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DRAFT SCIENTIFIC OPINION

Draft Scientific Opinion on the essential composition of infant and follow-on formulae [1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on the essential composition of infant and follow-on formula. This Opinion reviews the Opinion provided by the Scientific Committee on Food in 2003 on the essential requirements of infant and follow-on formulae in light of more recent evidence and by considering the Panel's Opinion of October 2013 on nutrient requirements and dietary intakes of infants and young children in the European Union. The minimum content of a nutrient in formula proposed in this Opinion is derived from the intake levels the Panel had considered adequate for the majority of infants in the first six months of life in its previous Opinion and an average amount of formula consumed during this period. From a nutritional point of view, the minimum contents proposed by the Panel cover the nutritional needs of virtually all healthy infants born at term and there is no need to exceed these amounts in formulae, as nutrients which are not used or stored have to be excreted and this may put a burden on the infant's metabolism. Therefore, the Panel emphasises that maximum amounts should not be interpreted as target values but rather as upper limits of a range which should not be exceeded.

KEY WORDS

infant formula, follow-on formula, composition, public consultation

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[1] On request from the European Commission, Question No EFSA-Q-2013-00264, endorsed for public consultation on 10 April 2014.

[2] Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on the essential composition of infant and follow-on formula. This Opinion reviews the Opinion provided by the Scientific Committee on Food (SCF) in 2003 on the essential requirements of infant and follow-on formulae in light of more recent evidence and by considering the Panel's Opinion of October 2013 on nutrient requirements and dietary intakes of infants and young children in the European Union.

There is scientific consensus that breast milk is the preferred food for all healthy infants and provides an adequate supply of all nutrients to support healthy growth and development (with the exception of vitamin K during the first weeks of life and of vitamin D).

All formulae intended for infants must be safe and suitable in meeting the nutritional requirements and to promote growth and development of infants born at term when used as a sole source of nutrition during the first months of life and when used as the principal liquid element in a progressively diversified diet after the introduction of appropriate complementary feeding. Nutrients and substances should be added to formulae for infants only in amounts that serve a nutritional or other health benefit. The addition in amounts higher than those serving a benefit or the inclusion of unnecessary substances in formulae may put a burden on the infant's metabolism and/or on other physiological functions, as substances which are not used or stored have to be excreted.

The minimum content of a nutrient in formula proposed in this Opinion is derived from the intake levels the Panel had considered adequate for the majority of infants in the first half of the first year of life in its previous Opinion and an average amount of daily energy intake from formula during this period (500 kcal/day). These minimum amounts should be understood as target values which cover the nutritional needs of virtually all infants born at term for optimal growth and development, whereas maximum amounts are driven by safety and also taking into account technological considerations and should not be interpreted as target values but rather as upper limits of a range, which should not be exceeded.

Specifications for the currently permitted maximum amounts of micronutrients in formulae were generally calculated as three to five times the minimum amounts established at the time and took into account established history of apparent safe use (Codex Stan 72-1981, Codex Stan 156-1987, the Directive 2006/141/EC, and the SCF) and were not based on scientific evidence for adverse effects owing to the lack of such evidence for most nutrients.

There are no reports on any adverse effects associated with the use of formulae complying with the current specifications for micronutrients as laid down in Directive 2006/141/EC, although there are no studies available which were designed to investigate the short or long-term health consequences of consumption of formulae containing the currently permitted maximum amounts of micronutrients in infant or follow-on formula. Assuming an energy intake from formula of 500 kcal/day (average of the average requirement for energy of boys and girls aged three to four months), regular consumption of a formula by an infant containing the currently permitted maximum amounts of zinc, iodine, vitamin A and folate (if the whole amount is provided in the form of folic acid) would imply that the Tolerable Upper Intake Levels is exceeded for these nutrients. Assuming an energy intake from formula of 700 kcal/day (highest observed mean energy intakes in infants below six months of age), also intakes of selenium would also exceed the Tolerable Upper Intake Level. The Panel acknowledges the Tolerable Upper Intake Levels used in this estimation were those derived for young children and there is uncertainty with respect to the extrapolation to infants.

Cow's milk, goat's milk and isolated soy protein are safe and suitable protein sources for use in infant and follow-on formula based on intact protein. The use of other protein sources in infant and follow-on formula and/or the introduction of new technologies need clinical evaluation and their safety and

suitability should be established in the target population prior to their general use in infant and follow-on formula.

Formulae containing protein hydrolysates are insufficiently characterised by the declared protein content even if they fulfil regulatory criteria concerning amino acid patterns; therefore the safety and suitability of each specific infant and follow-on formula containing protein hydrolysates has to be established by clinical evaluation in the target population.

The use of a default conversion factor of 6.25 is proposed to calculate the protein content from the total nitrogen content, irrespective of the protein source.

Infant and follow-on formula should provide on an energy basis indispensable and conditionally indispensable amino acids in amounts at least equal to the reference protein (i.e. breast milk), irrespective of the protein source.

There is no necessity to add arachidonic acid, eicosapentaenoic acid, non-digestible oligosaccharides, “probiotics” or “synbiotics”, chromium, taurine, nucleotides, to infant and follow-on formula. There is also no necessity to use phospholipids as a source of long-chain polyunsaturated fatty acids instead of triacylglycerols in infant and follow-on formula or to use triacylglycerols with palmitic acid predominantly esterified in the *sn*-2 position in infant and follow-on formula instead of triacylglycerols from other fat sources. For follow-on formula, contrary to infant formula, the addition of L-carnitine, inositol and choline is not necessary.

The Panel did not consider it necessary to propose specific compositional criteria for formulae consumed after one year of age, as formulae consumed during the first year of life can continue to be used by young children.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Directive 2009/39/EC on foodstuffs intended for particular nutritional uses lays down general rules[4] on the composition of such foods that are specially designed to meet the particular nutritional requirements of the persons to whom they are intended, including infants and young children in good health.

One of the measures adopted under that framework legislation is Commission Directive 2006/141/EC on infant formulae and follow-on formulae[5]. That Directive was adopted originally in 1991 and revised globally in 2006.

The Directive defines 'infants’ as "children under the age of 12 months" and 'young children’ as "children aged between one and three years".

The Directive also defines 'infant formulae' as "foodstuffs for particular nutritional use by infants during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding" and 'follow-on formulae' as "foodstuffs intended for particular nutritional use by infants when appropriate complementary feeding is introduced and constituting the principal liquid element in a progressively diversified diet of such infants".

The Directive sets essential requirements for the composition of infant formula and follow-on formula, which are based on a number of opinions of the Scientific Committee on Food, the latest one being the "Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae", adopted on 4 April 2003[6]. In the last ten years, scientific and technological developments on the essential composition of these products have progressed and there are increasing calls for a review of the legislation to reflect such developments.

The Commission's proposal for a Regulation of the European Parliament and the Council on foods intended for infants and young children and on food for special medical purposes[7] aims at revising the legal framework applicable to food for particular nutritional uses and, among others, at repealing Directive 2009/39/EC. Negotiations on the proposal are reaching their conclusion and it is expected that such Regulation will be adopted in the next months.

Once the new Regulation is adopted, the Commission will need to adopt delegated acts setting specific rules for the categories of food covered by the Regulation, including infant formulae and follow-on formulae.

In the last years, increasing numbers of milk-based drinks and similar products are marketed in different Member States with the denomination of 'growing-up milks' or 'toddlers' milks' or with similar terminology. The composition of these products varies with respect to the protein origin (they can be derived from protein of animal or vegetable origin such as cows' milk, goats' milk, soy or rice) and other ingredients. They are promoted as being particularly suitable for young children and, as such, under the current rules, may be considered as foodstuffs for particular nutritional uses. However, no composition requirements for these products are set in EU legislation.

Different views exist in the scientific community and among stakeholders on whether these products are necessary to satisfy the nutritional requirements of young children or have any nutritional benefits when compared to other foods that can constitute the normal diet of young children. In this context,

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[4] Directive 2009/39/EC of the European Parliament and of the Council of 6 May 2009 on foodstuffs intended for particular nutritional uses, OJ L 124, 20.5.2009, p. 21.

[5] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p. 1.

[6] Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae, adopted on 4 April 2003, SCF/CS/NUT/IF/65 Final, 18 May 2003.

[7] COM (2011) 353.

some would argue that, given the potential variability of weaning diets that may result in different nutrient intakes for this group of the population, these products are convenient, as a liquid element in the diet of young children, in contributing to meeting their nutritional requirements. Taking all these elements into account, the European Parliament and the Council agreed that these products should be subject of a specific reflection. Therefore, in the abovementioned revision of the legal framework, the Commission will be requested, after consulting the European Food Safety Authority, to draft a report on the necessity, if any, of special provisions for milk-based drinks and similar products intended for young children (hereinafter 'growing-up milks').

In the meantime, at international level, the Codex Committee on Nutrition and Food for Special Dietary Uses (CCNFSDU) agreed at its 34th session in December 2012 to revise their existing standard for follow-up formulae[8], which dates back to 1987 and applies to food intended for use as a liquid part of the weaning diet for the infant from the 6th month on and for young children up to three years of age. Such review will cover all aspects of the existing standard and will include consideration of issues such as technological and scientific developments in follow-up formula production and composition over the past 25 years, the age range of the intended population, product definition and the role of such products in the diet of infants and young children. Furthermore, following comments by WHO and some Codex Member Countries and observers, the review may also consider whether this standard is still necessary at all. The first discussion on this subject has taken place at the session of the CCNFSDU held on 4-8 November 2013.

Taking into account the developments described above, it is considered necessary to request the EFSA to provide a scientific opinion on all milk-based drinks and similar products intended for infants and young children.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1) (a) of Regulation (EC) No 178/2002[9], the European Commission asks EFSA to:

- Provide advice on the nutritional requirements of infants and young children and, in particular, on those requirements that may be satisfied by breast milk, milk-based drinks and similar products. In this context it will also be important to provide advice to the Commission on how these nutritional requirements evolve during the age period 0-3 years.

- Provide advice on the essential composition requirements of infant formulae and follow-on formulae by updating the relevant opinions of the SCF on the matter.

- Provide advice on the importance of the role that 'growing-up milks' may have as a liquid element in the diet of young children, with respect to elements such as the pattern of consumption, the nutritional intake and any other relevant aspect related to exposure to substances that may be present in their diet. In this context it would be useful to take into account that different products are on the market which may have a considerably varied composition.

- Provide advice on whether 'growing-up milks' are necessary to satisfy the nutritional requirements of young children or have any nutritional benefits when compared to other foods that may be included in the normal diet of young children (such as breast milk, infant formulae, follow-on formulae, cows' milk and other similar products).

- If considered appropriate, advise the Commission with respect to the appropriate age range and the essential composition of 'growing-up milks'.

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[8] CODEX STAN 156-1987.

[9] OJ. L 31, 01.02.2002. p. 1.

ASSESSMENT

1. Introduction

The period of infancy is characterised by special needs in nutrition, with respect to requirements for energy and for nutrient amounts per kilogram body mass, which must not only maintain the body but also support a rapid growth rate and the appropriate synthesis and deposition of body tissue. A special feature of young infancy is that, as a rule, one liquid food is the sole source of nutrition and must supply appropriate amounts of energy, water and nutrients.

Comparative studies in affluent countries have indicated important health advantages of breast-feeding over formula-feeding for the recipient infants, such as lower incidence rates of gastrointestinal and respiratory tract infections (Ip et al., 2007; Agostoni et al., 2009; Hörnell et al., 2013b), otitis media (Hörnell et al., 2013b) and a lower risk of overweight and obesity (von Kries et al., 1999; Toschke et al., 2002; Owen et al., 2005; Hörnell et al., 2013b).

Infant formulae (IF) and follow-on formulae (FOF) have been regulated as foods for particular nutritional uses under Directive 2009/39/EC[10] and its implementing Directive 2006/141/EC[11] based upon a series of reports from the Scientific Committee on Food (SCF, 1983, 1989, 1991, 1993b, 1995, 2003a) and EFSA (EFSA, 2005e; EFSA NDA Panel, 2012c). No revision of the SCF reports in the light of new available evidence has been undertaken since then, and such review in the context of the revision of the implementation of Regulation (EU) No 609/2013[12] is part of the present Terms of Reference (ToR).

Owing to the limited time frame given, the Panel has decided, in agreement with the European Commission, to produce two separate Opinions. Of the five parts of the ToR:

- nutritional requirements of infants and young children and their coverage by human milk and milk-based products,
- advice on the necessity of a revision of the essential composition of IF and FOF as laid down in Directive 2006/141/EC,
- the potential role of milk-based drinks designed, manufactured and advertised to be used in the diets of infants and young children other than IF and FOF,
- a comparison of the nutritional role of such other milk-based drinks in the diet of young children with other formulae, human milk or cow's milk,
- eventually advice on the essential composition of such other milk-based drinks and their target groups,

the Panel will provide in this second Opinion

1) advice on the necessity of a revision of the essential composition of IF and FOF as laid down in Directive 2006/141/EC, and

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[10] Directive 2009/39/EC of the European Parliament and of the Council of 6 May 2009 on foodstuffs intended for particular nutritional uses, OJ L 124, 20.5.2009, pp. 21–29.

[11] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, pp. 1–33.

[12] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, pp. 35–56.

2) advice on the appropriateness to propose compositional requirements for formulae consumed after one year of life.

Advice on the dietary requirements of infants and young children, an evaluation of dietary intakes of infants and young children living in Europe in comparison with requirements, and advice on the potential role of milk-based drinks designed, manufactured and advertised to be used in the diets of infants and young children, including an evaluation whether they have any nutritional benefits when compared with other foods (such as breast milk, IF, FOF and cow's milk) that may be included in the normal diet of infants and young children has been given by the Panel in a previous Opinion (EFSA NDA Panel, 2013a).

This Opinion will not address compositional requirements of formulae intended for pre-term infants, for very low or low birth weight infants or for infants with specific nutritional requirements. Also the dietary management of cow's milk allergy in infants is outside the scope of this Opinion.

However, the general considerations and the specifications with respect to nutrients or other ingredients proposed in the present Opinion may serve as a basis for defining compositional requirements for foods for special medical purposes for infants, unless the disease conditions for which such foods are to be used necessitate other compositional aspects.

2. Definitions

For this Opinion the following definitions apply:

- Infants means children under the age of 12 months (Article 2(2)(a) of Regulation (EU) No 609/2013).

- Young children means children aged between one and three years (36 months) (Article 2(2)(b) of Regulation (EU) No 609/2013).

- IF means food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding (Article 2(2)(c) of Regulation (EU) No 609/2013 and Codex Stan 72-1981[13]).

- FOF means food intended use by infants when appropriate complementary feeding is introduced and which constitutes the principal liquid element in a progressively diversified diet of such infants (Article 2(2)(d) of Regulation (EU) No 609/2013).

- "Growing-up milk" or "toddlers' milk" are formulae intended specifically for young children. No compositional criteria have been laid down in EU legislation. They may or may not be based on milk. In the latter case they would have to contain other animal or plant protein. The Panel proposes not to use the term "growing-up milk" because this would imply a particular effect on growth. The Panel will also not use the term "toddlers' milk" because it considers that a "young child" is better defined by age. Young-child formula is the term proposed by the Panel for formulae intended for young children. This term includes also formulae based on protein sources other than cow's milk.

- Complementary feeding, as defined by WHO in 2002, is "the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants" so that "other foods and liquids are needed, along with breast milk" (WHO, 2002). In the Panel's Opinion on the appropriate age for the introduction of complementary food (EFSA NDA Panel, 2009) "complementary feeding" means the period, when complementary foods are

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[13] Codex-Stan 72-1981 (Codex Alimentarius), 2011. Standard for infant formula and formulas for special medical purposes intended for infants. Adopted 1981, amended 1983, 1985, 1987, revised 2007, amended 2011.

given together with either human milk or a breast milk substitute. The Panel notes that this definition differs from the definition of "complementary feeding" provided by WHO.

