lipid-based RUF the most important microbiological food safety programme goal. *Salmonella* spp. can persist in dry processing conditions and in low- a_w products for months and even years. It can cause illness at relatively small doses and causes severe illness in infants and young children at higher rates than it does in the rest of the population. The incidence of serious, life-threatening invasive non-typhoidal *Salmonella* infections in the African continent is ~100 times greater and in Asia is 1–7 times greater than elsewhere (Gordon, 2011). In addition, a number of *Salmonella* strains emerging from sub-Saharan Africa have been identified as highly virulent.

Recommendation: End-product specifications for *Salmonellae* should be enhanced with the development of an appropriate sampling strategy.

The risk that *Cronobacter* spp. pose to the population of concern could not be clearly established based on our current level of knowledge. Like *Salmonella*, *Cronobacter* spp. can persist in dry processing conditions and in low- a_w products for months and even years (Caubilla-Barron and Forsythe, 2007). Given the age of the consuming population, it cannot be assumed that the risk of *Cronobacter* meningitis would be elevated in this population; however, given the unknown status of the blood-brain barrier in malnourished children, it cannot at present be assumed that the risk is not elevated. Thus, it was concluded that *Cronobacter* spp. may not be any more problematic in this population than any of a range of other pathogens (Berkley *et al.*, 2005), but that greater understanding is needed of the risk of serious disease due to foodborne exposure to *Cronobacter* spp.

On balance, the experts believe that the current microbiological product standard for *Cronobacter* spp. is inappropriate and that more information on the safety of the product would be gained by replacing the narrow end-product *Cronobacter* spp. microbiological standard with broader quantitative standards for Enterobacteriaceae, which would provide better information on process control and overall hygienic conditions through trend analysis of quantitative Enterobacteriaceae data. When establishing microbiological criteria, it is critical that the sampling plan as well as all the other components as outlined in Codex guidelines are considered (CAC, 2013).

Recommendation: Control efforts should focus on the broader family of Enterobacteriaceae rather than on *Cronobacter* spp.

The expert meeting also noted the potential high level of concern associated with mycotoxins in these products, and recommended that these and other non-infectious hazards, e.g. heavy metals and pesticide residues, be considered in much more detail in the very near future.

6.2 FOOD SAFETY CONTROL

Existing microbiological specifications provide inadequate information on the potential level of hazards that may be present in lipid-based RUF for management of MAM and SAM. This deficiency, while influenced by the limitations of the existing sampling plan, is mostly a result of the statistical characteristics of end-product sampling. The meeting discussed at great length the limited inferences that can be derived from negative end-product test results compared with the levels of assurance that can be derived from indicators of process control, and concluded that:

- manufacturers must maintain control of microbiological hazards;
- microbiological specifications do not in any way replace control measures

during processing, but rather serve as a means of monitoring and verification; and

- inasmuch as no validated microbial kill step exists in the production of lipid-based RUF, their microbiological safety is completely dependent of the microbial content of the ingredients and the implementation of GHPs throughout to control ingress and survival or growth of pathogens.

Recommendation: Manufacturers should review their existing control programmes, including those aspects related to facility design, pre-requisite programmes (PRP) (e.g. environmental monitoring, GMPs, GHPs) and HACCP, with a specific focus on the hazards of concern identified during this meeting. External expert advice should be sought to ensure this is addressed in a manner appropriate to the control of the microbiological hazards associated with these products.

Recommendation: Purchasers of lipid-based RUF should require adequate environmental, product contact surface, in-line, and finished product surveillance, monitoring and investigation to assure adequate process control and hygiene. These plans will be facility specific and may require expert advice. Process validation by qualified external authorities (third-party body) should be required.

6.3 OPERATIONAL GUIDANCE

In the long-term approach to managing the safety of lipid-based RUF, the manufacturing process must be maintained under control by the manufacturer, and the organizations using these products must establish an effective system for safety assurance. Such comprehensive systems will require review of everything from bricks and mortar to final packaging. The objective of long-term efforts should be towards continuing improvement to move all manufacturing facilities toward the same level of performance. Until a rationale is developed for separating lipid-based RUTF used for SAM and RUSF used for MAM into different categories on the basis of product or consumer characteristics, they should be treated the same.

Recommendations to Manufacturers

- Each manufacturing facility, no matter how perfectly it is believed to be performing, should review its facility-specific PRP, including GHPs, GMPs, zoning, training, management of air flow, water use and flow (including drainage), cleaning and sanitizing, and maintenance procedures; this review may require expert consultation.
- Manufacturers should undertake ingredient, environment, in-line and end-product surveillance for Enterobacteriaceae and *Salmonella* spp., initially to establish baseline statistics (the first 12 months of data collection) and then,

in conjunction with other indicators, to monitor process control by reviewing trends.

- Manufacturers should continue to test technologies that may effectively control foodborne pathogens that threaten the safety of lipid-based RUF and, if microbial kill steps or effective combinations of microbial hurdles can be identified, they should be monitored as critical control points as part of efforts to establish, validate and monitor HACCP.
- Manufacturers should establish operational goals and specify reaction to marginal results and to failure to achieve microbial criteria, including root-cause investigations and written corrective action plans.
- Because the volume of product consumed by young children at one feeding is smaller than the volume of a sachet, and because the children are supposed to eat every 3 hours throughout the day, families are required to give their children sachets that have been open for hours under questionable hygienic conditions. It would be preferable to package the product into single-use sachets to minimize the risk of contamination in the home. Appropriate volume could be determined in consultation with implementing organizations.

Recommendations to FAO, WHO and Codex

In the context of some of the above conclusions and the need to provide internationally recognized guidance on the safety of these products, the meeting recommended that:

- FAO and WHO provide guidance on the methodology for analysing Enterobacteriaceae and *Salmonella* spp., and *Cronobacter* spp. as appropriate, so they can be used in purchase specifications.
- FAO and WHO provide technical support for establishing microbiological criteria for lipid-based RUF.
- FAO and WHO provide guidance for the analysis of baseline data (e.g. determining indicator values in Enterobacteriaceae data that identify a process that might be going out of control or a process that is already out of control) and creation of a long-term surveillance plan.
- Codex Alimentarius consider establishing international food safety standards for these products.

Recommendations to UNICEF, WFP, and MSF

The meeting made recommendations to UNICEF, WFP and MSF in three categories to highlight potential approaches to address the issue of product currently on hold, new product being produced in 2013, and then ongoing or longer-term measures to support an approach of continuous improvement, early identification and rapid response to any food safety issues.

For **short term** guidance on the best approach to managing product currently on hold because of positive *Cronobacter* spp. test results, the experts were in agreement that re-testing for *Cronobacter* spp. was not necessary or helpful, but due to the range of factors to be considered did not come to consensus on the final disposition of the retained product. Rather the expert meeting provided the following options for consideration (Table 2).

