ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

SAFE PREPARATION OF POWDERED INFANT FORMULA

1. The Department of Health (DH) is seeking further advice from the Food Standards Agency (FSA) on the safe preparation of powdered infant formula (PIF) with respect to microbiological risks. Preparing standard PIF using hot water (>70^{o}C) has been the recommended method in the UK for many years. The World Health Organisation (WHO) recommends hot water preparation for PIF and similar advice exists in a number of countries. The basis for the advice is the recognition that there is an increased risk to infants from harmful bacteria if PIF is prepared with water at lower temperatures which may permit survival and growth depending on the subsequent storage and feeding conditions. Preparation, storage and feeding are important factors in relation to risk from reconstituted PIF whether in the home or other settings and current advice seeks to take these into account in framing best practice for caregivers.

2. This paper is seeking the committee's views on the relative risk of different preparation, storage and feeding scenarios particularly those associated with reconstitution of PIF using water at different temperatures.

3. Information on the current advice on preparation of PIF and the impact of the current guidelines is provided at Annex A. The following sections set out rationale for the current advice and the findings from the application of an FAO/WHO risk assessment model (**http://www.fstools.org/esak/)** to examine the relative risk of different preparation scenarios for PIF.

Rationale for the current advice

The bacterial hazard(s)

4. Microbiological hazards associated with PIF have been extensively reviewed by expert groups and reported in the international literature [15,16,17,18,19]. The main microbiological hazards associated with PIF are *Cronobacter* spp. (formally *Enterbacter sakazakii*) and *Salmonella enterica* based on clear evidence of a causal association between their occurrence in PIF and illness in infants. *Cronobacter* has tended to present as sporadic cases of illness although outbreaks have been reported in hospitals. *Salmonella enterica* is usually associated with outbreaks [15,16,17]. A range of other potential microbiological hazards associated with PIF were considered by two FAO/WHO expert consultations concerning PIF [15,16]. There was insufficient evidence of causality for certain other bacteria although it was considered plausible, notably for certain Enterobacteriaceae other than *Cronobacter* and *Salmonella enterica.*

5. Although rare, infection with *Cronobacter* spp. in infants, particularly pre-term, low birth weight and immunocompromised individuals can be serious. Infection can include bacteraemia, meningitis, and necrotising enterocolitis and high mortality rates (40-80%) have been reported particularly amongst neonates [15,16,17,20,21,22,23]. The genus *Cronobacter* was proposed in 2007 and currently includes 7 species

which previously would all have been were classified as *Enterobacter sakazakii*. Information concerning these "new" species in PIF and human infections is beginning to emerge and not all *Cronobacter* spp. have been linked with human infections. *Cronobacter sakazakii* is one of the 7 species currently in the genus and multilocus sequence typing (MLST) has shown a predominance of sequence type 4 of this species in neonatal infections and also in isolates from powdered infant formula [24,25,25a]. There have been numerous documented cases of *Cronobacter* infection which have been linked to PIF contaminated with these organisms [15,16,26].

6. Because *Cronobacter* infections in infants are rare it is difficult to use laboratory notifications or case reports in infants to assess the impact of interventions in relation to PIF. In England and Wales between 1992 and 2012 there were 16 reports of isolation of *Cronobacter* from blood or CSF from infants aged <1 month and 20 from infants aged 1 to 11 months (Source HPA). It is also unclear whether *Cronobacter* can cause less severe illness in infants which may go undiagnosed.

Manufacturing PIF

7. PIF is not a sterile product and controlling *Cronobacter* during PIF manufacture is challenging because these organisms are able to contaminate various sites within the factory environment and in particular, dry processing areas [27,28,29,30]. These organisms can potentially gain access to the processing line and product, since current technology cannot completely eliminate them from the factory environment [15,16,17]. Environmental monitoring for microbiological contamination and in particular for *Cronobacter* is important in order to detect, track and control the occurrence of *Cronobacter* with the aim of preventing contamination of the finished product [15,16,17, 31].