- Complementary food in this Opinion comprises, therefore, all liquid, semisolid and solid foods other than breast milk and IF or FOF that are fed to infants. Complementary food can be beverages, spoon-fed foods or finger food (EFSA NDA Panel, 2009). Cereal-based foods and baby foods are regulated in Directive 2006/125/EC.[14]

3. General aspects of infant feeding

There is scientific consensus that breast milk is the preferred food for all healthy infants and provides an adequate supply of all nutrients to support healthy growth and development (with the exception of vitamin K during the first weeks of life and of vitamin D), besides providing protection against infection and immunostimulatory components (EFSA NDA Panel, 2013a). When complementary food is introduced into the infant's diet, breast milk remains the most appropriate liquid part of a progressively diversified diet (EFSA NDA Panel, 2009, 2013a).

4. Methodological considerations

All formulae intended for infants must be safe and suitable in meeting the nutritional requirements and to promote growth and development of infants born at term when used as a sole source of nutrition during the first months of life and when used as the principal liquid element in a progressively diversified diet after the introduction of appropriate complementary feeding. The safety and suitability of such formulae should be demonstrated by generally accepted scientific evidence.

Even though human milk composition of healthy, well-nourished mothers can provide guidance for the composition of formulae intended for infants, compositional similarity to human milk is not the only appropriate determinant or indicator of safety and nutritional suitability of such formulae. The mere presence of a substance in human milk does not necessarily indicate a specific benefit of this substance for the infant, nor do the concentrations of nutrients in human milk necessarily reflect infants' dietary requirements owing to the fact that they may mirror maternal intakes rather than infants' needs or because absorption efficiency of certain nutrients differ between breast milk and formula. A more suitable approach to evaluate the compositional suitability of formulae intended for infants is to relate health outcomes, including physiological parameters (including growth and development) and biochemical parameters, in formula-fed infants to those of healthy term infants who have been exclusively breast-fed for four to six months. The Panel also notes that nutrients and substances should be added to formulae for infants only in amounts that serve a nutritional or other health benefit. The addition in amounts higher than those serving a nutritional or health benefit or the inclusion of unnecessary substances in formulae may put a burden on the infant's metabolism or on other physiological functions, as substances which are not used or stored have to be excreted.

The compositional requirements of IF and FOF as laid down by Directive 2006/141/EC have been set by specifying the minimum and maximum content of nutrients and other substances in formulae as ready for consumption, including the contribution of water used to reconstitute powdered formulae. The Panel notes that, whereas minimum amounts should be understood as target values which cover the nutritional needs of the majority of infants born at term for optimal growth and development, maximum amounts are driven by safety while also taking into account technological considerations and should not be interpreted as target values but rather as upper limits of a range which should not be exceeded.

4.1. Minimum content of nutrients and other substances in IF and FOF

Minimum amounts of nutrients in formulae should be based on generally accepted scientific evidence which establishes the nutrient requirements of virtually all infants in the target population. The Panel

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[14] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children (Codified version). OJ L 339, 6.12.2006, pp. 16–35.

considers that the minimum content of a nutrient in formula can be derived from the intake levels the Panel had considered adequate for the majority of infants in the first half of the first year of life (EFSA NDA Panel, 2013a) and an average amount of formula consumed during this period. The average amount of formula consumed in the first six months of life is taken to be equivalent to 500 kcal/day by averaging the Average Requirements (ARs) for energy of boys and girls aged three to less than four months (i.e. 479 kcal/day) (EFSA NDA Panel, 2013a) and rounding up. The Panel notes that observed mean energy intakes at this age are generally above the AR. Therefore, the Panel considers observed mean energy intakes not to be a suitable basis for deriving the minimum content of nutrients in formulae. Bearing in mind that the levels the Panel had considered adequate for the majority of infants in the first half of the first year of life take into account inter-individual variability in nutrient requirements and are designed to cover the dietary requirements of at least 97.5 % of infants and that observed formula intakes are generally above the intakes assumed by the Panel, the minimum content derived on that basis can be assumed to be adequate for virtually all infants below six months of age and there is no necessity to provide nutrients in amounts higher than the amounts proposed by the Panel.

The Panel notes that while for a food which is the sole source of energy and nutrients, such as IF, compositional requirements can be based on energy and nutrient needs of the respective population, the evidence for proposing compositional requirements for foods which are not the sole source of energy and nutrients, such as FOF, is less strong, as other foods contribute to nutrient and energy intakes in variable amounts. For the present Opinion, the Panel assumes that energy and nutrient intakes from complementary foods will compensate for higher requirements of infants and for potentially lower feeding volume of formulae in infants receiving complementary foods, unless otherwise specified in the respective Section.

4.2. Maximum content of nutrients in IF and FOF

As the different protein and fat sources used in the manufacture of formula and the water used to reconstitute powdered formula contribute to the total nutrient content of a formula in varying amounts, maximum contents of nutrients have been established in order to ensure the safe use of formula while limiting technological alternations of the initial nutrient contents of food constituents used in the production of formulae.

The Panel notes that specifications for the currently permitted maximum amounts of micronutrients in formulae were generally calculated as three to five times the minimum amounts established at the time and took into account established history of apparent safe use (Codex Stan 72-1981, Codex Stan 156-1987, the Directive 2006/141/EC, and the SCF (2003a)) and were not based on scientific evidence for adverse effects owing to the lack of such evidence for most nutrients.

The Panel acknowledges that the scientific data available to derive Upper Tolerable Intake Levels (UL) for infants remain scarce for most micronutrients and there are no reports on any adverse effects associated with the use of formulae complying with the current specifications as laid down in Directive 2006/141/EC. However, there is a lack of studies designed to investigate the short or long-term health consequences of consumption of formulae containing the currently permitted maximum amounts of nutrients in IF or FOF. Whenever a UL has been established for a specific nutrient for infants or young children, the Panel will note if the continuous consumption of formulae containing the currently permitted maximum amount of that micronutrient could lead to intakes exceeding the UL.

5. Minimum and maximum content of energy and macronutrients in IF and FOF

5.1. Energy

5.1.1. Current compositional requirements of IF and FOF

Based on the Opinion of SCF (2003a), Directive 2006/141/EC lays down a minimum energy content of 60 kcal/100 mL and a maximum energy content of 70 kcal/100 mL. These minimum and maximum values apply both to IF and to FOF and were based on the energy content of breast milk.

5.1.2. Energy density of human milk

The average energy density of human milk has been shown by Butte et al. (2001) to be about 65 kcal/100 mL.

Since then, this value has been confirmed by several recent studies on donor breast milk or own mother's milk (mean ± standard deviation (SD)): 65 ± 11 kcal/100 mL for donor breast milk (Wojcik et al., 2009); 67.3 ± 6.5 kcal/100 mL for own mother's milk, 64.1 ± 5.9 kcal/100 mL for single-donor pooled breast milk, 63.6 ± 4.5 kcal/100 mL for multiple-donor pooled breast milk (de Halleux and Rigo, 2013); 62 ± 9.6 kcal/100 mL to 65 ± 9.1 kcal/100 mL for own mother's milk (Nielsen et al., 2011) and 66 ± 12 kcal/100 mL for donor breast milk (Cooper et al., 2013).

5.1.3. Energy requirements of infants

The energy content of human milk can provide some guidance for the composition of IF and FOF. However, the energy content of human milk changes within one feed. Because the lipid content increases markedly with emptying of the breast, hindmilk has a significantly higher energy content than foremilk (Stam et al., 2013), while formula is of stable composition. Therefore, the knowledge of energy requirements of infants is a key factor to determine the optimal composition of IF and FOF. Energy requirement is the amount of food energy needed to balance energy expenditure in order to maintain body mass, body composition, and a level of physical activity consistent with long-term good health. This requirement includes the energy needed for growth and development. Dietary reference values (DRVs) for energy are provided as ARs (EFSA NDA Panel, 2013b). Table 1 summarises the energy intakes considered adequate for infants in the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a).

Table 1: Intakes of energy considered adequate for infants (EFSA NDA Panel, 2013a).

Age (months)	**AR (MJ (kcal)/day)**		**AR (MJ (kcal)/kg body weight per day)**	
	Boys	**Girls**	**Boys**	**Girls**
0 to < 1	1.5 (359)	1.4 (329)	0.45 (109)	0.43 (103)
1 to < 2	2.1 (505)	1.9 (449)	0.47 (112)	0.45 (107)
2 to < 3	2.2 (531)	2.0 (472)	0.40 (95)	0.39 (92)
3 to < 4	2.1 (499)	1.9 (459)	0.33 (78)	0.33 (79)
4 to < 5	2.3 (546)	2.1 (503)	0.33 (78)	0.33 (79)
5 to < 6	2.4 (583)	2.3 (538)	0.33 (78)	0.33 (78)
6 to < 7	2.5 (599)	2.3 (546)	0.32 (76)	0.31 (75)
7 to < 8	2.7 (634)	2.4 (572)	0.32 (76)	0.32 (76)
8 to < 9	2.8 (661)	2.5 (597)	0.32 (77)	0.32 (76)
9 to < 10	2.9 (698)	2.6 (628)	0.32 (77)	0.32 (76)
10 to < 11	3.0 (724)	2.7 (655)	0.33 (79)	0.32 (77)
11 to < 12	3.1 (742)	2.8 (674)	0.33 (79)	0.32 (77)

These ARs are generally lower than the ones used by the SCF (2003a), with the exception of male infants at the age of one month (+1.9 %) and two months (+4.7 %), and of female infants at the age of two months (+4.7 %). From three months of age onwards, the differences range between -6.2 % and -3.2 %. This is a result of more refined equations used to calculate total energy expenditure, different assumptions made with respect to energy needs for growth and the use of updated reference body weights.

5.1.4. Energy intakes of infants

Data on energy intakes were available from four surveys for mostly formula-fed infants aged from zero to below six months (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) in which mean/median energy intakes of about 550-700 kcal/day were reported. In exclusively breast-fed infants mean energy intakes at 15 and 25 weeks of age were reported to be 590 kcal/day and 620 kcal/day, respectively (Nielsen et al., 2011). For infants in the second half of the first year of life mean/median energy intakes in the range of 650-980 kcal/day were observed (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

Only one study from a representative sample of French infants (Fantino and Gourmet, 2008) reported on the contribution of formula to energy intakes in formula-fed infants over the first year of life. Energy from formula intakes represented the following percentages of the total energy intakes (E%): 95.5 E% at one to three months; 91.0 E% at four months; 77.8 E% at five months; 63.8 E% at six months; 58.6 E% at seven months; 54 E% at eight to nine months; and 36.7 E% at 10-12 months of age.

5.1.5. Health consequences

Several studies have observed slight differences in growth patterns of formula-fed infants as compared to breast-fed infants, with formula-fed infants growing at a faster rate over the first year of life (Koletzko et al., 2009a; Hörnell et al., 2013a). A role for the higher energy and protein content of IF as compared to breast-milk has been suggested to explain these differences. A number of studies have found associations between a high growth velocity during the first months of life and an increased risk of non-communicable diseases later in life. Systematic reviews showed that upward percentile crossing for weight and length in infancy was associated with a higher risk of later obesity (Baird et al., 2005; Monteiro and Victora, 2005).

The Panel notes that the composition of IF has evolved over the last decade and energy and protein contents of current IF resemble more closely that of human milk. It should, however, also be noted that whereas composition of IF remains stable over time, breast-milk composition changes continuously and therefore IF cannot imitate breast-milk with respect to its energy and protein content.

5.1.6. Recommendations

IF and FOF should ensure that growth and development of infants fed IF are similar to that of infants who are exclusively breast-fed during the first four to six months of life and that growth and development of infants fed FOF in association with appropriate complementary feeding are similar to those of infants who continue to be breast-fed while complementary food is introduced into their diet. Since IF and FOF can be used instead of breast milk, there is no reason to set different minimum and maximum energy contents for IF and FOF.

There is no scientific evidence suggesting that the mean energy content of breast milk nor the energy requirements of infants up to the age of one year are markedly different from the values used by the SCF (2003a) to determine the minimum and maximum energy content of IF and FOF. An energy density of IF and FOF considerably higher than that of human milk may increase total energy intakes beyond the energy intakes considered adequate for infants and may play a role in the development of a higher than desirable weight gain.

The Panel therefore proposes a minimum energy content of IF and FOF of 60 kcal (250 kJ)/100 mL and a maximum energy content of 70 kcal (293 kJ)/100 mL. However, the Panel considers it desirable if IF and FOF are designed in a way that their energy content tends towards the lower bound of the range provided that infants are fed *ad libitum*.

5.2. Protein

5.2.1. Current compositional requirements of IF and FOF

Permitted sources of protein in IF and FOF as laid down in Directive 2006/141/EC include cow's milk protein, goat's milk protein, isolated soy protein (ISP) and protein hydrolysates of unspecified origin and unspecified degree of hydrolysis. Currently permitted minimum and maximum amounts of protein in IF and FOF as laid down in Directive 2006/141/EC and as compared to the recommendations by the SCF (2003a) are depicted in Table 2.

Table 2: Currently permitted minimum and maximum amounts of protein in IF and FOF from different sources as laid down by Directive 2006/141/EC in comparison to the recommendations by the SCF (2003a) and by the EFSA NDA Panel (2012c)

Formula with	**IF**				**FOF**			
	Directive 2006/141/EC		SCF (2003a)		Directive 2006/141/EC		SCF (2003a)	
g per 100 kcal	min	max	min	max	min	max	min	max
Cow's milk protein	1.80(a)	3.00	1.80	3.00	1.80	3.50	1.80	3.00
Goat's milk protein	1.80(a)	3.00	1.80	3.00	1.80	3.50	1.80	3.00
Protein hydrolysates	1.80(b)	3.00	2.25	3.00	1.80(b)	3.50	2.25	3.00
ISP	2.25	3.00	2.25	3.00	2.25	3.50	2.25	3.00

(a) Formulae with a protein content between 1.80 and 2.00 g per 100 kcal currently require that their safety and suitability is demonstrated by clinical evaluation.

(b) Formulae with a protein content between 1.80 and 2.25 g per 100 kcal currently require that their safety and suitability is demonstrated by clinical evaluation. To date only one specific formulation of whey protein hydrolysates that provides 1.86 g protein per 100 kcal is authorised for use in IF and FOF following an evaluation by the Panel (EFSA, 2005e).

5.2.2. Protein content of human milk

Protein concentrations in human milk change during the first days of life. In a meta-analysis of 21 studies reporting on energy and macronutrient composition of breast milk from mothers of healthy singleton infants born at term and who were exclusively breast-fed at the time of breast milk sampling (Hester et al., 2012), crude protein content expressed as mean (range) was reported for colostrum (1-5 days): 2.5 (1.4-6.5) g/100 mL (3.8 (2.2-10.0) g/100 kcal, n = 433), for transitional milk (6-14 days): 1.7 (1.3-2.5) g/100 mL (2.6 (2.0-3.8) g/100 kcal, n = 308) and for mature human milk (> 14 days): 1.3 (0.8-2.1) g/100 mL (2.0 (1.2-3.2) g/100 kcal, n = 415). Protein accounts for around 17 E% in colostrum and 7 E% in mature human milk (Räihä, 1994). The concentrations of different proteins also change with duration of lactation. Casein is low or absent in early lactation, then increases rapidly and subsequently decreases. The concentration of whey proteins decreases from early lactation and continues to fall. These changes result in a whey protein:casein ratio of about 90:10 in the first three to four days *post-partum*, 55:45 in mature milk and 50:50 in late lactation (at around six months) (Kunz and Lönnerdal, 1992).

5.2.3. Protein requirements of infants

Estimating true protein intakes from breast milk is difficult because of the non-protein nitrogen (NPN) fraction that represents about 25 % of total nitrogen, made up of urea (up to 50 % of NPN), free amino acids and other nitrogenous compounds. How and how much of NPN is utilised by the body is not entirely understood (WHO/FAO/UNU, 2007). Moreover, the composition of the protein fraction of breast milk changes with time and no data are available on the true digestibility of the different fractions. Therefore, in previous Opinions (EFSA NDA Panel, 2012d, 2013a) the Panel decided to derive an AR and subsequently a Population Reference Intake (PRI) for protein for infants based on a factorial approach as the sum of the requirement for maintenance and the requirement for growth adjusted for efficiency of dietary protein utilisation.