In the intermediate term (i.e. 2013 production), it was important to rapidly determine the current capacity of manufacturers to control their process relative to the presence of *Salmonella* spp. and the levels of Enterobacteriaceae in end products, and to use the information to establish temporary microbiological criteria for lot acceptance while indicators of process control were being established and applied. Mesophilic aerobic bacterial monitoring should be maintained as an indicator of adherence to GMPs while the final surveillance plan is being designed. It is thus recommended that:

- WFP, UNICEF and MSF establish a snap-shot of the current distribution of Salmonellae and Enterobacteriaceae in lipid-based RUF according the following approach:
 - select products for intensive testing to help establish microbiological criteria (Enterobacteriaceae and *Salmonella* spp.) for 2013 production. Randomly select 10 sachets of product being manufactured each day for 5 days for each product manufactured that week in each eligible manufacturing facility (n = 50 per product per facility);
 - contract with a certified laboratory to analyse the samples, without compositing, using methods specified in the contract;
 - liaise with FAO and WHO to obtain guidance on methodology for analysing Enterobacteriaceae and *Salmonella* spp. so they can be used in purchase specifications (see earlier recommendation to FAO and WHO). The distribution of *Cronobacter* spp. in lipid-based RUF is unclear and the epidemiology and clinical significance of its infections are poorly characterized in malnourished children, and sample collection for Enterobacteriaceae and *Salmonella* spp. provides an opportunity also to learn more about *Cronobacter* spp.; therefore, optional guidance on methodology for *Cronobacter* spp. also should be obtained. Isolates of *Salmonella* spp. and *Cronobacter* spp. should be retained for future analysis; and
 - liaise with FAO and WHO to obtain technical support for establishing microbiological criteria for lipid-based RUF (see earlier recommendation to FAO and WHO).

TABLE 2. Alternative options for managing on-hold product

Options	Rationale	Positive Considerations	Negative Considerations
Option 1 – Release product without further testing	The confidence interval around the <i>Cronobacter</i> spp. test results makes it likely that contamination of test negative and test positive lots are the same; test negative lots have been used and no <i>Cronobacter</i> infections have been diagnosed among consumers.	The retained product is sufficient to manage MAM for many months in hundreds of thousands of children who appear to be at low risk of <i>Cronobacter</i> infection	<i>Cronobacter</i> is a recognized foodborne pathogen for infants less than 6 months of age and it is not appropriate to feed product known to be contaminated, even at low levels, without some additional measure of its safety for its intended use in malnourished children whose susceptibility to infection with <i>Cronobacter</i> spp. is poorly characterized. Although <i>Cronobacter</i> infections have not been diagnosed among the population fed contaminated lots, diagnoses among these children are typically made clinically rather than through laboratory testing.
Option 2 – Test according to the microbiological criteria established by Codex for FUF	FUF is intended to be consumed by the same age group, likely with the same age-dependent development of a blood-brain barrier, and has a stringent <i>Salmonella</i> criterion and Enterobacteriaceae criterion, but no <i>Cronobacter</i> criterion.	Lipid-based RUF that pass the FUF standards should be considered to be safe for their intended purpose without further consideration of <i>Cronobacter</i> spp. that may be present. It is potentially holding existing product to a higher standard for a well-recognized pathogen, <i>Salmonella</i> , which can be considered appropriate for a lot which has tested positive for <i>Cronobacter</i>	In the time required for sample collection, laboratory analysis, review of results, and distribution of the sub-lots that are in compliance with FUF standards, at least some of the product will be beyond its useful shelf life. It is potentially holding existing product to a higher standard than new production.
Option 3 – Test according to the new product criteria for <i>Salmonella</i> and Enterobacteriaceae to be established in 2013	The retained product was manufactured under conditions similar to those that will pertain at least during early 2013 and it will be distributed and used at the same time as new production in 2013, so holding it to the same standards makes sense.	Holding the retained product to the same standard as 2013 production does not require using more than one standard for lipid-based RUF used in 2013.	Inasmuch as the retained product has been held because of potential pathogen contamination, it should be held to a higher standard of safety than product that has not been found to be contaminated.

A brief microbiological survey that included product from 15 manufacturers in 10 countries and which was consistent with these recommendations was carried out in the first quarter of 2013 (Appendix 2).

The microbiological criteria established for 2013 production of lipid-based RUF would follow the definitions and include the components specified in CAC/GL 21-1997, 'Principles for the Establishment and Application of Microbiological Criteria for Foods' (CAC, 2013).

- A microbiological criterion for food defines the acceptability of a product or a food lot, based on the absence, presence or number of micro-organisms per unit of mass or volume.
- A microbiological criterion consists of a statement of the micro-organisms of concern and/or their toxins/metabolites and the reason for that concern, the analytical methods for their detection and/or quantification, a plan defining the number of field samples to be taken and the size of the analytical unit, microbiological limits considered appropriate to the food at the specified point(s) of the food chain, the number of analytical units that should conform to these limits, the food to which the criterion applies, the point(s) in the food chain where the criterion applies, and any actions to be taken when the criterion is not met.

In terms of continuous improvement for the long term, particularly with regard to early identification and rapid response to any food safety issues, the expert meeting recommended the following actions.

- Continued research by manufacturers and academic food scientists on practical, effective microbial control and kill steps that can be integrated into the manufacturing process.
- Periodic review of the hazard analyses to accommodate a changing hazard landscape.
- Periodic re-evaluation of microbial criteria, including sampling plans, in the technical specifications for lipid-based RUF.
- Routine recording of lot codes of products that are distributed to individual patients to facilitate targeted interventions, case-finding and product trace-back in the event of product quality concerns.
- As appropriate product specifications are determined and implemented, and as risk messaging is developed, it would be useful to learn more about actual use of these products. Mothers of children enrolled in outpatient therapeutic and supplemental feeding programmes in different settings should be interviewed and feeding observed to help clarify actual usage patterns, permitting more accurate risk assessments and more targeted messaging.

- If a setting with reasonable clinical and laboratory support can be identified, it may be useful to conduct enhanced surveillance for illness that could be associated with contaminated lipid-based RUF. This would involve culturing patients when they present with diarrhoea, fever, etc., and possibly also conducting surveillance urine cultures.
- It is likely that the majority of children enrolled in community-based therapeutic feeding programmes do not have consistent access to safe drinking water. Water in such settings is often contaminated with high levels of faecal organisms and is associated with an increased risk for diarrhoea and death (Fewtrell et al., 2005). In therapeutic feeding programmes, poor access to safe drinking water may also be associated with prolongation of malnutrition and enrolment in the programme (Dorion, 2012). Providing information on the importance of safe drinking water alone typically is insufficient to drive changes in water treatment behaviours; thus, it may be useful for programmes to explore the acceptability of different water treatment methods (including silver-impregnated filters, sodium hypochlorite products, flocculent-disinfectant products or solar disinfection) and to consider routinely distributing acceptable water disinfection products with lipid-based RUF, or fostering social marketing of such products in areas of need (Marino, 2007).