8. Manufacturers of PIF are expected to test their products against food safety criteria for *Salmonella* and *Cronobacter* according to the microbiological criteria regulations (EC 2073/2005). Testing for process hygiene criteria is also undertaken in PIF manufacture. *Cronobacter* has been found in PIF at retail in various countries and at varying frequencies [15,16,17,32]. It is recognised that contamination of PIF with *Cronobacter* can occur at very low levels including in PIF that has been linked to cases of *Cronobacter* infection [15,16,17]. The detection of the organism in large volumes of PIF is challenging even when stringent sampling and testing programmes are employed. It is therefore not possible to give 100% assurance that PIF placed on the market is not contaminated with *Cronobacter*. Jongenburger et al. [33,34] examined the distribution of *Cronobacter* spp. in industrial batches of powdered infant formula looking at the consequences for performance of sampling strategies and the methodology used for enumeration. The organism has been reported to survive in PIF for at least 2.5 years [35] indicating that it is likely to survive during the shelf-life of these products although recovery of these organisms from PIF may decrease over time [36].

Caregiver practices

9. Even if PIF were free of *Cronobacter,* preparation of feeds takes place in environments where cross contamination of the powder or feeding bottle is possible. This could occur from other foods, equipment, surfaces, utensils and the caregiver etc. *Cronobacter* is widespread in the environment and has been isolated from a wide range of foods [15,16,37,38]. In one recent study in the US, *Cronobacter* was recovered from 27% of domestic kitchens which were sampled [39].

10. Microbial contamination and organic soiling of feeding bottles both prior to and after cleaning has been reported. In one study, Enterobacteriaceae were found in 15% (11/75) of "unclean" bottles but none of 75 clean, ready to use bottles [40]. Disinfection methods used for the decontamination of bottles used for feeding powdered infant formula have been shown to be effective if there is adherence to recommended procedures combined with good hygiene [41].

11. Because most cases of infection tend to be sporadic and rare it is difficult to assess the significance of infants ingesting low numbers of *Cronobacter.* Indications from some incidents and outbreaks are that the organism may have grown to high levels prior to or during feeding or perhaps that an infectious dose was acquired through multiple feeds from the same source [15,16].

12. Feeds are not always consumed straight away. There are practical constraints which influence caregiver decisions about immediate feeding such as when they are travelling, sending feeds with infants to nurseries, where there are multiple infants to feed or when the feed is consumed slowly by the infant. The FSA has funded research concerning perceptions about the non-sterile nature of PIF [42]. Caregivers felt that advice to make up bottles as required with freshly boiled water was difficult to put into practice and many made them up in advance. This is reflected in the findings from the more recent infant feeding surveys [13,14].

13. *Cronobacter* has been reported to grow in the temperature range 5.5^{o}C to 45^{o}C and in reconstituted infant formula between 6°C and 45°C with optimum growth being between 37°C and 43°C [15,16]. A more recent study with a 6 strain cocktail reported growth between 6.5^{o}C and 51.4^{o}C and with a very short lag phase for non-heat treated cells [42a]. In the event that reconstituted formula is contaminated with *Cronobacter* from the powder or environment then keeping the feed at between 37°C and 43°C carries a significant risk of growth. The risk and extent of growth occurring is lower at refrigeration temperatures but even here growth may be possible depending on the temperature of the fridge, the temperature and volume of the reconstituted formula and the storage time.

14. Research conducted for WRAP in 2009 [43] examined the temperatures of 50 domestic refrigerators in UK homes. The majority of these refrigerators operated at a mean air temperature of 7°C although it was found that 29% (14/50) were operating at a mean air temperature of 9°C or above with only 29% (14/50) with a mean air temperature of 5°C or less. Clearly a refrigerator with an air

temperature of 6°C or more, which would correspond to 68% (34/50) of refrigerators in the WRAP study, could potentially permit growth of *Cronobacter*, albeit slowly.

Cronobacter risk assessment for PIF

15. We are fortunate that a risk assessment model for *Cronobacter* in PIF has been developed by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) **http://www.fstools.org/esak/** as part of the work to inform the revision of the Codex Code of Hygienic Practice for PIF [17]. The risk assessment addresses PIF that is intrinsically contaminated with *Cronobacter* and considers the impact of preparation, storage and feeding of reconstituted PIF to infants. The model describes the effect that each of the preparation and storage stages has upon the intrinsic microbiological quality of the PIF in terms of *Cronobacter.* The model is available on line and was presented and reviewed at a technical meeting convened by FAO/WHO [16]. A document is available which provides an overview of the risk assessment model together with selected key assumptions and data (**ftp://ftp.fao.org/ag/agn/jemra/r_a_overview.pdf)**.