Table 3 summarises the protein intakes considered adequate for the majority of infants in the Panel's previous Opinion on nutrient requirements and dietary intakes in infants and young children in the European Union (EFSA NDA Panel, 2013a).

Table 3: Intakes of protein considered adequate for the majority of infants (EFSA NDA Panel, 2013a).

Age (months)	**PRI (g/kg body weight per day)**	**Body weight (kg) [(a)]**		**PRI (g/day)**	
		Boys	**Girls**	**Boys**	**Girls**
1 to < 2	1.77	4.5	4.2	8	7
2 to < 3	1.50	5.6	5.1	8	8
3 to < 4	1.36	6.4	5.8	9	8
4 to < 5	1.27	7.0	6.4	9	8
5 to < 6	1.21	7.5	6.9	9	8
6 to < 7	1.15	7.9	7.3	9	8
7 to < 8	1.27	8.3	7.6	11	10
8 to < 9	1.23	8.6	7.9	11	10
9 to < 10	1.19	8.9	8.2	11	10
10 to < 11	1.16	9.2	8.5	11	10
11 to < 12	1.14	9.4	8.7	11	10

(a): 50th percentile of WHO Growth Standards.

No PRI has been proposed by the Panel for the age group zero to less than one month owing to the lack of data for the first month of life. However, the Panel considers it safe to assume that requirements for protein intakes in the first month of life do not differ significantly from those of the second month of life.

5.2.4. Protein intakes of infants

Protein intakes in mostly formula-fed infants in Europe were reported to be around 9-10 E% in infants less than six months of age (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and around 10-15 E% in infants in the second half of the first year of life (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

5.2.5. Health consequences

5.2.5.1. Protein intakes to ensure adequate growth and development

Several studies which investigated the safety and suitability of IF based on intact cow's milk protein with protein contents of 1.8-1.9 g/100 kcal have been reviewed by the Panel previously (EFSA, 2005e). These studies have generally shown that protein concentrations in formula of 1.8-1.9 g/100 kcal when derived from intact milk protein are adequate to promote normal growth when these formulae are fed *ad libitum*. In a study on infants (Koletzko et al., 2009b) consuming a low protein IF with 1.77 g protein per 100 kcal and subsequently FOF providing 2.2 g protein per 100 kcal for the first year of life and who were followed-up until 24 months of age, no statistically significant differences between the group consuming low protein formula and the breast-fed reference group with respect to weight-for-length and BMI was found at 24 months of follow up. Another study (Trabulsi et al., 2011) investigated the effect on infant growth of an IF with a protein content of 1.9 g/100 kcal as compared to an IF formula with a protein content of 2.2 g/100 kcal which was consumed for four months. There were no statistically significant differences between the two formula groups with respect to weight gain, length gain and head circumference at the end of the study at four months of age.

No studies which evaluated the safety and suitability of lower than currently permitted (i.e. 2.25 g/100 kcal) protein contents in formulae containing ISP were published after the report by the SCF (2003a). Also, no evidence is available to suggest that this protein content would be inadequate to ensure adequate growth and development.

The Panel considers that based on the available evidence a minimum protein intake of 1.8 g/100 kcal from IF and FOF based on intact milk protein and 2.25 g/100 kcal from formula containing ISP are sufficient to ensure adequate growth and development. Adequate minimum protein intakes from IF and FOF containing protein hydrolysates need to be established for each specific IF or FOF containing hydrolysed proteins following clinical evaluation, as outlined in Section 5.2.5.4.

5.2.5.2. High protein intakes

In infants, a very high protein intake (around 20 E%) can impair the water balance, particularly when no other liquids are consumed and/or extrarenal water losses are increased (EFSA NDA Panel, 2012d). It has been suggested that high protein intakes contribute to higher insulin secretion, and to a higher release of insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-1 (Axelsson, 2006). It has also been suggested to be associated with increased growth (Koletzko et al., 2009b; EFSA NDA Panel, 2012d; Hörnell et al., 2013a) and a higher body mass index (BMI) in childhood (Hörnell et al., 2013a; Thorisdottir et al., 2013; Weber et al., 2014). Whether protein plays a role in the observed increased growth rate and higher BMI in childhood is still matter of debate and requires more research.

5.2.5.3. Plant proteins as protein sources for IF and FOF

Some plant proteins are deficient in certain indispensable amino acids and the digestibility of plant proteins can be less than that of milk proteins. Therefore, a higher minimum protein content is usually recommended for formulae with intact proteins other than milk proteins. Also, when setting minimum amounts for the contents of certain minerals in IF and FOF based on plant proteins, the increased phytic acid content of plant proteins that can reduce the availability of minerals has to be taken into account (SCF, 2003a).

Currently, for formulae containing intact proteins the only permitted source of plant proteins is ISP. ISP is low in sulphur containing amino acids. It contains around 1-2 % phytate and is rich in nucleotides and isoflavones (SCF, 2003a). Soy protein also contains trypsin inhibitors and lectins (Bhatia et al., 2008). Reducing phytic acid content in formulae by around half from around 600 mg/kg to around 270 mg/kg or completely as compared to around 250-400 mg/kg ready-to-feed formula has been shown to improve zinc absorption and to a lesser extent also iron absorption (Lönnerdal et al., 1984; Davidsson et al., 1994; Davidsson et al., 2004). Concerns have been raised with respect to potential negative effects of soy isoflavones on sexual, reproductive and neurobehavioural development, immune function and thyroid function. The American Academy of Pediatrics (AAP) Committee on Nutrition concluded in its review that the evidence for adverse effects of dietary soy isoflavones on human development, reproduction or endocrine function is not conclusive (Bhatia et al., 2008). The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition acknowledged the lack of evidence from human studies, but recommended the reduction of soy isoflavones in soy-based formulae as precautionary approach (ESPGHAN Committee on Nutrition et al., 2006). Trypsin inhibitors and lectins may interfere with protein digestion and nutrient absorption. Enzyme inhibitors and lectins are inactivated under heat treatment, although some residual activity can be found when proper heating is not achieved (Lajolo and Genovese, 2002). It is technologically possible to remove isoflavones, trypsin inhibitors, lectins and phytic acid from formula.

The Panel considers that concentrations of isoflavones, trypsin inhibitors, lectins and phytic acid in IF and FOF should be kept as low as is feasible.

The Panel notes that the main indications for the use of formulae exclusively based on ISP in place of milk-based formula are congenital lactase deficiency and galactosaemia, provided the formula is lactose-free according to the criteria laid down in Directive 2006/141/EC (i.e. 0.01 g/100 kcal) (EFSA NDA Panel, 2010b), and infants for whom caregivers chose a vegetarian diet.

5.2.5.4. Protein hydrolysates as protein sources for IF and FOF

According to Directive 2006/141/EC, formulae containing hydrolysed protein may be produced from any suitable protein source and by different enzymatic or chemical means provided that the compositional criteria laid down by the Directive are met. In its Opinion, the SCF (2003a) concluded that there is a need for clinical evaluation of formulae containing protein hydrolysates with respect to their safety and suitability.

The Panel emphasises that the safety and suitability of each specific formula containing protein hydrolysates has to be established by clinical studies. Information on protein sources and the technological processes applied should also be provided. In this context, the Panel notes that one particular formula containing partially hydrolysed whey protein has been evaluated for its safety and suitability by the Panel (EFSA, 2005e) and has been authorised for use by Directive 2006/141/EC.

Directive 2006/141/EC specifies criteria for formulae containing protein hydrolysates to be allowed to be marketed as reducing the risk of developing allergy to milk proteins. Attempts have been made to classify formulae containing hydrolysed protein into partially and extensively hydrolysed protein formulae according to the degree of protein fragmentation, but there is no agreement on the criteria on which to base this classification (Greer et al., 2008) and no regulatory definition exists as to what would constitute a partially or extensively hydrolysed protein formula. Formulae containing hydrolysed protein have been studied with respect to their potential to reduce the risk of developing allergic manifestations in at-risk-infants who are not breast-fed (Osborn and Sinn, 2006; Szajewska and Horvath, 2010; von Berg et al., 2013; de Silva et al., 2014). These studies indicate that the characterisation of a formula by molecular weight of protein cannot predict their potential to reduce the risk of developing allergic manifestations in genetically predisposed infants in the general population.

The Panel considers that the criteria given in Directive 2006/141/EC alone are not sufficient to predict the potential of a formula to reduce the risk of developing allergy to milk proteins. Clinical studies are necessary to demonstrate if and to what extent a particular formula reduces the risk of developing short and long-term clinical manifestations of allergy in at-risk-infants who are not breast-fed.

5.2.5.5. Protein quality

Amino acid reference patterns can be used in the assessment of protein quality by comparing the amino acid composition of a food to an amino acid reference pattern. Given that intakes of breast milk from a healthy well nourished mother are considered to satisfy the amino acid requirements for the first six months of life, the Panel considers the amino acid pattern of breast milk to be the best reference pattern for a product substituting for breast milk in infants.

The SCF (2003a) determined the amount of indispensable and conditionally indispensable amino acids per energy value in IF and FOF based on six studies of the amino acid content of human milk (Bindels and Harzer, 1985; Lönnerdal and Forsum, 1985; Janas et al., 1987; Darragh and Moughan, 1998; Villalpando et al., 1998; Räihä et al., 2002). A recent meta-analysis of 26 studies (Zhang et al., 2013) which investigated the total amino acid profile in human milk closely corroborated the amounts of indispensable and conditionally indispensable amino acids in human milk determined by the SCF (2003a) and which are also in line with the values proposed by the Codex Alimentarius in Codex Stan 72-1981 and by an ESPGHAN coordinated international expert group (Koletzko et al., 2005). The Panel, therefore, considers that the available evidence supports the amino acid pattern of human milk proposed by the SCF (2003a).

Based upon results indicating a lower formation of cysteine from cystathionine in the transsulfuration pathway, it has been considered that L-cysteine is a conditionally indispensable amino acid for neonates and that methionine cannot substitute for cysteine completely (White et al., 1994; Vina et al., 1995). These results were not confirmed in recent studies in parenterally fed infants (Courtney-Martin et al., 2008; Thomas et al., 2008; Courtney-Martin et al., 2010). However, as there is marked individual variability in the rate of transsulfuration, the Panel considers that it is appropriate to provide both cysteine and methionine in IF and FOF and the ratio of methionine to cysteine in IF shall not exceed two unless the safety and suitability of the formula has been demonstrated by clinical evaluation.

Tyrosine is synthesised by the hydroxylation of phenylalanine, via phenylalanine hydroxylase in the liver. Studies in human neonates have reported a substantial ability to hydroxylate phenylalanine (the first step in phenylalanine oxidation) (van Toledo-Eppinga et al., 1996; House et al., 1998). However, the extent to which neonates can accommodate high phenylalanine and low tyrosine intakes via phenylalanine hydroxylation remains unknown. Infants may require a pre-formed dietary source of tyrosine because the activity of phenylalanine hydroxylase in some neonates can be low, and hyperphenylalaninaemia tends to occur, whereas tyrosine tends to be deficient in these infants. Therefore, the Panel considers that it is appropriate to provide both tyrosine and phenylalanine in IF and FOF and the ratio of tyrosine and phenylalanine in IF shall not exceed two unless the safety and suitability of the formula has been demonstrated by clinical evaluation.

5.2.5.6. Effects of processing on nutritional value of protein

The nutritional value of protein is influenced by its amino acid composition, by protein hydrolysis but also by heat-treatment, especially in the presence of iron, vitamin C and lactose in these products. Heat processing is essential for the preservation of IF and FOF but induces a number of degradation reactions in milk, including Maillard reactions between lactose and protein and advanced glycation end products, as well as other direct modification reactions, which reduce the nutritional value of protein and could produce potentially active derivatives (Pischetsrieder and Henle, 2012). Among the Maillard reaction products the most important is lactulosyllysine, the reaction product of lactose and lysine side chains of the milk proteins (Fritsch and Klostermeyer, 1981; Langhendries et al., 1992; Henle et al., 1993). The presence of lactose is also an important prerequisite for extensive protein oxidation during the thermal treatment of milk (Meltretter et al., 2007) as the oxidation of other amino acid side chains can be promoted by reactive oxygen species, which are formed in the course of the Maillard reaction (Mossine et al., 1999). As a consequence of their specific formulation and processing, IF and FOF can show higher content of glycation markers than regular milk products. Liquid formulae contain around double the amount of advanced Maillard reaction products compared with formulae in powdered form (SCF, 2003a).

The Panel considers that the contents of Maillard reaction products and protein degradation products in IF and FOF should be kept as low as technologically possible owing to their potentially untoward effects on the nutritional value of protein.

5.2.6. Recommendations

5.2.6.1. Calculation of protein content

The SCF (2003a) proposed to use a default conversion factor of 6.25 to calculate the protein content from the total nitrogen content, irrespective of the protein source. The Panel is aware of the discussions with respect to the use of different conversion factors for different protein sources in order to reflect variations in the nitrogen content of different proteins (EFSA NDA Panel, 2012d). The Panel, however, proposes to retain the conversion factor of 6.25 mainly for practical considerations.

5.2.6.2. Protein sources

The Panel considers that cow’s milk, goat’s milk and ISP are safe and suitable protein sources for use in IF and FOF based on intact protein. The use of other protein sources in IF and FOF and/or the introduction of new technologies need clinical evaluation and their safety and suitability should be established in the target population prior to their general use in IF and FOF.

With respect to formulae containing protein hydrolysates, the Panel reiterates the conclusions of the SCF (2003a) that those formulae are insufficiently characterised by the declared protein content even if they fulfil regulatory criteria concerning amino acid patterns and contents and that the safety and suitability of each specific IF or FOF containing protein hydrolysates has to be established by clinical evaluation.

The Panel notes that the characterisation of protein hydrolysates by molecular weight of the protein cannot predict their potential to reduce the risk of developing allergic manifestations in genetically predisposed infants. Therefore, the Panel considers that the criteria given in Directive 2006/141/EC are not sufficient to predict the potential of a formula to reduce the risk of developing allergy to milk proteins.

5.2.6.3. Minimum and maximum protein content of IF and FOF

Human milk is a food of changing composition during the lactational period, during 24 hours and during one feed, whereas an IF is a product of constant composition and, therefore, must be a compromise on the safe side, both as to the amount and as to the quality of the protein.

Based on the studies which investigated the adequacy of IF containing around 1.8 g protein per 100 kcal, the Panel considers that a minimum protein content in IF and FOF of 1.8 g/100 kcal (0.43 g/100 kJ) for cow's and goat's milk-based formula is suitable to satisfy the nutritional requirements of infants. For IF and FOF containing ISP, the Panel proposes a minimum protein content of 2.25 g/100 kcal (0.54 g/100 kJ). A minimum protein content for IF and FOF containing protein hydrolysates cannot be proposed and the adequacy of protein content of a specific IF or FOF containing hydrolysed proteins needs to be established based on clinical evaluation.

There is no evidence of a physiological need for protein intakes at amounts of 3.0 g/100 kcal in infancy, which is the currently permitted maximum content of protein in IF. In addition, protein intakes of infants are generally well above the requirements, so the protein content of IF and FOF could be decreased. Therefore, the Panel proposes to reduce the currently permitted maximum protein content to 2.5 g/100 kcal (0.60 g/100 kJ) for IF and FOF based on cow's milk and goat's milk protein and to 2.8 g/100 kcal (0.67 g/100 kJ) for IF and FOF containing ISP and IF and FOF containing protein hydrolysates. The Panel, however, acknowledges that there are no scientific data available which allow the establishment of precise cut-off values for the maximum protein content in IF and FOF and the proposed values are based on expert judgement of what would constitute an upper bound of the adequate range of intake. Table 4 gives an overview of the proposed minimum and maximum amounts of protein in IF and FOF.