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Annexes

Annex 1

Objective	Management of Severe Acute Malnutrition	Management of Moderate Acute Malnutrition
Generic Term	Ready-to-Use Therapeutic Foods	Ready-to-use Supplementary Foods
	(RUTF)	(RUSF)
Products*		
		High quantity* (92 g)
Purpose	Management of uncomplicated severe acute malnutrition with continued breastfeeding. SAM is defined as presence of nutritional edema or middle-upper arm circumference < 115 mm or weight-for-height < -3Z by WHO 2006 growth tables	Supplement to manage moderate acute malnutrition with continued breastfeeding. MAM is defined as MUAC 115-124 mm or WHZ between -3 and -2 Z according to WHO 2006 growth tables
Target Group	Infants and children 6-59 months with uncomplicated SAM; and older patients with SAM	infants and children 6-59 months with WFH between -3 to -2 Z or MUAC < 125 mm
	Children may transition from F-75/F-100 in hospital settings to RUTF adults including those with HIV	Others such as HIV positive adults, pregnant and lactating women
	May also be used as convalescent feeding, for example 2 weeks ration following episode of measles or malaria	May also be used as convalescent feeding, for example 2 weeks ration following episode of measles or malaria

Micronutrient and chronic malnutrition prevention

Ready-to-use Supplementary Foods (RUSF)	Lipid-based Nutrient Supplements (LNS)	Lipid-based Nutrient Supplements (LNS)	Lipid-based Nutrient Supplements (LNS)
High quantity* (100 g)	Medium quantity* (50 g)	Medium quantity* (46 g)	Low quantity* (20 g)
			
Acha Mum	Wawa mum		
Supplement to manage moderate acute malnutrition with continued breastfeeding	Supplement to the local diet for prevention of acute malnutrition with continued breastfeeding and prevent micronutrient deficiency and stunting	Supplement to the local diet for prevention of acute malnutrition with continued breastfeeding and prevent micronutrient deficiency and stunting	Supplement to the local diet with continued breastfeeding to prevent micronutrient deficiency and stunting
Infants and children 6-59 months with WFH between -3 to -2 Z or MUAC < 125 mm	infants and children 6-23 months	infants and children 6-23 months	infants and babies 6-23 months

Objective	Management of Severe Acute Malnutrition	Management of Moderate Acute Malnutrition
Directions for use	Eaten directly from sachet, without dilution or cooking; drinking water must be available Indicated on the individual packaging	Eaten directly from sachet, without dilution or cooking; Indicated on the individual packaging
Sole Source of Food	yes (100% of daily energy and micronutrient requirements)	no (generally 25-50% of daily energy and up to 100% of micronutrient requirements)
Ingredients	Sugar, vegetable oil (palm, soy, canola), peanuts or peanut paste, skimmed milked, whole milk or whey powders, vitamin and mineral premix, stabilizer (hydrogenated fat), emulsifier (mono or diglycerides)	Vegetable fats, sugar, peanut paste, soy proteins, maltodextrin and whey, vitamin and mineral complex.
Energy / nutrients per 100g	520-550 kcal	500 kcal
	12.5g protein	12.5g protein
	32.9g fat	32.9g fat
	65g Carbohydrate	65g Carbohydrate
	Moisture 2.5% (0.6% Wa)	Moisture 2.5% (0.6% Wa)
	Vitamin and mineral premix: Vitamin A, B1, B2, B3, B5, B6, Folic acid, Vitamin C, D, E, K. Minerals Na, K, Ca, P, Mg, Fe (10-14mg) Zn (10 mg), Cu, Se, I.	Vitamin and mineral premix: Vitamin A, B1, B2, B3, B5, B6, Folic acid, Vitamin C, D, E, K. Minerals Na, K, Ca, P, Mg, Fe Zn, Cu, Se, I.

Micronutrient and chronic malnutrition prevention

Eaten directly from sachet, without dilution or cooking; Indicated on the individual packaging	Eaten directly from sachet, without dilution or cooking; Indicated on the individual packaging	Eaten directly from sachet, without dilution or cooking; Indicated on the individual packaging	Eaten directly from sachet, without dilution or cooking; Indicated on the individual packaging
no	no	no	no
Chickpeas, Vegetable Oil, Skimmed Milk Powder, Sugar, Vitamins (A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E, K), Minerals (Ca, Cu, I, Fe, Mg, P, K, Zn) and Emulsifier.	Chickpeas, Vegetable Oil, Skimmed Milk Powder, Sugar, Vitamins (A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E, K), Minerals (Ca, Cu, I, Fe, Mg, P, K, Zn) and Emulsifier.	blended vegetable oil, (palm, soybean, canola) peanuts, sugar, non-fat milk powder, whey, maltodextrin, vitamin and mineral premix, cocoa, (optional) emulsifier)	penut paste, sugar, vegetable oil, (palm, soy, canola), non fat milk powder, whey, maltodextrin, vitamin and mineral complex, emulsifier, lecethin.
500 kcal	240 kcal approx	247kcal	108kcal
13 g protein	6.5 g protein	5.9g protein	2.5g protein
29 g fat	14.5 g fat	16g fat	7g fat
		65g Carbohydrate	65g Carbohydrate
Moisture 2.5% (0.5% Wa)	Moisture 2.5% (0.5% Wa)	Moisture 2.5% (0.6% Wa)	Moisture 2.5% (0.6% Wa)
520 kcal, 13g protein (10%), 29g fat (50%). Contains EFA, meets RNI and PDCAAS. Vitamin and mineral premix: Vitamin A, B1, B2, B3, B5, B6, Folic acid, Vitamin C, D, E, K. Minerals Na, K, Ca, P, Mg, Fe, Zn, Cu, Se, I.	260 kcal, 6.5g protein (10%), 14.5g fat (50%). Contains EFA, meets RNI and PDCAAS.	Vitamin and mineral premix: Vitamin A, B1, B2, B3, B5, B6, Folic acid, Vitamin C, D, E, K. Minerals Na, K, Ca, P, Mg, Fe, Zn, Cu, Mn, Se, I.	