16. Documentation describing how to run the model including descriptors to aid parameterisation of the key steps of preparation, holding/cooling of formula, active re-warming/cooling and feeding period are also available **http://www.fstools.org/ESAK/RunModel_UsingTheModel.aspx** **http://www.fstools.org/ESAK/RunModel_PrepGuidance.aspx**

Application of the FAO/WHO risk assessment model

17. The FSA has used the model to explore the impact of several key scenarios with regard to preparation, storage and feeding of PIF. The model also enables different *Cronobacter* contamination levels and sampling plans to be included as a risk reduction approach for manufacturers prior to placing product on the market. The mean log concentration was left at the default value set by the model as the focus here was on the preparation and handling scenarios.

18. The outputs of the model are expressed as relative risk reduction compared to the selected baseline scenario which is set at 1.0. The outputs provide a comparison across the range of water temperatures used for reconstitution of PIF and all risk reductions are relative to a baseline preparation of 50°C. The scenarios explored in the work were reconstitution temperatures between 70°C and 20°C, storage of reconstituted PIF at ambient temperature for between 1 and 16 hours, storage of reconstituted PIF in a refrigerator for between 6 and 48 hours and feeding duration of between 2 and 12 hours at an ambient temperature. In all the scenarios cooling/rewarming to feeding temperature was deemed to be done quickly. Annexes B-E provide the outputs generated by the model for these scenarios which will be described in turn.

Reconstitution temperature

19. The temperatures used for mixing the powder were 70^{o}C, 60^{o}C, 50^{o}C, 40^{o}C and 20^{o}C. This cover the range most likely to be used for reconstitution of PIF and includes the currently recommended temperature (70^{o}C), temperatures perceived as presenting a higher risk (40^{o}C and 50^{o}C) and a temperature close to ambient which may be favoured for preparation by some caregivers from a practical point of view (20^{o}C). It was reported earlier that for those parents who made up the formula while out, the majority made up the formula with cold or cooled water. Although ambient (air) temperature was set at 20^{o}C for comparative purposes it is recognised that there are many situations where the ambient temperature may be much higher.

20. Applying the model looking at the impact of reconstitution temperature with no ambient storage, rapid cooling to feeding temperature and feeding within 2 hours the relative risk reduction compared to that using water at 50^{o}C (1.00) was 40^{o}C (+1.46 fold), 20^{o}C (+1.46 fold), 60^{o}C (+5.72 fold) and 70^{o}C (+ >10^{5} fold) (Annex B). Clearly the bactericidal effect of water at 70^{o}C results in a much greater risk reduction relative to the baseline than the other temperatures examined.

21. Preparation of PIF at temperatures of 70^{o}C or above is effective in reducing bacterial contamination in PIF. Studies have shown that reconstitution of PIF with water at 70^{o}C would result in a 4-6 log reduction in *Cronobacter* [15,16,45]. There is also the additional although unquantified benefit of hot water reducing contamination arising from preparation in the kitchen given that Cronobacter are ubiquitous bacteria so there is likelihood that they are present in the domestic environment [39].

Ambient storage

22. The impact of ambient (20^{o}C) storage of reconstituted formula was examined at 1, 2, 4, 8 and 16 hours. When made up formula is taken outside the home, the majority of parents (61%) do not keep it chilled. For this scenario the same reconstitution temperatures were used apart from varying the ambient storage duration at 20^{o}C. Results are presented in Annex C. The greatest risk reduction relative to the baseline was achieved by the 70^{o}C treatment (+ >10^{5} fold for all storage times examined 1-16 hours) relative to a baseline of 50^{o}C with 1 hour storage. Other reconstitution temperature/storage time conditions resulted in decreased risk (+ 43.67 fold for 20^{o}C, storage for 1 hour) to a significantly increased risk (4.37 x 10^{-4} for 40-60^{o}C, storage for 16 hours).

Refrigerated storage

23. The impact of refrigerated storage at 6^{o}C for 6, 12, 24 and 48 hours was examined. This is likely to be a relatively common practice particularly where multiple feeds are made. It should be noted that 6^{o}C is approximately the lowest temperature permitting growth of *Cronobacter* [15,16]. Results are presented in

Annex D. The greatest risk reduction relative to the baseline (50^{o}C with 6 hours storage at 6^{o}C) was achieved by the 70^{o}C treatment (+ >10^{5} fold for all storage times examined 6-48 hours). Most other reconstitution temperature/storage time conditions resulted in decreased risk (+14.76 to 43.20 fold for 20^{o}C, storage for 6-48 hours) to a slightly increased risk (0.3-0.67 for 50^{o}C, storage for 24-48 hours). Kandhai *et al.* [36a] looked at the temperature conditions occurring during cooling of reconstituted PIF and found that heat transfer coefficients were highly variable depending on volume of formula being cooled and that this could be reduced by limiting the volume to portion size only.