Table 4: Proposed minimum and maximum content of protein in IF and FOF.

Formulae with	**Minimum content**		**Maximum content**	
	g/100 kcal	g/100 kJ	g per 100 kcal	g/100 kJ
Cow's milk protein	1.80	0.43	2.50	0.60
Goat's milk protein	1.80	0.43	2.50	0.60
ISP	2.25	0.54	2.80	0.67
Protein hydrolysates[a]	---	---	2.80	0.67

[a] The safety and suitability of formulae containing protein hydrolysates, including their minimum protein content, should be established based on clinical evaluation.

5.2.6.4. Amino acid reference pattern

As IF is considered as breast milk substitute and FOF can be used as the principal liquid element of a progressively diversified diet of infants in place of breast milk, the Panel considers that IF and FOF should provide indispensable and conditionally indispensable amino acids in amounts on an energy basis at least equal to the reference protein (i.e. breast milk), irrespective of the protein source.

Given that a recent meta-analysis (Zhang et al., 2013) closely corroborated the findings of the SCF (2003a) with respect to the total amino acid content of human milk, the Panel proposes to base the amino acid reference pattern for IF and FOF on the analysis of indispensable and conditionally indispensable amino acids in human milk by the SCF (2003a). The proposed reference pattern is depicted in Table 5.

Table 5: Proposed amino acid reference pattern for human milk protein using a conversion factor of 6.25.

Amino acid	mg/100 g protein	mg/100 kcal	mg/100 kJ
Cysteine	2.1	38	9
Histidine	2.2	40	10
Isoleucine	5.0	90	22
Leucine	9.2	166	40
Lysine	6.3	113	27
Methionine	1.3	23	5
Phenylalanine	4.6	83	20
Threonine	4.3	77	18
Tryptophan	1.8	32	8
Tyrosine	4.2	76	18
Valine	4.9	88	21

The sum of methionine and cysteine and the sum of tyrosine and phenylalanine in IF may be used for calculation purposes. If the ratio between methionine to cysteine and/or the ratio between tyrosine and phenylalanine, respectively, exceeds two, this must be justified by clinical evaluation. For FOF the Panel considers that no restrictions with respect to amino acid ratios need to apply, owing to the fact that complementary foods will contribute to amino acid intakes and the metabolism of older infants is more mature with respect to the capacity to convert methionine to cysteine and phenylalanine to tyrosine.

5.3. Fat

5.3.1. Current compositional requirements of IF and FOF

Current compositional requirements of IF and FOF with respect to total fat, fatty acids and phospholipids as laid down by Directive 2006/141/EC are depicted in Table 6. These compositional requirements differ from the Opinion of the SCF (2003a) with respect to the minimum content of alpha-linolenic acid (ALA, 18:3n-3) and the maximum content of phospholipids (PL) in IF and FOF. These differences are highlighted in the Table as footnotes.

Table 6: Current compositional requirements of IF and FOF with respect to total fat, fatty acids and PL as laid down by Directive 2006/141/EC.

	IF				FOF			
	g per 100 kcal		**FA%**		**g per 100 kcal**		**FA%**	
	min	max	min	max	min	max	min	max
Compulsory composition								
Total fat	4.40(a)	6.00(b)			4.00(c)	6.00(b)		
Trans FA				3.0				3.0
Lauric acid + myristic acid				20.0				20.0
Erucic acid				1.0				1.0
LA (18:2, n-6)(d)	0.30	1.20			0.30	1.20		
ALA (18:3, n-3)(d)	0.05(e)	0.24(f)			0.05(e)	0.24(f)		
Voluntary addition								
Total n-3 LCPUFA				1.0				1.0
Total n-6 LCPUFA				2.0				2.0
ARA (20:4, n-6)				1.0				1.0
DHA (22:6, n-3)	shall not exceed total n-6 LCPUFA				shall not exceed total n-6 LCPUFA			
EPA (20:5, n-3)	shall not exceed DHA				shall not exceed DHA			
Phospholipids	2 g/L(h)				2 g/L(h)			

(a) 40 E%, (b) 55 E%, (c) 35 E%.

(d) with a ratio of LA to ALA of ≥ 5 and ≤ 15.

(e) the SCF (2003) proposed a minimum content of 0.05 mg/100 kcal for formulae supplemented with ARA and DHA and 0.10 mg/100 kcal for formulae not supplemented with ARA and DHA.

(f) calculated from the lowest permitted LA:ALA ratio of 5 and the highest permitted LA concentration.

(h) the SCF (2003) proposed a maximum content of 1 g/L.

Abbreviations: LA: linoleic acid, ALA: alpha-linolenic acid, ARA: arachidonic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, FA%: percent of total fatty acids, LCPUFA: long-chain polyunsaturated fatty acids.

Conjugated-linoleic acid (CLA) is currently not permitted to be added to formulae in addition to the CLA naturally present in the fat ingredients, as it is considered to be a novel food ingredient in this context. Also, the use of sesame oil and cotton-seed oil is not permitted in IF and FOF.

5.3.2. Fat composition of human milk

Breast milk has an average total fat content of 24-59 g/L (3.7-9.1 g/100 kcal, around 50 E%), but the fat content varies markedly with pregnancy weight gain and during the feed as the fat content increases as the breast is emptied (Michaelsen et al., 1994). Most of the fat in breast-milk is triacylglycerol (TAG, > 98 %), but it also contains some cholesterol (around 0.25 g/L) and PL (around 0.24 g/L), predominantly sphingomyelin, phosphatidylethanolamine and phosphatidylcholine (Abrahamse et al., 2012; Giuffrida et al., 2013).

The main saturated fatty acid (SFA) in human milk is palmitic acid (16:0), which accounts for around 26 % of total fatty acids (FA%), and the main monounsaturated fatty acid (MUFA) is oleic acid (18:1, n-9), which typically accounts for approximately 35 FA% (Abrahamse et al., 2012). The composition of polyunsaturated fatty acids (PUFAs) in human milk varies depending on the dietary intake of the mother with milk from vegans having the highest content of linoleic acid (LA, 18:2, n-6) and ALA (Sanders and Reddy, 1992; Davis and Kris-Etherton, 2003). Inuit and other populations with a high intake of marine animals have the highest breast-milk content of docosahexaenoic acid (DHA, 22:6n-3). The concentrations of DHA in breast milk, however, are also influenced by polymorphisms in the fatty acid desaturase (FADS) gene cluster (Moltó-Puigmarti et al., 2010). In general, DHA concentrations are the most variable of all fatty acid concentrations in human milk, while the content of arachidonic acid (ARA, 20:4, n-6) is much more stable (Brenna et al., 2007). Breast-milk usually has a low content of *trans*-fatty acids (TFA), around 2-5 FA% (Larqué et al., 2001), and CLA, 0.2-0.6 FA% (Rist et al., 2007), but the content of these fatty acids varies depending on the maternal diet (Larqué et al., 2001; Rist et al., 2007).

Human milk contains little short-chain SFA (SCFA, with a carbon-chain length < 6), but usually contains 8-10 FA% as medium-chain SFA (MCFA, usually defined as fatty acids with a carbon length of 6-10) (EFSA NDA Panel, 2010d)). TAG containing SCFA, MCFA and to some extent also lauric acid with 12 carbon atoms are more rapidly hydrolysed by gastrointestinal lipases and the hydrolysis products are more easily absorbed as they bypass the enterocytes and chylomicrons and are taken to the liver directly via the portal vein (Novak and Innis, 2011). The ingestion of these fatty acids, therefore, could provide some benefit under conditions where fat absorption is a limiting factor. The MCFA content of human milk varies as it is increased by a high carbohydrate and low fat intake of the mother (Koletzko et al., 1992; Sauerwald et al., 2001; Novak and Innis, 2011).

About 70 % of the palmitic acid in human milk is esterified to the *sn*-2 position of the milk TAG (Innis, 2011), and as the endogenous lipases hydrolyse dietary TAG mainly at the *sn*-1,3 position, palmitic acid may be absorbed in part as glycerol-palmitate. It has been proposed that the absorption of unesterified palmitic acid is limited.

5.3.3. Requirement for total fat and essential fatty acids and Adequate Intakes (AI) of long-chain (LC) PUFA

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded on levels of intakes of fats, essential fatty acids and LCPUFA (unsaturated fatty acids with 20 or more carbon atoms) considered adequate for the majority of infants. These are depicted in Table 7.

Table 7: Intakes of fat, essential fatty acids and LCPUFA considered adequate for the majority of infants.

Age	RI[(a)] total fat	AI LA	AI ALA	AI DHA	AI ARA
	E%	E%	E%	mg/day	mg/day
0 to < 6 months	50-55	4	0.5	100	140
6 to < 12 months	40	4	0.5	100	---

(a) RI: Reference Intake range for macronutrients

5.3.4. Total fat and fatty acid intakes of infants

Mean total fat intakes in mostly formula-fed European infants below the age of six months were available from four studies (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and were between 42 and 46 E%. Intakes of SFAs, MUFAs and PUFAs were reported to be around 16-22 E%, 15-17 E% and 6.7-7.0 E%, respectively (Hilbig, 2005; Noble and Emmett, 2006; Lennox et al., 2013). Intakes of LA were 3.6-4.2 g/day (around 6-7 E%), of ALA 0.41-0.48 g/day (around 0.7-0.8 E%) and of DHA 57 mg/day (Fantino and Gourmet, 2008; Schwartz et al., 2010). Total fat intake usually decreases once breast-feeding or formula-feeding ceases (Niinikoski et al., 2007). In infants between 6 and < 12 months mean total fat intakes were reported between 26 and 40 E% (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013). Intakes of SFA, MUFA and PUFA were around 12-16 E%, 9-14 E% and 4.6-7.0 E%, respectively (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008). Intakes of LA were 3.4-6.8 g/day (around 3.4-4.4 E%), of ALA 0.40-0.65 g/day (around 0.5-0.9 E%) and of DHA 28-47 mg/day (Lagström et al., 1997; de Boer et al., 2006; Fantino and Gourmet, 2008; Schwartz et al., 2010). The Panel, however, notes the skewed distribution of LA, ALA and DHA intakes and that in the absence of information on median intakes the given values cannot be interpreted.

5.3.5. Fat sources for IF and FOF

The obvious and previously used staple sources of fat for use in the production of IF and FOF are cow’s milk, to a certain extent goat’s milk and different types of vegetable oils. Like human milk, the lipids in bovine milk are mainly present in globules as an oil-in-water emulsion. Most of the fat is saturated and around 11 % of the fatty acids are SCFA, almost half of which is butyric acid (4:0) (Månsson, 2008). The SCFA are esterified almost entirely at the *sn*-3 position of the TAG molecules, but similar to human milk, cow’s milk usually has palmitic acid and MCFA preferentially esterified at positions *sn*-2 and *sn*-1 and oleic acid in positions *sn*-1,3. Owing to the hydrogenation of PUFAs catalysed by the rumen bacteria, cow’s milk has a relatively high content of *t*FA, typically 2.6-3.9 FA%, of which *cis*-9, 11-*trans* CLA and 11-*trans* vaccenic acid (18:1*t*) are the major ones, constituting 0.3-0.5 FA% and 2-3.3 FA%, respectively and a low content of PUFA (Slots et al., 2009).

The average total fat content in goat’s milk is similar to that found in other ruminant species as it usually ranges from 3 to 6 % (Chilliard and Ferlay, 2004). The fatty acids are arranged in TAG in accordance with the milk pattern of other ruminants and the percent of unsaturated fatty acids do not differ from that found for cow’s milk. The major difference between caprine and bovine milk fat is the distribution among specific SFAs, as goat’s milk has a lower content of SCFA and more MCFA, specifically a higher content of capric acid (10:0) and caprylic acid (8:0) (Strzałkowska et al., 2009).

There are many different vegetable oils that could be used in the production of IF, but most of the vegetable oils that are used have a high content of PUFA and less SFA. The TAG positioning of SFA in vegetable oils furthermore differs from that in breast-milk, as they will usually have more unsaturated fatty acids in the *sn*-2 position and the SFA in position *sn*-1,3. An overview about the typical fatty acid composition of human milk and other potential fat sources for IF and FOF is given in Table 8.

Table 8: Typical fatty acid composition of breast-milk and potential sources of fat for IF and FOF.

FA	Human milk(a)	Cow’s milk(b)	Goat’s milk(c)	Soybean oil(d)	Canola oil(d)	Sunflower oil(d)	Palm oil(d)
	% FA	% FA	% FA	% FA	% FA	% FA	% FA
SFA	45-46	53-84	62-79	16	7	10	49
MUFA	35-40	13-42	17-29	23	63	20-45	37
PUFA	14-19	2-4	3-6	58	28	40-66	9
LA	10-15	1-2	1.5-4	50	18	40-66	9
ARA	0.7-1.1	0.1					
ALA	0.1-2.0	0.2-1.3	0.25-1.3	7	9	0-0.2	0.2
DHA	0.2-0.5						

(a) from Greek and Finnish mothers (Antonakou et al., 2013; Mäkelä et al., 2013).
(b) Kliem et al. (2013); Ferrand-Calmels et al. (2014).
(c) Ferrand-Calmels et al. (2014).
(d) USDA (online).

As neither cow’s milk nor vegetable oils contain LCPUFAs, oil sources other than those discussed above are needed to supply LCPUFAs. LCPUFA sources currently used in IF and FOF are fish oil, ARA-rich fungal oil from *Mortierella alpina* and egg PL (lecithin/phosphatidylcholine from egg yolk).

5.3.6. Health consequences

5.3.6.1. Overall fat intake

The content of fat in IF and FOF is determined by the need for energy for growth and for the supply of essential fatty acids. Moreover, fat facilitates the absorption of the fat-soluble vitamins. TAG are the predominant source of energy for breast-fed and formula-fed infants. Major changes in body size and composition take place during early life and early growth pattern may have both beneficial and adverse long-term effects on health and obesity risk. The concern about excessive weight gain in infancy has increased as childhood obesity becomes increasingly more prevalent. The role of high fat intakes as determinants of adiposity in infancy and childhood has been poorly studied and results are inconclusive (Macé et al., 2006; Agostoni and Caroli, 2012).

5.3.6.2. Fatty acid composition

The background for the concern about the use of myristic and lauric acid in IF and FOF expressed by the SCF (2003a) is their cholesterol-increasing effects in adults. However, palmitic acid is by far the most dominant SFA in breast-milk and also increases cholesterol. Furthermore, plasma cholesterol is higher in breast-fed compared to formula-fed infants and there is no evidence that this has any long-term adverse health effects (Owen et al., 2008; Owen et al., 2011). With respect to MCFA, the SCF (2003a) concluded that there was no necessity to add MCFA to IF or FOF. The main purpose of adding MCFA would be to increase fat absorption (as would lauric acid), but healthy infants do not appear to have any limitations with respect to fat absorption. Furthermore, MCFA may have potential negative health effects, as high MCFA intakes may lead to diarrhoea and dicarboxylic-acid-uria (Borum, 1992; Tserng et al., 1996; Odle, 1997). In infants, TFA may interfere with PUFA metabolism (Larqué et al., 2001), but no studies have been able to link TFA intake to any growth or developmental outcomes in infants. CLA, evaluated in the form of CLA-rich oils (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 in a mixture 1:1), has been suggested to have negative health effects (EFSA NDA Panel, 2010a, 2010c). Both TFA and *cis*-9, *trans*-11 CLA are present in milk and therefore are contained in formula in which milk fat has been used as a fat source and are not of safety concern in amounts which are naturally introduced to formula from milk fat.

Of importance when it comes to the fatty acid composition of IF is that the PUFA content is high enough to provide the required amount of essential fatty acids.