Objective	Management of Severe Acute Malnutrition	Management of Moderate Acute Malnutrition
Packaging	1 Sachet = 92g printed aluminum foil sachet. Often nitrogen flushed	Sachet = 92g printed aluminum foil sachet often nitrogen flushed
Shelf life	24 months from manufacturing date	24 months from manufacturing date
Ration/dose	According to weight: 6-59m: 200kcal/kg/day; Practically: 2-3 sachets/day, or 184-276 g/day	One sachet per day 92g/day (approx 75kcal/kg/day)
Approximate duration of Intervention	6-8 weeks	1 to 3 months
Medical consultation	yes	no
Concomitant medication	Vitamin A (single dose 50 000IU - 200 000IU depending on age) Amoxicillin 3 times per day for 5-7 days; albendazole/ Mebendazole	no, other than an opportunity for de-worming and 6 monthly vitamin A supplementation

Micronutrient and chronic malnutrition prevention

Sachet = 100g printed PET sachet often nitrogen flushed	Sachet = 100g printed aluminium foil sachet often nitrogen flushed	325 gm polypropelene pots or sachets of different quantities. Printed aluminium foil sachet or polypropelene tubs	Aluminium and PET sachet = 20g
6 months from manufacturing date	6 months from manufacturing date	24 months from manufacturing date	18 months from manufacturing date
47-50g/day Doses administered by spoon and added to meals. Tub / sachet lasts 1 week (aprox)	20g/day		
1 to 3 months	3 - 18 months	3- 18 months	Up to 18 months
no	no	no, unless used in growth promotion program	no, unless used in growth promotion program
no	no	no	

Proposed interim purchase specifications for microbiological hazards for lipid-based ready-to-use foods for management of moderate acute malnutrition and severe acute malnutrition

Summary

The FAO/WHO technical meeting on the microbial safety of lipid-based nutrient supplements, convened in December 2012, recommended the revision of the microbiological specifications for ready-to-use therapeutic foods (RUTF) and ready-to-use supplementary foods (RUSF), with particular focus on *Salmonella* spp. and Enterobacteriaceae. Before any new specifications could be proposed it was necessary to gain a better understanding of the prevalence and levels of these organisms in the products of concern. A snapshot study of a week's production of RUTF and RUSF was implemented in the first quarter of 2013, which provided data on *Salmonella* spp. and Enterobacteriaceae in a range of RUTF and RUSF products produced by 15 manufacturers in 10 countries (Table A1). These data were considered together with other relevant information on the products, and the conclusions and recommendations of the aforementioned technical meeting. A range of potential sampling plans were identified and an assessment of their performance and potential impact in terms of product rejection was undertaken as part of the process of proposing interim microbiological criteria for *Salmonella* spp. and Enterobacteriaceae. Product safety is primarily achieved through the implementation and monitoring of effective control measures throughout the food production process. End-product testing can only provide a specified degree of certainty with regard to the safety of the product and serves as one of the measures of effectiveness of the control measures in place. In this context, FAO and WHO propose interim criteria for each lot or batch of absence in 25 samples of 25 g for *Salmonella* spp. and testing 10 samples of 10 g for Enterobacteriaceae, 8 of which should contain 10 or less cfu/g and 2 of which may contain up to 100 cfu/g. It is

recommended that batches of product exceeding these criteria would not enter the human food chain. These proposed criteria are of an interim nature and should be reviewed after an implementation period of about one year. They are intended to drive improvement in the industry and the Enterobacteriaceae criteria in particular should gradually become stricter.

Preparation of this annex

The scientific advice in this annex has been prepared by FAO and WHO, with input from selected experts, particularly in terms of the statistical analysis of the performance of sampling plans.

1. BACKGROUND

The safety of foods is principally achieved through the implementation of control measures that address the quality of the ingredients, the design of the product, the design of the manufacturing facility and the production process and its control. Such controls are based upon the consistent application of good hygiene practices (GHPs), good manufacturing practices (GMPs) and hazard analysis critical control point (HACCP) principles. This preventive approach offers more control than microbiological end-product testing, which is limited by the number of samples tested, the available methodology and the cost, among others, and can only ever provide a specified degree of confidence in the result. In a modern food safety management system, microbiological testing is optimally used for validation and verification procedures in HACCP, and assessing adherence to GMP/GHP and the suitability of a food or ingredient for its intended purpose, while end-product testing is only a final control step to check if the measures put in place are effective in producing safe products. Microbiological criteria are developed taking into account the susceptibilities of the anticipated consumers, and these criteria should, therefore, be the same for all similar products to be consumed by the same consumer groups. One needs a reasonable level of sampling to achieve a reasonable level of confidence, and the degree of confidence one needs is determined by the impact of being wrong: one would need to be more confident of being correct when dealing with a pathogen that causes severe disease at a low dose and that, in turn, increases the number of samples that must be tested. Similarly, if contamination is not uniformly distributed within the batch, a larger number of samples is required to achieve the same level of confidence on the acceptability of the batch.

Assuring the safety of RUTF and RUSF requires moving away from a dependence on end-product testing for lot or batch release, and towards a comprehensive system that will establish and document process control. Microbiological testing, which is part of that documentation, will indicate, at some required level of confidence, that

the desired level of control has or has not been achieved. This includes end-product testing as verification of process control, but will not make the incorrect assumption that safety can be assured by end-product testing alone.

2. ESTABLISHMENT OF END-PRODUCT SPECIFICATIONS

Given the above context, the establishment of a microbiological testing programme will take into account the hazards of greatest concern in the product to be tested; the potential to use one organism or group of organisms as an indicator of the level of control of a range of potential hazards; the availability, sensitivity and specificity of the analytical tests and the cost of sampling and analysis; as well as some understanding of the prevalence of the hazards of concern in the product and the homogeneity or heterogeneity of distribution.

In this context the following text focuses on proposed interim microbiological criteria to be used by purchasers of RUTF and RUSF as part of their product specifications for procurement of these products. They were developed to be used in conjunction with documented implementation of controls throughout the production of these products provided by the manufacturer. These criteria are the targets which the supplier needs to meet. In order for the supplier to consistently meet these criteria it will be necessary to implement a high level of process control, from the ingredients used in manufacturing to the end of the production process. The effectiveness of the measures taken may be monitored using microbiological criteria; however, the criteria used by the processor should be more stringent than those of the programmes that purchase RUSF and RUTE, to minimize the risk of exceeding the purchasers' requirements. Suppliers should also be aware that since the production process does not include a kill step, the ingredients will also need to meet the same criteria so the final product can meet the specifications of the purchaser.

The implementation of microbiological testing is a costly endeavour and only provides a limited amount of information about the safety of a particular product. The level of confidence one can have in the results depends on numerous factors including the number of samples, the size of the sample actually tested, the specificity and sensitivity of the analytical test, and the distribution of the organism in the product.

The technical meeting convened by FAO and WHO in December 2012 recommended that the purchaser of these products focus on two microbiological criteria: (i) *Salmonella* spp. and (ii) Enterobacteriaceae. The following outlines the aspects

taken into consideration in proposing microbiological criteria for *Salmonella* spp. and Enterobacteriaceae.

These criteria are intended for use by the purchaser. It should be further noted that these criteria are only being recommended for use on an interim basis, and should be reviewed after one year and adjusted as appropriate to take into account the additional information that will have been collected in the course of the year, as well as any other information from facility inspections, possible surveillance of illnesses in consumers, etc.