Feeding duration

24. Feeding duration of 2, 4, 6, 8 and 12 hours at an ambient temperature of 20^{o}C were examined. Results are presented in Annex E. The greatest risk reduction relative to the baseline (50^{o}C with 2 hours feeding time) was achieved by the 70^{o}C treatment (+ >10^{5} fold for all feeding times examined 2-12 hours). Prolonged feeding periods in excess of 2 hours tended to increase risk relative to the baseline scenario particularly if there was a very long duration of feeding (>6 hours).

Other information concerning the model

25. The FAO/WHO model was considered in detail in the report of the expert consultation in 2006 and further scenarios are explored in the report [16]. In the report it is stated that "*reconstitution of PIF with liquid of 70°C was evaluated to be an effective risk mitigation strategy for all scenarios investigated. The highest risk scenarios were associated with reconstitution at temperatures of 40° and 50°C, when the formula is not consumed immediately. As a result, quick cooling to lower temperatures to minimize growth is essential. When PIF reconstituted at temperatures of 10° or 20°C was evaluated, minimal growth and inactivation was observed, but subsequent holding for long periods at room temperatures, including extended feeding periods, can result in growth and therefore increased risk."*

26. As with all risk assessment models they should be subject to review/revision when significant new data becomes available. A number of key data assumptions /interpretations were made at the time the *Cronobacter* and PIF model was developed for example, parameter values used in the risk assessment model to estimate the growth and decline of the organism in PIF (e.g. lag, optimum growth temperature, *D* and *z* values). There is now further data for some of these parameters in the peer reviewed literature [42a,44-48] which might have an impact on the current model although this would need to be assessed.

Summary

- Although illness in infants associated with PIF is very rare the impact of infection can be severe particularly in pre-term, low birth weight or immunocompromised individuals.

- PIF is not sterile and environmental contamination in the manufacturing environment can lead to occasional contamination of PIF with low levels of potentially harmful organisms notably *Cronobacter.*

- The FAO/WHO risk assessment model shows that reconstitution of PIF with hot water (at 70^{o}C) results in a substantially reduced risk from *Cronobacter* relative to reconstitution with water at lower temperatures. The choice of reconstitution temperature impacts on the relative risk associated with subsequent handling parameters (ambient storage, refrigerated storage, feeding duration) with reconstitution at 40-50^{o}C generally leading to a higher relative risk. Risk reduction due to reconstitution with hot water (at 70^{o}C) is also likely to have an impact on other hazards including *Salmonella enterica* and other Enterobacteriaceae.

- Studies have shown that feeding bottles can be contaminated with bacteria and hot water preparation is likely to have some impact in reducing microbiological contamination arising from the equipment or environment during the preparation of feed. There are few studies which have looked at the extent and likelihood of contamination from this source during reconstitution and subsequent handling.

- The FAO/WHO risk assessment indicates that caregiver compliance with feeding straight away is likely to be more critical with feeds prepared at <50^{o}C than for feeds reconstituted at 70°C or more. Caregiver practices and duration are likely to have an important influence on bacterial multiplication in reconstituted feed. There are indications from recent surveys in the UK that that a proportion of caregivers continue to make up multiple rather than single feeds and subsequent storage (ambient, refrigeration) and feeding duration will influence risk. When taking made up formula outside the home, the majority of parents did not keep it chilled. Duration of ambient storage and feeding are important considerations particularly if the feed is reconstituted with water at temperatures with little or no bactericidal effect.

Action

27. The committee is asked to comment on the following areas:

    a) The information provided regarding microbiological hazards associated with powdered infant formula, its preparation and use.

    b) The risk reduction achieved by different preparation scenarios using the FAO/WHO model and their relative importance.

    c) The conclusion that the reconstitution of PIF with water at 70^{o}C can make a significant contribution to risk reduction from i) intrinsic contamination of PIF and, ii) extrinsic contamination arising from equipment and the preparation environment.

Secretariat
June 2013

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Annexes

Annex A - Current advice on preparation of PIF and the impact of the guidelines

Annex B - Risk assessment output from the FAO/WHO model – Effect of different reconstitution temperatures.

Annex C - Risk assessment output from the FAO/WHO model – Effect of different reconstitution temperatures and ambient storage for 1-16 hours.