ALA is essential in human nutrition as precursor for n-3 LCPUFA. LA, when incorporated into skin ceramides, is essential for maintaining the water-permeability barrier of the skin and thereby avoiding excessive trans-epidermal water loss and the accompanying energy loss from water evaporation. Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and to a lesser degree DHA are synthesised from ALA. DHA is a component of membrane structural lipids, especially of PL in nervous tissue and the retina. The developing brain accumulates large amounts of DHA both pre- and post-natally, particularly during the first two years of life. DHA is predominantly acquired from the mother via placental transfer and from breast milk or formula, although the capacity of the brain to synthesise DHA increases with gestational age (EFSA NDA Panel, 2010d). Biochemical changes of n-3 PUFA deficiency include a decrease in plasma and tissue DHA concentrations. There is no accepted cut-off concentration of plasma or tissue DHA concentrations below which functions ascribed to n-3 PUFA such as visual or neurological functions are impaired (IoM, 2005b).

The effects of the addition of fatty acids to formula, such as effects on visual acuity, brain development, growth, and immune function, have mainly been studied for ALA and DHA.

A meta-analysis of the effect of ALA on growth and development of pre-term and term infants (Udell et al., 2005) concluded that ALA supplementation had a statistically significant effect on plasma and erythrocyte PL DHA concentrations but that there was a lack of convincing evidence for the effects of ALA supplementation of formula on infant growth and development. The meta-analysis did not find any effects of ALA on growth of pre-term infants and the small differences in weight and length between term infants fed ALA enriched formula and controls which were observed at 12 months of age were not sustained at 24 months of age. There was a transient improvement in retinal function in pre-term but not in term infants and no effect on any of the other developmental indices.

Most studies have compared IF with and without the addition of LCPUFA and more recent studies have also supplemented lactating mothers with fish oil, which generally result in far higher infant intakes of especially DHA than those added to IF (around 0.5-1.0 FA% vs. 0.2-0.4 FA%). Because of the large number of studies in this area, the Panel has chosen to summarise the evidence in this area based on meta-analyses and large randomised controlled trials (RCTs).

DHA is accreted in the brain during the first two years of life and data mainly from *in vitro* and animal studies have shown effects of DHA on neuronal cell growth, rhodopsin function and levels of neurotransmitters (Lauritzen and Carlson, 2011). Studies generally show that the most predictable way to increase tissue DHA is to supply DHA rather than ALA (Arterburn et al., 2006).

Meta-analyses assessing various functional effects of LCPUFA-intake on cognitive outcomes in RCTs in both formula-fed and breast-fed term infants have consistently concluded that there are no clear long-term benefits or harms of addition of LCPUFA to IF and FOF during infancy on neurodevelopmental outcomes (Delgado-Noguera et al., 2010; Schulzke et al., 2011; Simmer et al., 2011; Qawasmi et al., 2012). However, these meta-analysis pool studies that vary greatly in intake and source of LCPUFAs and in the duration of supplementation. The majority of studies have only measured outcomes in infancy when the range of cognitive functions that can be tested is limited. Furthermore, the specific cognitive and behavioural properties one would expect to be affected by LCPUFA and the most appropriate tests and age at testing to detect such effects is still a matter of debate (Cheatham et al., 2006). Thus, no firm conclusions with respect to the short as well as the long-term consequences of early LCPUFA supplementation on cognitive function can be drawn.

Some studies have reported negative effects of LCPUFA intakes on infant growth. Two studies on maternal n-3 LCPUFA-supplementation during lactation and/or pregnancy found that this was associated with increased head circumference and a decrease in length of the breast-fed infants (Delgado-Noguera et al., 2010). However, a meta-analysis on the effect of LCPUFA in IF on infant growth in 14 RCTs with a total of 1 846 infants did not show any significant positive or a negative effects of LCPUFA on growth (weight, length, or head circumference) (Makrides et al., 2005).

One meta-analysis concluded that post-natal n-3 LCPUFA intake (from fish, fish oil and breast-milk) decreases childhood asthma (Yang et al., 2013), whereas a systematic review of RCTs found an effect on asthma and the response to skin prick test only after supplementation during pregnancy, and no significant effect was seen after post-natal intake (Klemens et al., 2011).

Studies have suggested that polymorphisms in the fatty acid desaturase (FADS) gene cluster that determine the endogenous conversion of LA and ALA to LCPUFAs alter the effect of breast-feeding on cognitive outcomes (Caspi et al., 2007; Steer et al., 2010; Martin et al., 2011; Morales et al., 2011; Steer et al., 2013) and risk of atopy (Rzehak et al., 2010; Standl et al., 2011; Standl et al., 2012). Different FADS single-nucleotide polymorphisms (SNPs) may have different effects on LCPUFA synthesis and these may vary with age (Harsløf et al., 2013). The presence of these polymorphisms could influence the effect of LCPUFA addition and thus further complicate the evaluation of the effects of addition of LCPUFA to IF and FOF.

The Panel considers that DHA should be added to IF and FOF, even though there is currently no conclusive evidence for any effects beyond infancy of DHA supplementation on any of the health outcomes studied. The basis for proposing this addition is that: (1) DHA is an essential structural component of the nervous tissue and the retina and is involved in normal brain and visual development (EFSA, 2009); (2) the developing brain has to accumulate large amounts of DHA in the first two years of life; (3) although DHA can be synthesised in the body from ALA, the intake of pre-formed DHA generally results in an erythrocyte DHA status more closely resembling that of a breast-fed infant than is achieved with ALA alone (Brenna et al., 2009); (4) whereas to date there is no convincing evidence that the addition of DHA to IF and FOF has benefits beyond infancy on any functional outcomes , there is also a lack of long-term follow-up data on specific aspects of cognitive and behavioural function from adequately powered RCTs of DHA addition to IF and FOF to demonstrate any assumed biologically plausible effect of DHA on these aspects. Considering all of these factors, it seems prudent to provide pre-formed DHA to formula-fed infants in similar amounts as breast-fed infants, even though benefits beyond infancy of this practice cannot be established based on the currently available data.

With respect to the EPA:DHA ratio, the SCF (2003a) recommended the ratio should be kept below one. However, there are no studies which investigated any potential health risk of high intakes of EPA in infants, but the content of EPA in breast-milk is usually low, typically in the range of 0.05-0.4 FA% (Lauritzen and Carlson, 2011).

5.3.6.3. Molecular speciation of fatty acids

In breast-milk, PL that constitute the fat globule membrane have a high content of LCPUFA compared to the TAG molecules in the core of the milk globules (Abrahamse et al., 2012). The PL supplied by breast-milk are expected to play a role together with bile PL in the emulsification of the fat in the infant gut and thus promote digestion, absorption and transport (Ramirez et al., 2001). It has been proposed that specifically LCPUFA may be better utilised if supplied in PL owing to increased absorption and tissue incorporation (Abrahamse et al., 2012). Furthermore, as constituents of the membranes of all cells in the infant, PL are involved in a variety of physiological processes, but these are usually not expected to be influenced by dietary intake of PL (German, 2011; Küllenberg et al., 2012; Oosting et al., 2012; Tanaka et al., 2013). Few studies have looked at functional consequences of inclusion of PL in IF. A meta-analysis that specifically evaluated the effect of formula LCPUFA on infant growth did not find any differences depending on whether these were added as PL or TAG (Makrides et al., 2005). The Panel considers that there is no convincing evidence for a beneficial effect of LCPUFA supplied as PL instead of TAG in IF or FOF. There are no adverse effects reported of the use of PL to supply LCPUFA in IF and/or FOF instead of TAG. Lecithins are authorised by Regulation (EC) No 1333/2008[15] to be added to IF and FOF as emulsifier in an amount of 1 g/L.

5.3.6.4. TAG with palmitic acid predominantly in the *sn*-2 position

Structured triglycerides in which palmitic acid has been predominantly esterified in the *sn*-2 position in order to imitate breast-milk have been studied for a number of health outcomes in healthy term infants, including mineral absorption and retention, bone mineral density (BMD), growth, stool consistency, blood lipid profiles and infant crying time.

In these studies no effects on infant growth (Kennedy et al., 1999; Nelson and Innis, 1999; Litmanovitz et al., 2013) or on phosphorus and magnesium absorption (Carnielli et al., 1996) were observed. Also, no cause and effect relationship could be established between the feeding of formula high in *sn*-2 palmitate and stool consistency (EFSA NDA Panel, 2014d). The studies investigating calcium absorption (EFSA NDA Panel, 2011) and infant crying time (Litmanovitz et al., unpublished; Zhong et al., unpublished) reported inconsistent results. The only study which used a relevant measure of BMD did not report consistent results on BMD (Kennedy et al., 1999). A small study (Yaron et al., 2013) evaluated the effect of formula with high *sn*-2 palmitate as compared to a formula with low *sn*-2 palmitate on infant gut microbiota composition, reporting increases in *Clostridium*, *E. coli*, *Pseudomonas* and *Staphylococcus* numbers, but only by plate counting and without providing further characterisation of the bacterial groups analysed to infer possible physiological/clinical consequences of the changes reported. One study which investigated the effect of *sn*-2 palmitate on blood lipids found lower HDL-cholesterol and apolipoprotein A1 concentrations and higher apolipoprotein B concentrations in the group being fed high *sn*-2 palmitate formula (Nelson and Innis, 1999) but the relevance of this finding in infants is unknown. The Panel considers that there is no convincing evidence for a beneficial effect of the use of TAG with palmitic acid predominantly esterified in the *sn*-2 position in IF and/or FOF instead of other TAG. There are no adverse effects reported of the use of TAG with palmitic acid predominantly esterified in the *sn*-2 position in IF and/or FOF instead of other TAG.

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[15] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31/12/2008, p. 16–33.R

5.3.7. Recommendations

5.3.7.1. Total fat

According to Directive 2006/141/EC and in line with the SCF (2003a), IF have to provide fat in the range of 40-55 E% and FOF in the range of 35-55 E%. The Panel had concluded in its previous Opinion (EFSA NDA Panel, 2013a) on intakes of fat considered adequate for the majority of infants from birth to below six months of 50-55 E% for the first and of 40 E% for the second half year of life. The Panel considers that there is no scientific reason to differentiate the fat content of IF and FOF and that complementary foods that contribute to the dietary intake in the later half of infancy have a low fat content.

Therefore, the Panel proposes a minimum fat content of IF and FOF of 40 E% (i.e. 4.4 g/100 kcal (1.1 g/100 kJ)) and a maximum fat content of 55 E% (i.e. 6.0 g/100 kcal (1.4 g/100 kJ)).

5.3.7.2. LA

The range of LA concentrations in IF and FOF can be derived based on the level of LA intakes (4 E%) which the Panel had considered to be adequate for the majority of infants (EFSA NDA Panel, 2013a) and the highest concentrations of LA observed in human milk (24 FA%) (Sanders and Reddy, 1992). These derivations translate into a lower bound of the range of 500 mg/100 kcal (120 mg/100 kJ, equivalent to 4.5 E%) and an upper bound of the range of 1 200 mg/100 kcal (300 mg/100 kJ, equivalent to 10.8 E%), in line with what has been recommended previously by the SCF (2003a).

5.3.7.3. ALA

In line with the approach for LA, a lower bound for ALA in IF and FOF can be derived based on the level of ALA intakes (0.5 E%) which the Panel had considered to be adequate for the majority of infants (EFSA NDA Panel, 2013a) and an upper bound can be derived based on the highest ALA concentrations observed in human milk (2 FA%) (Mäkelä et al., 2013). These derivations translate into a lower bound of the range of 50 mg/100 kcal (12 mg/100 kJ, equivalent to 0.5 E%) and an upper bound of the range of 100 mg/100 kcal (24 mg/100 kJ, equivalent to 0.9 E%).

The Panel considers that there is no necessity to set a specific ratio for LA:ALA in the presence of LCPUFA in IF and FOF.

5.3.7.4. LCPUFA

In line with the approach for LA and ALA, a lower bound for DHA in IF and FOF can be derived based on the level of DHA intakes (100 mg/day) which the Panel had considered to be adequate for the majority of infants (EFSA NDA Panel, 2013a) and an upper bound can be derived based on the highest observed DHA concentrations in human milk (around 1 FA%) (Brenna et al., 2009). These derivations translate into a lower bound of the range of 20 mg/100 kcal (4.8 mg/100 kJ) and an upper bound of the range of 50 mg/100 kcal (12 mg/100 kJ).

The Panel considers that there is no necessity to set a specific minimum content of ARA or EPA in IF or FOF nor a specific ratio for DHA:ARA or DHA:EPA. The Panel notes that ARA and EPA will, however, be introduced to IF and FOF via sources of LCPUFA.

The Panel also notes that there is no convincing evidence that the addition of DHA to IF and FOF has benefits beyond infancy on any functional outcomes. However, the proposal of the Panel to add DHA to IF and FOF is based on its structural role in the nervous tissue and the retina and its involvement in normal brain and visual development, the need of the developing brain to accumulate large amounts of DHA in the first two years of life and the consideration that the intake of pre-formed DHA generally results in an erythrocyte DHA status more closely resembling that of a breast-fed infant than is achieved with ALA alone.

5.3.7.5. SFA and TFA

The Panel considers that there is no evidence which would allow lower or upper bounds of a range for specific types of SFA (MCFA or lauric, myristic or palmitic acid) to be proposed in IF or FOF. For TFA, the Panel considers the current specifications for TFA content in IF and FOF (< 3 FA%) to be adequate. These specifications allow for the use of milk as a source of fat for formulae.

5.3.7.6. Vegetable oils

The Panel considers that the vegetable oils used in the production of IF and FOF should be safe from a toxicological point of view (e.g. regarding the content of eruric acid, cyclopentene fatty acids, etc.). The use of partially hydrogenated vegetable oils in IF and FOF should be avoided owing to the production of some TFA.

5.3.7.7. PL

Taking into account the lack of convincing evidence for a beneficial effect of LCPUFAs supplied as PL instead of TAG in IF or FOF, the Panel considers that the use of PL as a source of LCPUFA instead of TAG in IF and FOF is not necessary. PL are naturally present in milk and ISP, and may be added to IF and FOF for technological purposes, for example as an emulsifier or as a source of LCPUFA.

5.3.7.8. TAG with palmitic acid predominantly in the *sn*-2 position

Taking into account the lack of convincing evidence for a benefit of use of TAG with palmitic acid predominantly esterified in the *sn*-2 position in IF and/or FOF, the Panel considers that there is no necessity to use TAG with palmitic acid predominantly esterified in the *sn*-2 position in IF and FOF instead of TAG from other fat sources.

Table 9 provides an overview of the proposed minimum and maximum content of total fat and an adequate range of fatty acids in IF and FOF.

Table 9: Proposed minimum and maximum content of fat and adequate range of fatty acids in IF and FOF

	Minimum content		**Maximum content**	
	mg/100 kcal	mg/100 kJ	mg/100 kcal	mg/100 kJ
Total fat	4 400	1 052	6 000	1 434
	Lower bound		**Upper bound**	
LA	500	120	1 200	300
ALA	50	12	100	24
DHA	20	4.8	50	12
TFA	---	---	3 FA%	3 FA%

5.4. Carbohydrates

Nutritionally, two broad categories of carbohydrates can be differentiated: “glycaemic carbohydrates”, i.e. carbohydrates digested and absorbed in the human small intestine with a substantial subsequent rise in blood glucose, and “dietary fibre‟, i.e. non-glycaemic carbohydrates passing undigested to the large intestine and with no rise in blood glucose. The absolute dietary requirement for glycaemic carbohydrates is not known because there is no indispensable carbohydrate. For practical purposes recommendations for glycaemic carbohydrates will depend on the amount of fat and protein ingested. The main glycaemic carbohydrates are monosaccharides, disaccharides, malto-oligosaccharides, and starch. Dietary fibre is defined to include all non-digestible carbohydrates (plus lignin) (EFSA NDA Panel, 2010e), but for IF and FOF only resistant oligosaccharides (fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides), and resistant starch and chemically and/or physically modified starches are of relevance.