2.1 MICROBIOLOGICAL CRITERIA FOR SALMONELLA

The FAO/WHO technical meeting in December 2012 determined that *Salmonella* is the pathogen of greatest concern for RUTF and RUSF, and that its control should be the focus of efforts to ensure the microbiological safety of RUTF and RUSF. A snap-shot study of RUTF and RUSF undertaken during the first quarter of 2013 to gain insight into the possible degree of contamination with *Salmonella* spp. and Enterobacteriaceae confirmed that *Salmonella* can indeed be found in lipid-based nutrient products (Table A1).

Salmonella spp. is a well-recognized foodborne pathogen and has caused outbreaks of foodborne disease at low exposure doses associated with a variety of foods, including contaminated low-moisture foods (Beuchat *et al.*, 2013), as well as some low-moisture foods that contained the same types of ingredients as used in the manufacture of RUTF and RUSF. *Salmonella* spp. causes infections, illness, serious illness and death in people of all ages, genders, races and underlying states of health, and poses a particularly severe risk to infants and children that are mal-nourished (Majowicz *et al.*, 2010; Gordon, 2011).

TABLE A1. Collated results of the snapshot study of RUTF and RUSF for *Salmonella* spp and Enterobacteriaceae.

No of products tested	No of samples tested	<i>Salmonella</i> positive	Enterobacteriaceae </=10 cfu/g	Enterobacteriaceae >10cfu/g
20	1061	2	1034	23*

*16/23 were also tested for *Cronobacter* spp. and all 16 with an Enterobacteriaceae count of >10cfu/g also tested positive for *Cronobacter* spp.

Efficacy of current sampling approach

As *Salmonella* spp. may be present at low numbers in the manufacturing environment or the product itself, it can be difficult to find by testing a small number of samples. To date, the approach to end-product testing for *Salmonella* in these products has been to take 1 sample per batch. The amount of information provided by such a sampling approach is statistically insensitive and does not provide a good understanding of the extent of potential contamination in these products, and hence only provides a low degree of confidence in the safety of the product.

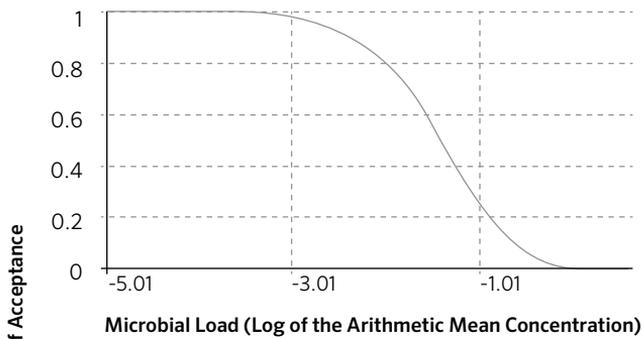
For example, if testing just one sample per production batch for *Salmonella* spp., there is a 5% probability of releasing product with 333 cfu/kg, given a standard deviation of 0.4 and 4000 cfu/kg, given a standard deviation of 0.8. In other words, there is a 95% probability of rejecting product with approximately 333 cfu/kg (or 3 cfu/10 g) of *Salmonella* spp. at a standard deviation of 0.4, and with approximately 4000 cfu/kg (or 40 cfu/10 g) at a standard deviation of 0.8 (Figure A1). Considering that *Salmonella* is an organism that can cause illness at very low doses, the current sampling approach clearly gives no assurance that the product has been produced under conditions that minimize the potential presence of *Salmonella*.

In comparison, the current Codex criterion for *Salmonella* in powdered formula for infants and young children (CAC, 2009) means that there is a 5% probability of releasing product that only has 2 cfu/kg when the standard deviation is 0.4, or approximately 3 cfu/kg if the standard deviation is 0.8. In other words, there is a 95% probability of rejecting product with approximately 2 cfu/kg *Salmonella* spp. at a standard deviation of 0.4, and with approximately 3 cfu/kg at a standard deviation of 0.8 (Figure A2).

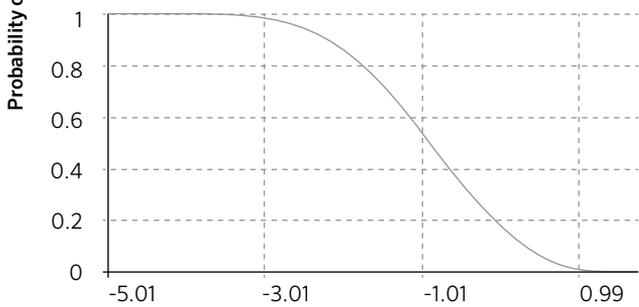
Assessment and selection of an alternative microbiological criterion

A range of potential sampling plans for *Salmonella* spp. were evaluated in terms of their performance, considering two different within-lot standard deviations (0.4 and 0.8) in order to propose a microbiological criterion that would provide more information than the existing one, and act as a driver for suppliers to improve their process control. The number of samples and the sample mass were varied as part of this analysis.

Increasing the number of samples could be compared to increasing the impact in terms of risk reduction. The greater the number of samples tested, the greater the degree of confidence you can have in your results. Increasing sample size also increases the cost. Explicit consideration was given to the impact of sampling plans with sample numbers ranging between 20 and 60 samples, taking into consider-

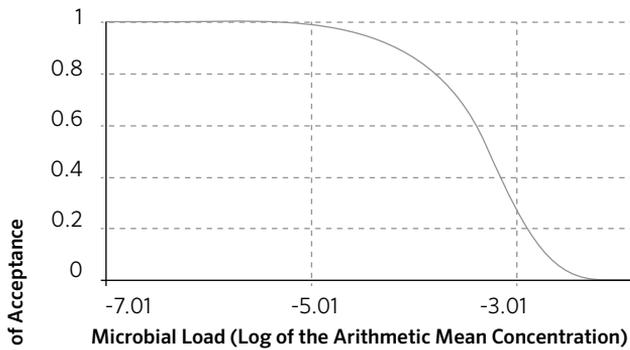


a: 95% Probability of Rejection at -0.451 log₁₀ cfu/g; 99% Probability of Rejection at -0.094 log₁₀ cfu/g; within-lot SD = 0.4

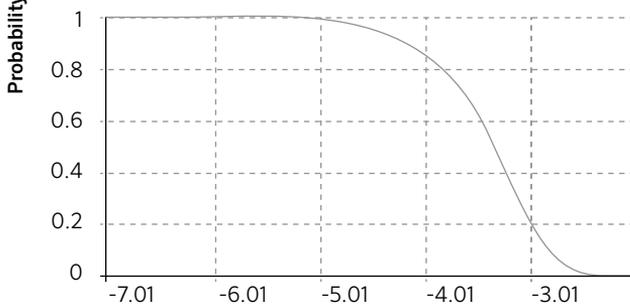


b: 95% Probability of Rejection at 0.636 log₁₀ cfu/g; 99% Probability of Rejection at 1.24 log₁₀ cfu/g; within-lot SD=0.8

FIGURE A1. Operating Characteristic (OC)-Curve for *Salmonella* sampling plan n = 1 (25 g)



a: 95% Probability of Rejection at -2.69 log₁₀ cfu/g; 99% Probability of Rejection at -2.49 cfu/g; within-lot SD = 0.4



b: 95% Probability of Rejection at -2.57 log₁₀ cfu/g; 99% Probability of Rejection at -2.34 cfu/g; within-lot SD = 0.8

FIGURE A2. OC-Curve for *Salmonella* sampling plan n = 60 (25 g) c = 0.

ation the need to increase confidence in the safety of the product, encourage the production of a safer product and minimize the impact on the availability of the product. Sensitivity analysis on the impact of varying the number of samples is presented in Table A2 and Figure A3.