Annex D - Risk assessment output from the FAO/WHO model – Effect of different reconstitution temperatures and subsequent refrigerated storage at 6^{o}C for 6-48 hours.

Annex E - Risk assessment output from the FAO/WHO model – Effect of different reconstitution temperatures and feeding times between 2 and 12 hours at ambient temperature.

Annex A

Current advice on preparation of PIF

1. DH and the FSA issued advice concerning safe preparation of PIF in December 2006 and further advice was issued in January 2010 [1,2]. The advice has been incorporated into various documents and publications including the NHS "Birth to Five" book [3] and advice on the NHS choices website [4]. Reconstitution of PIF with hot water (at least 70^{o}C) is integral to this advice. The advice does not advocate cooling boiled water specifically to 70^{o}C but seeks to attain a water temperature of at least 70^{o}C after the water has been boiled and left to cool. Caregivers are encouraged to make up single rather than multiple feeds then cool to feeding temperature and discard any unused feed after 2 hours feeding. If feeds are made up and stored in the refrigerator they should be used within 24 hours. The purpose of the advice is to give caregivers in the home and other care settings, including nurseries and childminders, practical advice that can be followed.

2. A guideline for making up special feeds for infants and children in hospitals was published by the Pediatric Group of the British Dietetic Association in 2007 [5]. This included detailed advice about the safe preparation and storage of powdered infant formula for health professionals in hospitals, especially intensive care units. Specific advice in relation to formula for special medical purposes has not been published by the FSA although it is recognised that in some cases preparation of these products with water at >70^{o}C is not possible. No specific FSA advice has been published with regard to powdered infant formula containing probiotics.

3. The WHO advice on preparation of PIF with water at a temperature of at least 70^{o}C is effective in reducing bacterial contamination in general and importantly *Cronobacter* and *S. enterica* in the unlikely event that these organisms are present in the powder [15,16,37]. Reconstitution of PIF with water at 70^{o}C or more is effective in destroying *Cronobacter* [15,16,45] whereas preparation at lower temperatures will be much less effective, or even ineffective in the event that the feed becomes contaminated from the preparation environment.

4. The European Food Safety Authority (EFSA) Biohazards panel has published an opinion related to the microbiological risks in infant formulae and follow-on formulae [10]. The opinion indicated that guidelines should be developed for reconstitution, handling, storage and use in the home and in hospitals. The opinion indicated that for the home PIF should be reconstituted in hot water (>70°C) or water that has been boiled and cooled, avoiding recontamination. Where PIF was reconstituted in hospitals it was recommended to always use hot water (>70°C) again avoiding recontamination [10]. Guidance from Ireland, the USA and Canada recommends use of pre-boiled hot water (i.e. 70^{o}C or more) for some preparations of PIF [7,8,9,10]. However adoption of hot water preparation is not universal with advice in some countries recommending lower temperatures for reconstitution[11].

5. The FSA has worked with the British Specialist Nutrition Association Ltd (BSNA) to encourage members selling PIF products in the UK to adopt a more consistent approach to labelling of their products with respect to preparation, storage and use. This includes the adoption of hot water preparation (at least 70^{o}C) for PIF other than for certain formulae intended for special medical purposes where hot water preparation would be detrimental to the intended use. The BSNA has recently indicated which products comply with DH guidance on best practice [12].

Impact of the guidelines

6. Findings from UK infant feeding surveys show that there has been a marked reduction in caregivers making up several feeds at a time from 69% in 2005 to 26% in 2010 [13]. In 2010, 71% of caregivers followed the guidelines on preparation with hot water compared with 59% in 2005 [13]. More recently in the Diet and Nutrition Survey of Infants and Young Children, 2011 it was found that the majority of parents feeding their child infant formula in the home followed the recommendations for preparation of PIF with 79% reporting making up the formula as needed and 68% using water that had been boiled and left to cool for no longer than 30 minutes [14]. A higher proportion (23%) of parents of children aged 4 to 6 months made up several feeds at once compared to 14% of parents with children aged seven months or over [14].

7. When reconstituted formula was taken outside the home, the majority of caregivers (61%) did not keep it chilled and the proportion was slightly higher for parents of infants aged 4 to 6 months (64%) than older ones [14]. For those who made up feeds away from home, the majority used cold or cooled water, which is not in line with current recommendations [14]. The proportion making up the feed with hot water either obtained while out of the home or carried in a flask, was less than 50% for all age groups [14].