5.4.1. Current compositional requirements of IF and FOF

Permitted carbohydrates in IF are lactose, maltose, sucrose, glucose, maltodextrins, glucose syrup (or dried glucose syrup), pre-cooked starch and gelatinised starch free from gluten. These carbohydrates can be used under the conditions outlined in Table 10. For FOF there are no restrictions with respect to the type of carbohydrates that can be used as long as they are free from gluten. If honey is used (for FOF only), it has to be treated in order to destroy spores of *Clostridium botulinum*.

Table 10: Current compositional requirements for IF and FOF with respect to glycaemic carbohydrates and FOS plus GOS according to Directive 2006/141/EC.

	IF with			**FOF with**		
g/100 kcal	**Milk protein**	**ISP**	**Protein hydrolysates**	**Milk protein**	**ISP**	**Protein hydrolysates**
Carbohydrates	9-14			9-14		
of which						
Lactose	≥ 4.5	NR(a)	≥ 4.5	≥ 4.5	NR(a)	≥ 4.5
Sucrose	not to be added		≤ 20 % of total carbohydrates(b),(c)	Σ sucrose, fructose, sugar from honey ≤ 20 % of total carbohydrates(b)		
Fructose	not to be added					
Glucose	not to be added		≤ 2(b),(c)	not to be added		≤ 2(b),(c)
Maltose, maltodextrins(b)	unrestricted within maximum amounts			unrestricted within maximum amounts		
Starches(b)	≤ 2 g/100 mL and ≤ 30 % of total carbohydrates(d)			unrestricted within maximum amounts as long as free of gluten		
FOS + GOS(b),(e)	≤ 0.8 g/100 mL			≤ 0.8 g/100 mL		

(a) not required (NR) if more than 50 % of the protein content is from ISP
(b) voluntary addition
(c) to mask the bitter taste
(d) pre-cooked or gelatinised starch only
(e) a combination of 90 % oligogalactosyl-lactose and 10 % high molecular weight oligofructosyl-saccharose only

5.4.2. Carbohydrate content of human milk

5.4.2.1. Digestible (glycaemic) carbohydrates

The Panel proposes to differentiate between glycaemic and non-glycaemic carbohydrates to underline the difference in their physiological function. The first source of glycaemic carbohydrates in infants is human milk, in which lactose, a disaccharide of glucose and galactose, is the primary sugar. Lactose occurs exclusively in milk and milk products. Human milk has the highest lactose content of all milks, about 55-70 g/L or 8.2-10.4 g/100 kcal (corresponding to 33-42 E%), whereas the content of monosaccharides is only about 1 % of total carbohydrates (Coppa et al., 1994). Human milk does not contain sucrose or fructose but small amounts of sugar alcohols, including inositol (Cavalli et al., 2006).

5.4.2.2. Non-digestible (non-glycaemic) carbohydrates in human milk

The third main component in human milk after lactose and fat are neutral and acid oligo- (and poly)saccharides in concentrations between around 5-10 g/L (Aggett et al., 2003). The structure of about 200 human milk oligosaccharides has been identified (Kunz et al., 2000) with the principal oligosaccharide in milk being lacto-N-tetraose. Neutral linear and branched-chain oligosaccharides consisting of 3-23 monosaccharide units are fucosylated to a varying degree and make up 90-95 % of the total amount. Oligosaccharides containing sialic acid are acidic. The production of oligosaccharides is genetically determined and the individual pattern of oligosaccharides differs between women (Ninonuevo et al., 2006).

The oligosaccharides of human milk are considered to be one of the principal growth factors, for example, for bifidobacteria in the infant gut and responsible for the composition of the gut microbiota found in breast-fed infants. The fermentation of non-digestible oligosaccharides leads to the generation of organic acids (lactic acid) and SCFA such as acetic, propionic and butyric acid. Butyrate is a main source of energy for the colonocytes and has effects on cell differentiation. Acetate and propionate are absorbed from the colon and thus provide energy to the host (Aggett et al., 2003). Fermentation products, i.e. SCFA, contribute to the energy content of the diet, but less than glycaemic carbohydrates. Human milk oligosaccharides are not considered in the estimation of the energy content of the milk.

5.4.3. Carbohydrate requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded on a Reference Intake range (RI) for total carbohydrates of 40-45 E% for infants below six months of age and of 45-55 E% for infants from 6 to < 12 months. Fibre intakes are usually not considered in recommendations up to one year of life.

5.4.4. Carbohydrate intake of infants

5.4.4.1. Digestible (glycaemic) carbohydrates

Assuming a human milk intake of 0.8 L/day and a lactose content of 70 g/L, a breast-fed infant would consume 56 g lactose per day (around 50 E%) during the first six months of life. Mean total carbohydrate intakes in mostly formula-fed infants were reported in the range of around 63-93 g per day or 46-50 E% (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013). In infants older than six months, mean total carbohydrate intakes amounted to around 80-140 g per day or 49-58 E% (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

5.4.4.2. Non-digestible (non-glycaemic) carbohydrates

Assuming an intake of 0.8 L/day of human milk and an oligosaccharide content of 5-10 g/L, a breast-fed infant would consume between 4 and 8 g per day of non-digestible oligosaccharides during the first half of the first year of life. There are no data on the oligosaccharide intakes in infants. Some European countries (Germany, France, Finland and the Netherlands) have reported the dietary fibre intake of infants older than six months of around 4-10 g/day (Lagström et al., 1997; Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008).

5.4.5. Health consequences

5.4.5.1. Type of carbohydrates

Currently only carbohydrates which are free of gluten can be used in IF and FOF. The risk of developing celiac disease (CD) and type 1 diabetes mellitus (T1DM) has been related to the timing of gluten introduction into the infant’s diet. In 2009, the Panel concluded, based on the available data, that the early (< 4 months) introduction of gluten might increase the risk of CD and T1DM, whilst the introduction of gluten between four and six months preferably while still breast-feeding might decrease the risk of CD and T1DM (EFSA NDA Panel, 2009). CD is a multi-organ disease, triggered by gluten and related prolamins in genetically pre-disposed subjects. The prevalence in Europe is in the range of 0.5-1 % (Fasano, 2001; Virta et al., 2009; Catassi et al., 2012).

A current European intervention study on 1 000 infants positive for HLA DQ2/DQ8 and randomised to receive about 100 mg of gluten per day or placebo will likely provide further evidence regarding the role of gluten introduction into the infant’s diet. However, detailed results of this study have not yet been published.

The Panel considers that the available data suggest that gluten containing complementary foods could be introduced between four and six months in small amounts preferably while the infant is still breast-fed. However, in the case of FOF feeding together with complementary foods, the use of gluten-containing starches in such formulae could result in amounts of gluten that are too high to be tolerated in genetically predisposed infants. Therefore, gluten containing starches should not be added to IF and FOF.

5.4.5.2. Glycaemic carbohydrates

Glycaemic carbohydrates provide carbohydrates to body cells, mainly in the form of glucose. Only cells in the central nervous system, red blood cells and some other cells dependent on anaerobic glycolysis have an absolute requirement for glucose. The body can in principle synthesise glucose from protein and glycerol, but this is not efficient and may lead to ketosis. Therefore, the DRV for carbohydrates is based on the energy gap between the energy provided by the sum of recommended protein and fat intakes and the total energy requirement.

5.4.5.2.1. Lactose

Lactose should be the preferred carbohydrate in IF and FOF although no absolute requirement of dietary galactose intake exists. This preference for lactose in formulae is justified by the predominance of lactose in human milk, the newborn’s capacity to hydrolyse lactose from human milk, and the absence of advantages that other glycaemic carbohydrates might have compared to lactose.

The capacity of the newborn to metabolise lactose was demonstrated in a study on 24 infants of > 36 weeks gestation, post-natal age 2-16 days, and taking full enteral feeding (n = 6 breast milk, n = 5 formula, n = 11 mixed) which showed that although lactose from formula or human milk provides glucose and galactose in equimolar amounts to the portal vein, galactose is cleared almost completely by the liver within 60 minutes after a meal and reaches only 1-2 % of the glucose concentration in plasma, whilst glucose increased in both the hepatic and systemic circulation without net hepatic uptake. There was a positive influence of post-natal age on hepatic galactose clearance that could be attributed to either maturation of enzymes or closure of a patent ductus venosus (shunting blood from the left portal vein to the vena cava) (Brown et al., 2008).

That there is no requirement for lactose is supported by two RCTs of 12 weeks duration with lactose-free formulae with maltodextrin and sucrose (Heubi et al., 2000; Lasekan et al., 2011) performed on healthy term infants in comparison to a standard lactose containing formula in which growth parameters did not differ. As soy does not contain digestible lactose or galactose naturally and formulae containing ISP may be used in infants with galactosaemia, these formulae should not contain any lactose.

5.4.5.2.2. Sucrose, glucose and fructose

The ingestion of sucrose and fructose by infants with fructose intolerance, a hereditary disease affecting approximately 1 in 26 000 infants in Central Europe (Santer et al., 2005), can lead to severe symptoms, including poor feeding, vomiting and overall failure to thrive (Coffee and Tolan, 2010). The consumption of sucrose and fructose by healthy infants does not have any advantages over the consumption of lactose and may, because of their greater sweetness, increase the preference for sweet tastes in infants. Sucrose may be added to IF containing hydrolysed protein to camouflage the taste of the hydrolysate. Because complementary food will provide other glycaemic carbohydrates than lactose, there is no reason to restrict their use in FOF as long as certain maximum levels are not exceeded.

Glucose is rapidly absorbed with a rapid rise in blood glucose and has, moreover, a higher osmotic activity than di-, oligo- and polysaccharides. Hyperosmolar feeds may lead to an increased incidence of diarrhoea. Small amounts of glucose may, however, help mitigate the disagreeable taste of IF and FOF containing protein hydrolysates.

5.4.5.2.3. Maltodextrins and starches

Maltodextrins and starches have the advantage of producing lower osmolality in products than mono- and disaccharides. The SCF (2003a) recommended that maltodextrins with 5-9 glucose units should be preferred because this corresponds to the chain-length specificity of the intestinal glucoamylase.

Pancreatic α-amylase concentrations in the infant's duodenum are lower than in adults (Christian et al., 1999). Shulman et al. (1983) compared the effects of feeding glucose, glucose polymers and precooked corn starch which were substituted for saccharose in the basal diet for one meal at a dose of 1 g/kg body weight in 16 healthy infants aged between three and four weeks on breath $^{13}CO_2$, breath hydrogen and stool ^{13}C abundance taking into account the natural ^{13}C abundance in the different formulae. The calculated oxidation rate was comparable for the different carbohydrates studied, but hydrogen production increased with carbohydrate complexity, indicating that more undigested carbohydrates reached the colon with increasing complexity of the carbohydrates. This finding is similar to the findings of another study (Shulman et al., 1986) in which it has been shown that long-chain glucose polymers are absorbed less, and with greater individual variation, than glucose or short-chain glucose polymers. Carbohydrates that are not digested and absorbed in the small intestine may be fermented by colonic bacteria. This fermentation increases the net utilisation of complex carbohydrates, but the capacity for bacterial fermentation can be exceeded by high intakes of complex carbohydrates (Shulman et al., 1983). Earlier studies reviewed by Fomon (1993) suggested that starch is tolerated up to daily intakes of 5.5-6 g/kg body weight per day and that most infants from 1-5 months of age are able to digest 10-25 g of starch per day.

5.4.5.3. Non-digestible (non-glycaemic) carbohydrates

Because of the variety, variability, complexity and polymorphism of human milk oligosaccharides the addition to IF and FOF of a mixture of oligosaccharides mimicking those found in breast milk is not feasible and oligosaccharides which are currently added to IF and FOF are not comparable to human milk oligosaccharides. Instead oligofructosyl-saccharose (oligofructose; FOS) and oligogalactosyl-lactose (oligogalactose; GOS) have been used in IF and FOF. FOS is not found in human milk and GOS is found only in trace amounts.

Cow's milk contains only traces of oligosaccharides (0.03-0.06 g/L), mostly sialylated derivatives, whilst the content of neutral and acidic oligosaccharides (AOS) in goat milk is 4-5 times higher than in cow's milk (Martinez-Ferez et al., 2006).

The SCF (2001d) had earlier assessed the safety of a formula with the addition of 0.8 g/100 mL of a mixture of 90 % GOS and 10 % high-molecular weight FOS and found no reason for concern nor conclusive proof of potential beneficial effects for infants in the second half of the first year of life. It found the evidence insufficient to establish the safety for infants below six months of age. It recommended that additional information on the suitability and safety of resistant short-chain oligosaccharides should be submitted, with particular attention to possible effects on water balance. When reviewing the available evidence in 2003, the SCF (2003a) concluded that the particular mixture of GOS and FOS which had been evaluated previously did not raise any safety concerns at concentrations used lboth in IF and FOF up to a maximum of 0.8 g/100 mL. The SCFalso reaffirmed its conclusions that further information on the safety and benefits of this combination as well as of other forms of oligosaccharides in IF and FOF should be gathered.

Since the report by the SCF (2003a), other oligosaccharides or combinations of oligosaccharides (e.g. GOS, inulin-type fructans or their combination or with mixtures of polydextrose and AOS) have been studied for a number of health outcomes, such as bowel function, gastrointestinal and respiratory tract infections, atopic dermatitis, eczema, urticaria and asthma. They have also been studied in relation to any potential untoward effects, such as delayed growth, diarrhoea and an increased risk of inadequate water balance.

Two recent systematic reviews on the effects of the addition of oligosaccharides to IF and FOF comprised 12 RCT (Mugambi et al., 2012) and 23 RCT (Braegger et al., 2011), respectively.

The systematic review by the ESPGHAN Committee on Nutrition (Braegger et al., 2011) builds upon and updates two earlier systematic reviews on "prebiotic" supplementation of full-term infants (Rao et al., 2009) and on "prebiotics" in the prevention of allergic disease and food hypersensitivity (Osborn and Sinn, 2007) and included 23 publications of RCTs performed in healthy term infants with durations between two weeks and six months. The "prebiotics" were added to IF and in two studies to FOF. In most studies a 9:1 mixture of short-chain GOS and long-chain FOS was used (Moro et al., 2002; Moro et al., 2003; Bakker-Zierikzee et al., 2005; Decsi et al., 2005; Haarman and Knol, 2005; Knol et al., 2005; Bakker-Zierikzee et al., 2006; Moro et al., 2006; Alliet et al., 2007; Costalos et al., 2008; Magne et al., 2008; Scholtens et al., 2008) four studies used GOS alone (Ben et al., 2004; Bakker-Zierikzee et al., 2005; Bettler and Euler, 2006; Ben et al., 2008), and one each AOS (Fanaro et al., 2005), GOS/FOS/AOS together (Fanaro et al., 2005), FOS plus inulin (Brunser et al., 2006) and polydextrose plus GOS (with or without lactulose) (Ziegler et al., 2007; Nakamura et al., 2009). The concentration in the formula ranged from 0.15 to 0.8 g/100 mL.

The review by Braegger et al. (2011) considered all studies included in the review by Mugambi et al. (2012) except for the studies by Bruzzese et al. (2009) and Moro et al. (2005). Therefore, the Panel has taken the review by Braegger et al. (2011) as a basis for the evaluation of data but has also considered the studies by Bruzzese et al. (2009) and Moro et al. (2005). The Panel is aware of a recent study which investigated the effect of the addition of a 50:50 mixture of FOS and long-chain inulin to an IF (Closa-Monasterolo et al., 2013).