TABLE A2. Sensitivity analysis on the impact of the number of samples tested on performance of the sampling plan (SD = 0.4)

Number of samples	Tolerated microbial load (95% rejection rate) Log ₁₀ cfu/g (cfu/kg)	Tolerated microbial load (95% rejection rate) 1 cfu per X g
20	-2.18 (7 cfu/kg)	151
30	-2.37 (4 cfu/kg)	234
40	-2.5 (3 cfu/kg)	316
50	-2.6 (2.5 cfu/kg)	398
60	-2.69 (2 cfu/kg)	490

Table A2 indicates that with an increasing sample size, one increases the probability of rejecting contaminated product, so, for example, with 20 samples taken, product with approximately 7 cfu/kg will be rejected 95% of the time; with 40 samples taken, product with 3 cfu/kg will be rejected 95% of the time; and with 60 samples taken, product with 2 cfu/kg will be rejected 95% of the time. Thus these sampling plans present quite a different scenario from the current sampling plan of taking 1 sample where one is tolerating product where the 95% rejection cut off is 333 cfu/kg. Considering that for most of these products a serving size is 90 g, accepting product with up to 333 cfu/kg means that, with homogenous distribution of the organism, a 90 g sachet or serving could contain approximately 33 cfu. In comparison with 20 samples, the contamination rate would be less than 1 cfu per sachet, indicating that the organism would be absent in some sachets. Using a dose-response of approximately 0.25% probability of illness for 1 cfu, one should be aware that accepting product with approximately 1 cfu per sachet indicates a willingness to tolerate 1 potential illness in every 400 sachets consumed.

However, a greater degree of confidence in the safety of the product comes with a price in terms of product rejection (Figure A3). As the level of contamination of the product decreases, the difference between the sampling plans in terms of their capacity to distinguish between contaminated and non-contaminated product decreases. Similarly if the level of contamination of the product is sufficiently high, all sampling plans are equally effective in rejecting such badly contaminated product. The differences lie in their effectiveness to reject product contaminated at

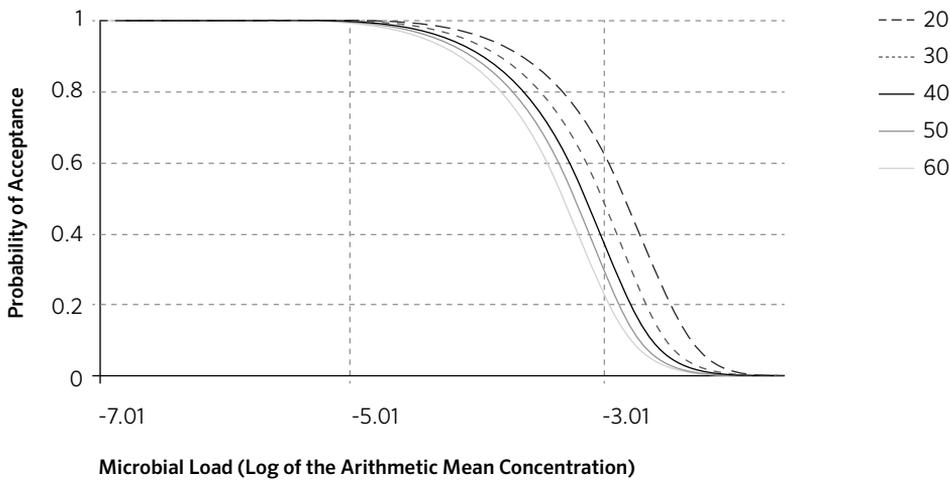


FIGURE A3. Impact of number of samples on acceptance and rejection of product

levels of between approximately 0.3 and 3 cfu/kg. Outside of these levels the plans begin to exhibit similar performance characteristics.

Given what we know from the snap-shot study, where *Salmonella* spp. was only detected in 2 out of 1061 samples analyzed (1 sample out of 51 samples of product X, and 1 out of 50 samples of product Y), it is assumed that in general the potential level of contamination in the product is low. Therefore, the sampling plan should be able to detect these low levels. However, at a level of contamination of -3.01 log₁₀ cfu/g, which is approximately 1 cfu/kg product, a sampling plan of n = 60 will result in rejection of product contaminated at this level approximately 80% of the time. In comparison n = 20 will mean that the same product will only be rejected 40% of the time. Both of these scenarios assume a standard deviation of 0.4.

Recommending a sampling plan for *Salmonella* spp. therefore cannot be based on risk alone, but needs also to consider cost in terms of the analytical testing and product rejection, confidence in suppliers, and the level of risk or uncertainty that the purchaser is willing to accept. Other factors to be considered include the fact that the organism is unlikely to multiply in this product. In comparison, there is a potential for multiplication of the pathogen in powdered infant formula after reconstitution, and in this case Codex recommends n = 60. This suggests that for products that are not further handled before consumption in a manner which would allow growth of organisms such as *Salmonella* spp., consideration of a less stringent sampling plan would be appropriate.

Bearing in mind that there is no such thing as zero risk, sampling only provides a specified degree of confidence in the safety of the product, and safety can only be assured through control of ingredients and the production process, and that these interim criteria should also serve to drive improvement in terms of the safety of these products, the proposed sampling approach for *Salmonella* spp. is based on the number of samples required to ensure that the plan will detect <1 cfu per serving/sachet (90 g) 95% of the time, while minimizing the amount of product to

Purpose of the Microbiological criterion	To monitor the safety of RUTF/RUSF and determine if product should be released for human consumption
The food, process or food safety control system to which the microbiological criterion applies	Production of RUTF/RUSF
The specified point in the food chain where the microbiological criterion applies	End product
The micro-organism(s) and the reason for their selection	<i>Salmonella</i> spp.
The microbiological limits	Absence in 25 g
A sampling plan defining the number of sample units to be taken (n), the size of the analytical unit and where appropriate, the acceptance number (c);	n = 25 Analytical sample size: 25 g c = 0
The statistical performance of the sampling plan	This sampling plan will reject product at a level of 5 cfu/kg 95% of the time with a standard deviation of 0.4, and 8 cfu/kg 95% of the time with a standard deviation of 0.8 (see Figure A4)
Analytical methods and their performance parameters	ISO or equivalent (if pooling samples, it is critical that the method is validated for this purpose so that the level of detection is not adversely affected, as this would otherwise affect the performance of the sampling plan)
Action to be taken when the criterion is not met	A batch of product not meeting the above criterion should be rejected and should not enter the human food chain

be rejected and considering a standard deviation of both 0.4 and 0.8. As a result, a sampling plan is $n = 25$ (25 g samples) and $c = 0$ is proposed. The operating characteristics of this sampling plan are provided in Figure A4.

Basis for the proposal for a microbiological criterion for Enterobacteriaceae

The microbiological family Enterobacteriaceae consists of a large group of bacteria, some of which (like *Salmonella*) are well-known human pathogens, some are opportunistic human pathogens, and some generally do not infect and cause illness in people. Enterobacteriaceae are widespread in nature and commonly contaminate foods and food ingredients. Enterobacteriaceae are useful indicators of the microbiological quality of ingredients and the state of hygiene of the production environment.

The technical meeting in December 2012 considered that more information on the safety of the product would be gained by replacing the narrow end-product *Cronobacter* species microbiological standard with broader quantitative standards for Enterobacteriaceae. This would provide information on the possible levels of all members of the family, including but not limited to *Cronobacter* spp., while at the same time providing better information on process control through trend analysis of quantitative Enterobacteriaceae data.

In proposing a microbiological criterion for Enterobacteriaceae, the following aspects were taken into account:

- The results of the snapshot study and the potential ability of suppliers to meet any proposed criteria.
- The ability to detect and reject any product with high Enterobacteriaceae counts (while no explicit correlation studies exist for the product of concern on the correlation between Enterobacteriaceae and members of the Enterobacteriaceae family which are potentially pathogenic, in general the higher the Enterobacteriaceae counts the greater the risk that the product is contaminated with a potential pathogen).
- The desire to drive improved process control by the suppliers without strong punishment for a small number of samples slightly exceeding the proposed limit.
- The desire to progressively move towards a more stringent criterion for Enterobacteriaceae, reflective of optimal control measures during production.

Based on the above considerations, a criterion of $n = 10$, $c = 2$, $m = 10$, $M = 100$ is proposed for Enterobacteriaceae. This is clearly an interim criterion. Given the results available from the snapshot study, it is something that should be achievable

by many of the suppliers, but nonetheless should identify those with high levels of Enterobacteriaceae and that are therefore not in control of their manufacturing process or the safety of the product they are producing.

In the medium term the objective should be to move towards more stringent criteria for Enterobacteriaceae. For example, the end target could be to move towards the absence of Enterobacteriaceae in finished product, as is currently required for powdered formulae for infants and young children. Such a sampling plan would

Purpose of the Microbiological criterion	To monitor the safety of RUTF/RUSF and determine if product should be released for human consumption
The food, process or food safety control system to which the microbiological criterion applies	Production of RUTF/RUSF
The specified point in the food chain where the microbiological criterion applies	End product
The micro-organism(s) and the reason for its selection	Enterobacteriaceae
The microbiological limits	$m = 10$, $M = 100$,
A sampling plan defining the number of sample units to be taken (n), the size of the analytical unit and where appropriate, the acceptance number (c);	$n = 10$ Analytical sample size: 10 g $c = 2$
The statistical performance of the sampling plan	This sampling plan will allow acceptance of product with 15 cfu/g 5% of the time with a standard deviation of 0.4 ; and 50 cfu/g 5% of the time with a standard deviation of 0.8 (see Figures A5a and A5b)
Analytical methods and their performance parameters	ISO or equivalent
Action to be taken when the criterion is not met	A batch of product not meeting the above criterion should be rejected and should not enter the human food chain. While up to 2 marginally acceptable samples are accepted, the supplier should be informed when such a result is received, as this may be an initial indication of a loss of process control.

allow acceptance of product with 1 cfu/10 g 5% of the time, with a standard deviation of 0.4, and approximately 3 cfu/10 g 5% of the time, with a standard deviation of 0.8 (see Figures A6a and A6b). Thus, this plan is much more stringent and requires a high level of process control. As a result, it also gives a higher degree of confidence in the safety of the product. The implementation of such a standard at this stage would likely lead to a high level of product rejection, and therefore moving towards such a criterion will require an incremental approach.

3. IMPLICATIONS OF THE PROPOSED MICROBIOLOGICAL CRITERIA FOR THE CONTROL OF *CRONOBACTER* SPP.

According to Codex guidelines (CAC, 2013) the need for a microbiological criterion should be demonstrated, usually either by epidemiological evidence that the food under consideration may represent a significant public health risk and that a criterion is meaningful for consumer protection, or as the result of a risk assessment. The current data available for *Cronobacter* spp. do not provide such a justification and there is no indication that the existing criterion of absence of

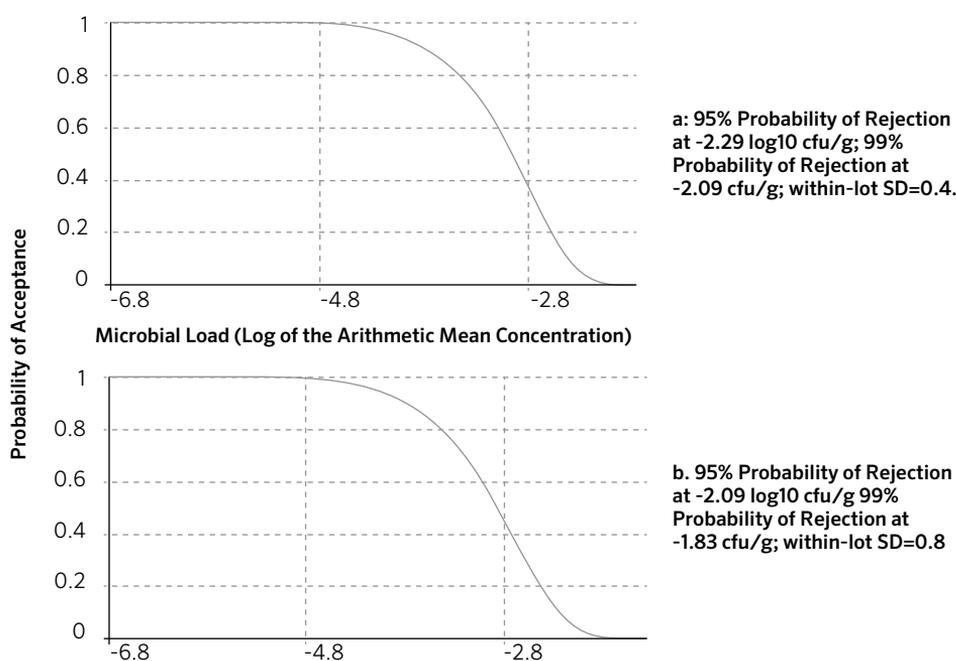
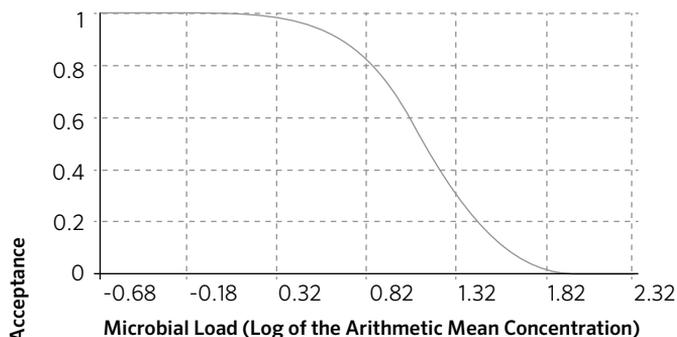
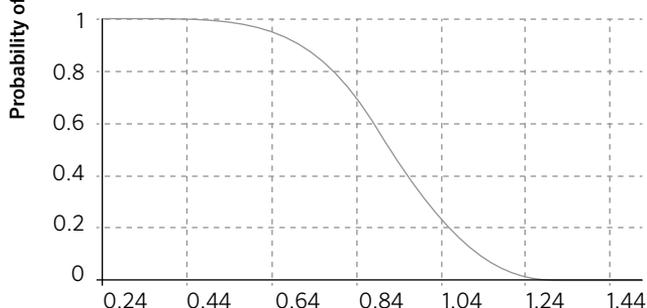