Some of the studies reviewed assessed the effect of different oligosaccharides on the reduction of stool pH or the number of bifidobacteria or lactobacilli in stools. Even if some of the studies reported significant effects on these outcomes for some of the oligosaccharides used, the relevance of these effects for infant health is unclear. None of the studies which investigated the effects on numbers of potentially pathogenic microorganisms in stools reported significant effects on this outcome (GOS/FOS: five studies (Moro et al., 2002; Moro et al., 2003; Ben et al., 2004; Decsi et al., 2005; Alliet et al., 2007; Costalos et al., 2008); GOS/FOS/AOS: one study (Fanaro et al., 2005))

The studies which examined the impact of supplementing formula with GOS/FOS (three studies) (Moro et al., 2002; Moro et al., 2003; Moro et al., 2006; Costalos et al., 2008) or with a mixture of FOS and long-chain inulin (Closa-Monasterolo et al., 2013) on stool frequency reported statistically significant effects on stool frequency in infants, whereas the study (Brunser et al., 2006) which used a combination of FOS and inulin did not. The Panel notes that all these studies had considerable methodological limitations which severely hamper the conclusions which can be drawn from them.

The effect of GOS/FOS on stool consistency was examined in four studies. Three of these studies reported statistically significant effects on these outcomes (Moro et al., 2002; Moro et al., 2003; Moro et al., 2006; Costalos et al., 2008), while one did not (Knol et al., 2005). The studies which used a combination of GOS/FOS/AOS (Fanaro et al., 2005), polydextrose plus GOS (with or without lactulose) (Ziegler et al., 2007), or a mixture of FOS and long-chain inulin (Closa-Monasterolo et al., 2013) reported effects on stool consistency, but not the study by Brunser et al. (2006) which also used a combination of FOS and inulin. The Panel notes that all these studies had considerable methodological limitations which severely hamper the conclusions which can be drawn from them.

The effect of oligosaccharides on the incidence of infections has been studied for GOS/FOS in two studies (one reported in three publications) (Moro et al., 2006; Arslanoglu et al., 2007; Arslanoglu et al., 2008; Bruzzese et al., 2009) and for a combination of FOS and inulin in one study (Brunser et al., 2006). For the administration of GOS/FOS a statistically significant reduced cumulative incidence of fever and of infectious episodes of the upper respiratory tract requiring antibiotic treatment was reported in one study (Arslanoglu et al., 2007; Arslanoglu et al., 2008), whilst there was no difference in the incidence of lower respiratory tract, gastrointestinal and urinary tract infections. In contrast, Bruzzese et al. (2009) did not report any effect of GOS/FOS on upper respiratory tract infections, but on gastrointestinal infections. For the administration of a combination of FOS and inulin (Brunser et al., 2006) there was no effect reported on the incidence of gastrointestinal infections. The Panel notes that these studies are inconsistent and had considerable methodological limitations which severely hamper the conclusions which can be drawn from these studies.

GOS/FOS have also been studied with respect to the occurrence of allergic manifestations. A reduced cumulative incidence of atopic dermatitis, of recurrent wheezing and of allergic urticaria as been reported in one study (Arslanoglu et al., 2007; Arslanoglu et al., 2008). The Panel notes that this study had considerable methodological limitations which severely hamper the conclusions which can be drawn from it.

The Panel notes that most of the studies which investigated the effect of non-digestible oligosaccharide addition to formula had considerable limitations, including a high drop-out rate, lack of consideration of missing values, unclear sequence generation, unclear achievement of allocation concealment and/or blinding. All these limitations and the resulting uncertainties greatly limit the conclusions which can be drawn from these studies. None of the studies gave rise to concerns on any of the studied non-digestible oligosaccharides with respect to growth and adverse effects.

On the basis of the data available and in consideration of the modest quality of the available studies, the Panel considers that there is insufficient evidence for beneficial effects on infant health of the non-digestible oligosaccharides that have been tested to date in RCTs when added to IF or FOF.

5.4.6. Recommendations

5.4.6.1. Total carbohydrates

The minimum and maximum content of carbohydrates in IF and FOF can be calculated based on the residual energy in formulae that contain the permitted minimum and maximum amounts of protein and fat, and converting this energy into g of carbohydrates (4 kcal/g). The corresponding calculations are given in Table 11.

Table 11: Calculation of total minimum and maximum carbohydrate content in IF and FOF.

	IF and FOF with			
	Milk protein		**ISP and hydrolysed protein**	
Proposed amounts	min	max	min	max
Protein (g/100 kcal)	1.8	2.5	2.25	2.8
Protein (E%)	7.2	10	9.0	11.2
Fat (g/100 kcal)	4.4	6.0	4.4	6.0
Fat (E%)	39.6	54	39.6	54
Corresponding calculated amounts	max	min	max	min
Carbohydrates (E%)(a)	53.2	36	51.4	34.8
Carbohydrates (g/100 kcal)	13.3	9	12.85	8.7

(a) calculated as: 100 – E% protein – E% fat

Rounding up, the Panel proposes a minimum carbohydrate content in IF and FOF of 9 g/100 kcal (2.2 g/100 kJ) and a maximum content of 14 g/100 kcal (3.3 g/100 kJ) for all types of formulae.

The Panel considers that only carbohydrates free of gluten should be used in IF and FOF.

5.4.6.2. Glycaemic carbohydrates

5.4.6.2.1. Lactose

The Panel considers that lactose should be the preferred carbohydrate in IF and FOF. In line with the Opinion of the SCF (2003a), the Panel proposes a minimum content of lactose in IF and FOF based on milk protein and in IF and FOF containing hydrolysed protein of 4.5 g/100 kcal (1.1 g/100 kJ), unless the formulae are intended to be labelled as “lactose-free”. In such case, IF and FOF should comply with the existing criterion of a “lactose-free” formula and provide at most 0.01 g/100 kcal (0.0024 g/100 kJ) lactose.

The Panel notes that the minimum lactose content has its origin in the traditional practice of diluting cow’s milk to make it more suitable for infant feeding with respect to protein. IF and FOF containing ISP were traditionally manufactured without lactose which made such formulae suitable for feeding infants that could not metabolise lactose.

5.4.6.2.2. Sucrose, glucose and fructose

The Panel considers that sucrose, glucose and fructose, irrespective of their sources, should not be added to IF, as sucrose and fructose do not have any advantage over lactose for healthy infants, but may pose a risk to infants with fructose intolerance and saccharase deficiency and as the addition of glucose may increase the osmolality of the formula. For FOF, the use of sucrose and fructose can be tolerated since an infant will receive both from complementary foods. The sum of sucrose, fructose and sugars from honey in FOF should not constitute more than 20 % of total carbohydrates. Honey should be treated in order to destroy spores of *Clostridium botulinum*.

However, for gustatory reasons sucrose and glucose are currently permitted to be added to IF containing protein hydrolysates and glucose is currently permitted to be added to FOF containing protein hydrolysates in order to mask the bitter taste of these formulae. The maximum concentrations which may currently be added are ≤ 20 % of total carbohydrates for sucrose and ≤ 2 g/100 kcal (≤ 0.5 g/100 kJ) for glucose. The Panel proposes to retain these values.

5.4.6.2.3. Maltose and maltodextrins

In line with the Opinion by the SCF (2003a), the Panel does not consider it necessary to propose any minimum and maximum amounts for maltose and maltodextrins in IF and FOF, as long as the maximum content of carbohydrates in IF and FOF is not exceeded.

5.4.6.2.4. Starches

Based on the evidence described in Section 5.4.5.2.3 and taking that an infant could tolerate starch in amounts of around 5.5 g/kg body weight per day and an average body weight at birth of 3.25 kg (WHO Multicentre Growth Reference Study Group, 2006), this would translate into a daily starch intake of 18 g/day which could theoretically be tolerated by newborns. However, lower tolerances have also been reported. Assuming an average formula consumption of 500 kcal/day, a daily starch intake of 18 g/day would be equivalent to a starch content in formula of 2.2-2.5 g/100 mL. The Panel notes that this calculated theoretical value is not much higher than the maximum content of starch which had been proposed by the SCF (2003a) (i.e. 2 g/100 mL). The Panel also notes that there are considerable uncertainties about the amount of starch which can be tolerated by newborns and that at current amounts of starch in IF no adverse effects have been reported.

Therefore, the Panel proposes in line with the SCF (2003a) that starches should not be added in concentrations higher than 2 g/100 mL (2.9-3.3 g/100 kcal (0.7-0.8 g/100 kJ)) and that they should not constitute more than 30 % of total carbohydrates. For FOF no restrictions need to apply. The Panel agrees with the SCF (2003a) that only pre-cooked and gelatinised starches free of gluten are suitable for use in IF and FOF.

5.4.6.3. Non-digestible (non-glycaemic) carbohydrates

In the absence of convincing evidence of any beneficial effects of non-digestible oligosaccharides on infant health, the Panel considers that the addition of non-digestible oligosaccharides to IF or FOF is not necessary. The Panel considers that there is no evidence to change the previous conclusions of the SCF (2001d) that a mixture of 90 % GOS and 10 % of high-molecular weight FOS is safe under the current conditions of use (i.e. ≤ 0.8 g/100 mL) in IF and FOF. The safety of any other oligosaccharides or any new mixture of oligosaccharides in IF and FOF should be established by clinical evaluation.

Table 12 summarises the conclusions of the Panel regarding the composition of IF and FOF with respect to glycaemic carbohydrates.

Table 12: Proposed composition of IF and FOF with respect to glycaemic carbohydrates.

	IF with			**FOF with**		
g/100 kcal	**Milk protein**	**ISP**	**Protein hydrolysates**	**Milk protein**	**ISP**	**Protein hydrolysates**
Carbohydrates	9-14(f)			9-14(f)		
of which						
Lactose	≥ 4.5(a),(g)	NR(b)	≥ 4.5(a),(g)	≥ 4.5(a),(g)	NR(b)	≥ 4.5(a),(g)
Sucrose	not to be added		≤ 20 % of total carbohydrates(c),(d)	Σ sucrose, fructose, sugar from honey ≤ 20 % of total carbohydrates(c)		
Fructose	not to be added					
Glucose	not to be added		≤ 2(c),(d),(h)	not to be added		≤ 2(c),(d),(h)
Maltose, maltodextrins(c)	unrestricted within maximum amounts			unrestricted within maximum amounts		
Starches(c)	≤ 2 g/100 mL and ≤ 30 % of total carbohydrates(e)			unrestricted within maximum amounts as long as free of gluten		

(a) not applicable to formulae declared as “lactose-free”; in such case the lactose content should not exceed 0.01 g/100 kcal (0.0024 g/100 kJ)
(b) not required (NR) if more than 50 % of the protein content is from ISP
(c) voluntary addition
(d) to mask the bitter taste
(e) pre-cooked or gelatinised starch only, free of gluten
(f) 2.2-3.3 g/100 kJ
(g) 1.1 g/100 kJ
(h) 0.5 g/100 kJ

6. Minimum content of micronutrients in IF and FOF

From a nutritional point of view, the minimum contents proposed by the Panel cover the nutritional needs of virtually all healthy infants born at term and there is no need to exceed these amounts in formulae, as nutrients which are not used or stored have to be excreted and this may put a burden on the infant’s metabolism and/or other physiological functions. Therefore, the Panel emphasises that the proposed minimum contents should be understood as target values for micronutrient contents of IF and FOF.

6.1. Calcium

6.1.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum calcium contents in IF and FOF of 50 mg/100 kcal and 140 mg/100 kcal with a calcium-to-available phosphorus-ratio between 1 and 2.

6.1.2. Calcium content of human milk

Calcium in breast milk was found to be in the range of 200-300 mg/L (31-46/100 kcal) (Rodriguez Rodriguez et al., 2002; Hicks et al., 2012; Olausson et al., 2012). The ratio of calcium to phosphorus in human milk is about 2:1 on a weight basis or about 1.6:1 on a molar basis (Specker et al., 1991; Steichen and Koo, 1992).

6.1.3. Calcium requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a calcium intake of 200 mg/day and 400 mg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.1.4. Calcium intakes of infants

Assuming a human milk intake of 0.8 L/day and a calcium content of 250 mg/L, an exclusively breast-fed infant would consume 200 mg calcium per day during the first six months of life. Mean/median calcium intakes of mostly formula-fed infants below six months of age were reported to be around 370-560 mg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013). Mean/median calcium intakes in infants aged 6 to < 12 months were in the range of 450-730 mg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.1.5. Health consequences

Calcium is an integral component of the skeleton where it has a structural role and is needed for bone rigidity, strength and elasticity. Calcium deficiency in children leads to inadequate growth and bone deformity. No UL for calcium was set for infants owing to insufficient data. The UL for adults of 2 500 mg per day has been based on the absence of adverse effects in long term human intervention studies in which 2 500 mg calcium per day were administered (EFSA NDA Panel, 2012a).

The concept of maintaining a certain calcium-to-phosphorus ratio in the diet has little relevance in adults but may have some utility under conditions of rapid growth. An absorbed calcium-to-phosphorus molar ratio of around 1.3:1 is assumed to be sufficient to support the sum of bony and soft tissue growth in infants (IoM, 1997). In order to derive an intake ratio, this value has to be corrected for the fractional absorption of calcium and phosphorus. Assuming an absorption efficiency of 60 % for calcium and 80 % for phosphorus the US Institute of Medicine has suggested a calcium-to-phosphorus molar intake ratio of 2:1 for infants. However, fractional absorption may vary with age and type of formula consumed and the ratio by itself is of limited value if the consumption of absolute quantities of both nutrients is insufficient to support adequate growth (IoM, 1997). The currently permitted lower calcium-to-phosphorus ratio of 1:1 reflects the calcium-to-phosphorus molar ratio of cow's milk which does not change if cow's milk is diluted for the manufacturing of formula. There are no reports which indicate that the currently permitted calcium-to-phosphorus ratio together with the current minimum content of calcium and phosphorus in IF and FOF is insufficient to ensure adequate growth of infants.

6.1.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of calcium considered adequate by the Panel for this age group of 200 mg/day based on calcium intakes from breast milk, this would convert into a required minimum calcium intake of 40 mg/100 kcal.

The SCF (2003a) considered mean calcium absorption from cow's milk to be around 20 percentage points lower than from human milk and therefore corrected the theoretical minimum level derived based on breast-milk content by this factor, yielding a minimum calcium content of 50 mg/100 kcal. Calcium absorption efficiency was assumed to be about 58 % from human milk and about 38 % from milk-based IF during the first four months of life. More recent studies have reported calcium absorption efficiencies from IF based on milk protein and IF containing hydrolysed protein of around 60 % (Abrams, 2010; Hicks et al., 2012; Leite et al., 2013). One of these studies (Hicks et al., 2012) also assessed fractional calcium absorption from human milk and reported an absorption efficiency from breast-milk of 76 %. No information on calcium absorption from formula containing ISP is available. However, no reports are available that current amounts of calcium in formulae containing ISP would be insufficient for infants.

Considering the potential difference in absorption efficiency of calcium between human milk and formula, the Panel considers it prudent to maintain the recommendations of the SCF (2003a) with respect to the minimum calcium content in IF and FOF of 50 mg/100 kcal.

Therefore, the Panel proposes a minimum calcium content in IF and FOF of 50 mg/100 kcal (12 mg/100 kJ).

With respect to the molar ratio of calcium-to-available phosphorus (based on measured bioavailability, or calculated as 80 % of total phosphorus in milk protein based formulae or formulae containing protein hydrolysates and as 70 % of total phosphorus in formulae containing ISP), the Panel proposes a calcium-to-available phosphorus molar ratio of not less than 1.0 and not greater than 2.0.

6.2. Phosphorus

6.2.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum phosphorus contents in IF and FOF based on milk protein and IF and FOF containing hydrolysed protein of 25 mg/100 kcal and 90 mg/100 kcal. For IF and FOF containing ISP the minimum content should be 30 mg/100 kcal and the maximum 100 mg/100 kcal. The calcium-to-available phosphorus-ratio should be between 1 and 2.

With respect to the determination of the amount of available phosphorus, the SCF (2003a) concluded that the amount of available phosphorus should be either measured or calculated as 80 % of total phosphorus for milk protein or as 70 % for ISP and that at least 20 mg/100 kcal and at most 70 mg/100 kcal of available phosphorus should be contained in the formula.