FIGURE A4. OC-Curve for *Salmonella* sampling plan $n = 25$; $c = 0$.

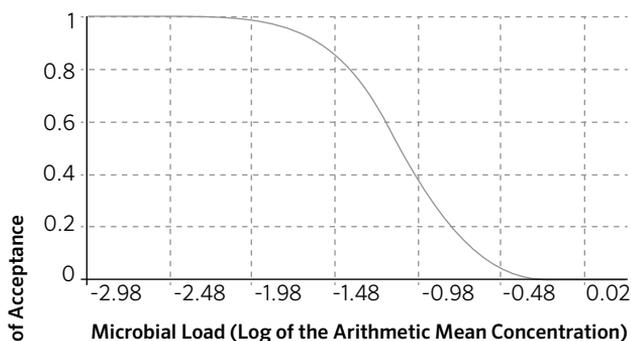


a. 95% Probability of Rejection at 1.19 log₁₀ cfu/g; 99% Probability of Rejection at 1.30 log₁₀ cfu/g; within-lot SD = 0.4.

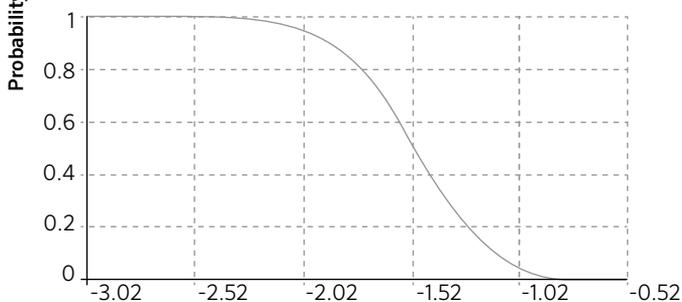


b. 95% Probability of Rejection at 1.69 log₁₀ cfu/g, 99% Probability of Rejection at 1.90 cfu/g; within lot SD=0.8.

FIGURE A5. OC-Curve for Enterobacteriaceae sampling plan $n = 10$; $m = 10$ cfu/g; $M = 100$ cfu/g; and $c = 2$.



a: 95% Probability of Rejection at -1 log₁₀ cfu/g; 99% Probability of Rejection at -0.83 log₁₀ cfu/g; within-lot SD = 0.4



b: 95% Probability of Rejection at -0.47 log₁₀ cfu/g 99% Probability of Rejection at -0.22 log₁₀ cfu/g; within-lot SD = 0.8

FIGURE A6. OC-Curve for Enterobacteriaceae sampling plan $n = 10$; $m =$ presence; and $c = 2$

Cronobacter spp. or even a criterion of 10 cfu/g is meaningful for consumer protection. This does not override the fact that the consumer should be protected from the potential risk posed by *Cronobacter* spp., but highlights that, based on our current knowledge, the establishment of a microbiological criterion is not the most effective way to control the safety of the product. As noted in the report of the December 2012 technical meeting, more stringent controls during processing and of the ingredients will decrease any potential risk associated with the presence of *Cronobacter* spp.. Furthermore, one can infer from elevated Enterobacteriaceae levels an increased risk of the presence of *Salmonella* spp., pathogenic *E. coli*, *Klebsiella* spp., *Cronobacter* spp. and other potential pathogens, but the presence of none of those pathogens will track exactly with Enterobacteriaceae. Any data collection efforts that are implemented will be useful to address the many unknowns that remain about the presence of *Cronobacter* spp. in the products and the possible role of the products in the transmission of *Cronobacter* spp. to consumers, and may in the future indicate whether it is indeed justified to establish a microbiological criterion for *Cronobacter* spp. Thus, the absence of a recommended criterion for *Cronobacter* spp. does not imply the absence of health risk for the consumers of RUTF and RUSE, or suggest that safe levels of exposure to *Cronobacter* spp. are known. Rather, the absence of recommended criteria for *Cronobacter* spp., and replacement with the general criteria for Enterobacteriaceae for these interim criteria, is an admission of inadequate data to establish a basis for making a specific *Cronobacter* spp. recommendation.

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Globally, approximately 180 million children suffer from malnutrition, of which an estimated 40 million annually develop severe, acute malnutrition. Efforts to mitigate severe acute malnutrition and prevent moderate acute malnutrition from developing further need to be multifaceted, given the range of contributors to malnutrition. Providing foods with optional nutrients and free from pathogens and toxins to the extent feasible is an important part of this. The development of lipid-based ready-to-use foods for the management of severe acute malnutrition and moderate acute malnutrition, which provide necessary energy, fatty acids, protein, and micronutrients tailored to the needs and tastes of the children, has become an important tool in community-based management of acute malnutrition, and has facilitated expanded coverage of programmes dealing with malnutrition. However, the detection of *Cronobacter* spp in these products in 2012 highlighted the importance of also giving more attention to the safety of these products for the intended population of young children.

This is the first report to look into the microbiological safety of these products. It highlights the organisms of concern and the actions that need to be taken by producers and purchasers of these products in order to minimise the food safety risk for the intended consumer.

This volume and others in this Microbiological Risk Assessment Series contains information that is useful to both risk assessors and risk managers, at both national and international level including international agencies which purchase and distribute lipid-based ready-to-use foods, the Codex Alimentarius Commission and national regulatory authorities, as well as the producers and suppliers of these products.

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