6.2.2. Phosphorus content of human milk

The phosphorus content of human milk has been reported to be in the range of 107-164 mg/L (17-25 mg/100 kcal) (Fomon, 1993), peaking in early lactation and decreasing as lactation progresses (Fomon, 1993; Atkinson et al., 1995) with an average concentration of around 120 mg/L (19 mg/100 kcal) (Atkinson et al., 1995). Motil et al. (1997) reported average ± SD concentrations falling from 184 ± 16 mg/L (28 ± 2.4 mg/100 kcal) at six weeks of lactation to 155 ± 17 mg/L (24 ± 2.6 mg/100 kcal) at 24 weeks.

6.2.3. Phosphorus requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a phosphorus intake of 100 mg/day and 300 mg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.2.4. Phosphorus intakes of infants

Assuming a human milk intake of 0.8 L/day and a phosphorus content of 120 mg/L, an exclusively breast-fed infant would consume around 100 mg phosphorus per day during the first six months of life. Mean/median phosphorus intakes of mostly formula-fed infants below six months of age were reported to be between around 210 mg/day and 330 mg/day (Hilbig, 2005; Fantino and Gourmet, 2008). Mean/median phosphorus intakes in infants aged 6 to < 12 months were in the range of around 360-700 mg/day (Hilbig, 2005; de Boer et al., 2006; Fantino and Gourmet, 2008; Thorsdottir et al., 2008).

6.2.5. Health consequences

Although phosphorus in the form of phosphate ions is essential for numerous body functions, its metabolism is intricately linked to that of calcium because of the actions of calcium-regulating hormones. Adequate phosphorus and calcium intakes are needed not only for skeletal growth and maintenance, but also for many cellular roles, such as energy production (i.e. adenosine triphosphate (ATP)). Too much phosphorus, in relation to too little dietary calcium, may contribute to bone loss, and too little phosphorus along with too little dietary calcium may not adequately maintain bone mass (Anderson, 2005). Data were insufficient to establish a UL for phosphorus (EFSA, 2005a).

6.2.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of phosphorus considered adequate by the Panel for this age group of 100 mg/day based on phosphorus intakes from breast milk, this convert into a required minimum intake of available phosphorus of 20 mg/100 kcal. Assuming that 80 % of total phosphorus from milk protein and 70 % from ISP is available, this translates into a minimum phosphorus content in IF and FOF based on milk protein of 25 mg/100 kcal and for IF and FOF containing ISP of 28.6 mg/100 kcal (rounded up to 30 mg/100 kcal).

Therefore, the Panel proposes a minimum phosphorus content in IF and FOF based on milk protein and IF and FOF containing protein hydrolysates of 25 mg/100 kcal (6.0 mg/100 kJ) and in IF and FOF containing ISP of 30 mg/100 kcal (7.2 mg/100 kJ).

The molar ratio of calcium-to-available phosphorus (based on measured bioavailability, or calculated as 80 % of total phosphorus in milk protein based formulae or formulae containing protein hydrolysates and as 70 % of total phosphorus in formulae containing ISP) should be not less than 1.0 and not greater than 2.0.

6.3. Magnesium

6.3.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum magnesium contents in IF and FOF of 5 mg/100 kcal and 15 mg/100 kcal.

6.3.2. Magnesium content of human milk

Reported concentrations of magnesium in breast milk vary over a wide range (15-64 mg/L (2.3-9.8 mg/100 kcal)), with a median value of 31 mg/L (4.8 mg/100 kcal) and 75 % of reported mean concentrations below 35 mg/L (5.4 mg/100 kcal) (Dorea, 2000).

6.3.3. Magnesium requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a magnesium intake of 25 mg/day and 80 mg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.3.4. Magnesium intakes of infants

Assuming a human milk intake of 0.8 L/day and a magnesium content of 31 mg/L, an exclusively breast-fed infant would consume 25 mg magnesium per day during the first six months of life. Mean/median magnesium intakes of mostly formula-fed infants below six months of age were reported to be between around 43 and 70 mg/day (Hilbig, 2005; Fantino and Gourmet, 2008; Lennox et al., 2013). Mean/median magnesium intakes in infants aged 6 to < 12 months were in the range of around 75-140 mg/day (Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Lennox et al., 2013).

6.3.5. Health consequences

Magnesium is the second most abundant intracellular cation after sodium and is a critical cofactor in several enzymatic reactions. Severe magnesium deficiency is rare and causes neuromuscular manifestations (Feillet-Coudray and Rayssiguier, 2005). No UL for magnesium normally present in foods could be established by the SCF. A UL related to readily dissociable forms of magnesium was set at 250 mg/day for children aged from four years upwards and adults (SCF, 2001a).

6.3.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of magnesium considered adequate by the Panel for this age group of 25 mg/day based on magnesium intakes from breast milk, this converts into a required minimum magnesium content in formula of 5 mg/100 kcal.

Therefore, the Panel proposes a minimum magnesium content in IF and FOF of 5 mg/100 kcal (1.2 mg/100 kJ).

6.4. Sodium

6.4.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum sodium contents in IF and FOF of 20 mg/100 kcal and 60 mg/100 kcal, respectively.

6.4.2. Sodium content of human milk

The average sodium concentration in human milk has been reported to be in the range of 140-160 mg/L (22-25 mg/100 kcal) (IoM, 2005a).

6.4.3. Sodium requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a sodium intake of 120 mg/day was adequate for the majority of infants in the first half year of life. The Panel also concluded that a sodium intake of 170-370 mg/day was adequate for the majority of infants from 6 to < 12 months of age.

6.4.4. Sodium intakes of infants

Assuming a human milk intake of 0.8 L/day and a sodium content of 150 mg/L, an exclusively breast-fed infant would consume 120 mg sodium per day during the first six months of life. Mean/median sodium intakes in mostly formula-fed infants from 0 to < 6 months of age were reported to be in the range of around 180-240 mg/day (Fantino and Gourmet, 2008; Lennox et al., 2013) and in the second half of the first year of life of 270-730 mg/day (DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Lennox et al., 2013).

6.4.5. Health consequences

Cell membrane potentials in cells throughout the body are controlled by the concentrations of sodium and potassium. Their concentration gradients are tightly regulated as they provide the potential for neural transmission, muscle contraction and vascular tone as well as the drive for active transport of nutrients (e.g. glucose). Sodium deficiency arising from inadequate dietary intakes is unlikely because of the ubiquity of this element (SCF, 1993a). The major adverse effect of increased sodium chloride intake is elevated blood pressure. It has also been suggested that taste preferences later in life are influenced by salt intakes in early life (Stein et al., 2012). No UL for sodium could derived by the Panel owing to insufficient data (EFSA, 2005d).

6.4.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake level of sodium considered adequate by the Panel for this age group of 120 mg/day based on sodium intakes from breast milk, this converts, into a required minimum sodium content of formula of 24 mg/100 kcal (after rounding 25 mg/100 kcal).

Therefore, the Panel proposes a minimum sodium content in IF and FOF of 25 mg/100 kcal (6.0 mg/100 kJ).

6.5. Chloride

6.5.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum chloride contents in IF and FOF of 50 mg/100 kcal and 160 mg/100 kcal.

6.5.2. Chloride content of human milk

The average chloride content in human milk has been reported to be 0.4 g/L (IoM, 2005a).

6.5.3. Chloride requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a chloride intake of 300 mg/day was adequate for the majority of infants in the first half year of life. The Panel also concluded that a chloride intake of 270-570 mg/day was adequate for the majority of infants from 6 to < 12 months of age.

6.5.4. Chloride intakes of infants

Assuming a human milk intake of 0.8 L/day and a chloride content of 0.4 g/L, an exclusively breast-fed infant would consume 320 mg chloride per day during the first six months of life. No information on chloride intakes in infants living in the Europe is available.

6.5.5. Health consequences

Chloride is the most abundant anion in the extracellular fluid and counterbalances the intracellular negative charges provided by proteins. Chloride also plays a major role as a constituent of hydrochloric acid excreted in the gastric juice. Chloride deficiency arising from inadequate dietary intakes is unlikely because of the ubiquity of this element (SCF, 1993a). The major adverse effect of increased intake of chloride, as sodium chloride, is elevated blood pressure. No UL for chloride was derived by the Panel owing to insufficient data (EFSA, 2005c).

6.5.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake level of chloride considered adequate by the Panel for this age group of 300 mg/day based on chloride intakes from breast milk, this converts into a minimum chloride content of formula of 60 mg/100 kcal.

Therefore, the Panel proposes a minimum chloride content in IF and FOF of 60 mg/100 kcal (14.3 mg/100 kJ).

6.6. Potassium

6.6.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum potassium contents in IF and FOF of 60 mg/100 kcal and 160 mg/100 kcal.

6.6.2. Potassium content of human milk

The average content of potassium in human milk has been reported to be around 500 mg/L (80 mg/100 kcal) (IoM, 2005a).

6.6.3. Potassium requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a potassium intake of 400 mg/day was adequate for the majority of infants in the first half year of life. The Panel also concluded that a potassium intake of 800 mg/day was adequate for the majority of infants from 6 to < 12 months of age.

6.6.4. Potassium intakes of infants

Assuming a human milk intake of 0.8 L/day and a potassium content of 0.5 g/L, an exclusively breast-fed infant would consume 400 mg potassium per day during the first six months of life. Mean/median potassium intakes of mostly formula-fed infants in the first half year of life ranged from around 490-900 mg/day (Hilbig, 2005; Noble and Emmett, 2006; Lennox et al., 2013) and in the second half year of life they ranged from around 1 000-1 400 mg/day (Noble and Emmett, 2001; Hilbig, 2005; DGE, 2008; Marriott et al., 2008; Lennox et al., 2013).

6.6.5. Health consequences

Cell membrane potentials in cells throughout the body are controlled by the concentrations of sodium and potassium. Their concentration gradients are tightly regulated as they provide the potential for neural transmission, muscle contraction and vascular tone as well as the drive for active transport of nutrients (e.g. glucose). Potassium deficiency arising from inadequate dietary intakes is unlikely because of the ubiquity of the element (SCF, 1993a). No UL for potassium was derived by the Panel owing to insufficient data (EFSA, 2005b). Prolonged high potassium intake can lead to high concentrations of blood potassium that may affect cardiac function, especially with impaired kidney function.

6.6.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake level of potassium considered adequate by the Panel for this age group of 400 mg/day, based on potassium intakes from breast milk, this converts into a required minimum potassium intake of 80 mg/100 kcal.

Therefore, the Panel proposes a minimum potassium content in IF and FOF of 80 mg/100 kcal (19.1 mg/100 kJ).

6.7. Iron

6.7.1. Current compositional requirements of IF and FOF

Currently permitted minimum and maximum content of iron in IF and FOF as compared to the recommendations by the SCF (2003a) are depicted in Table 13.

Table 13: Currently permitted minimum and maximum content of iron in IF and FOF as laid down in Directive 2006/141/EC in comparison to the recommendations by the SCF (2003a).

Formula	**IF**				**FOF**			
	Directive 2006/141/EC		SCF (2003a)		Directive 2006/141/EC		SCF (2003a)	
mg per 100 kcal	min	max	min	max	min	max	min	max
Cow's milk	0.30	1.30	0.30	1.30	0.60	2.00	0.60	1.70
Goat's milk	0.30	1.30	0.30	1.30	0.60	2.00	0.60	1.70
Protein hydrolysates	0.30	1.30	0.30	1.30	0.60	2.00	0.60	1.70
ISP	0.45	2.00	0.45	1.90	0.90	2.50	0.90	2.50

6.7.2. Iron content of human milk

The iron concentration in human milk is around 0.2-0.4 mg/L (0.03-0.06 mg/100 kcal). The average concentration given by the US Institute of Medicine is 0.35 mg/L (0.05 mg/100 kcal) (IoM, 2001). In line with previous published values is a new analysis showing concentrations of iron in breast-milk to be 0.28 mg/L (0.054 mg/100 kcal) (Concha et al., 2013). The absorption of iron from human milk is high. Absorption efficiencies up to 50 % have been reported but have been observed to vary down to 25 % with infant age and total dietary iron intake (Domellöf et al., 2002; Domellöf, 2007; Quinn, 2014).

6.7.3. Iron requirements of infants

Full-term infants have iron stores sufficient to cover their needs for a couple of months and when exclusively breast-fed no extra iron is needed for up to six months of age for most healthy term infants (Domellöf et al., 2002; Domellöf, 2007, 2011; Jonsdottir et al., 2012).

Most of the body iron in healthy term newborns is in haemoglobin and about one fourth is in iron stores. Haemoglobin falls from an average of 170 g/L to about 120 g/L during the first six weeks of life and as iron from erythrocytes is recycled the size of the body's iron stores grows. In the coming months iron is moved back from stores to red blood cells (Domellöf, 2007). Together with the intake of iron from breast milk or other foods these iron stores serve as back-up for the growing infant for increasing blood volume and for meeting other needs. Exclusive breast-feeding during this period can meet the infant's additional iron requirements despite the low concentration of iron in breast milk as iron absorption from breast milk is high, and the newborn's iron needs are also supported through the iron stores at birth and the recycling.

The Panel considered in its Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union that observed mean iron intakes from breast milk of 0.3 mg/day were generally sufficient to ensure that iron status in the first half year of life remains within the normal range for most healthy term infants in industrialised countries (Jonsdottir et al., 2012; EFSA NDA Panel, 2013a). This advice was based on an assumed iron content in breast milk of 0.35 mg/L assuming an average consumption of breast milk of 0.8 L/day, equal to around 500 kcal/day, leading to iron intakes of 0.28 mg/day (rounded up to 0.3 mg/day). With the absorption efficiency of iron in human milk taken as 50 %, around 0.15 mg of iron per day is thus supplied to the body. This value would be lower if a lower absorption efficiency and a lower iron content of breast milk is assumed. Therefore, the estimate of 0.15 mg/day of absorbed iron could be considered a high estimate.

When iron is provided through formula, in which it is assumed to be less available than in breast milk, a daily iron intake of 0.3 mg cannot be considered to be adequate anymore for the majority of infants and additional dietary iron needs to be provided in these formulae in order to ensure a sufficient iron supply to formula-fed infants. Assuming that on average 0.15 mg/day of iron has to be absorbed from formula in the first four to six months of life and that under a conservative assumption the absorption efficiency is around 10 % (range 7-14 %) (Quinn, 2014) an iron intake from formula of 1.5 mg/day in the first four to six months of life would ensure a sufficient iron supply to the infant.

For the second half year of life, the Panel considered an iron intake of 8 mg/day adequate for the majority of infants (EFSA NDA Panel, 2013a).

6.7.4. Iron intakes of infants

Mean/median iron intakes of infants below six months of age were reported to be between 0.3 and 8 mg/day in mostly formula-fed infants (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013). Mean/median iron intakes in infants aged 6 to < 12 months were in the range of around 4-10 mg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.7.5. Health consequences

Iron has the biological ability to donate and accept an electron and change between two oxidation states, the ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron, respectively. Iron has many functions in the body such as in the oxygen transporting haemoglobin and myoglobin and in enzymes in many metabolic pathways in the liver, brain and endocrine organs. The growth and development of the central nervous system is rapid during early childhood and iron is critical for this process. Iron deficiency and iron deficiency anaemia can have a serious impact on infants' and children's health and later development, i.e. alteration of the immune status, adverse effects on morbidity, delayed behavioural and mental development, below average school achievements and growth retardation, as well as adverse effects on cognition that may or may not be reversible with iron supplementation (Moffatt et al., 1994; Iannotti et al., 2006; Hermoso et al., 2011). Even severe iron deficiency anaemia in infancy may pass unnoticed, as symptoms such as pallor, fatigue, and developmental or behavioural disturbances are quite subtle.

Active excretion of iron in humans is minimal. An overload of iron in the body is a risk for those with hereditary haemochromatosis, a relatively common disorder especially in Northern Europe, with a reported frequency of homozygosity for the C282Y mutation of around 0.7 % (Thorstensen et al., 2010), but overload may also occur in infancy without this hereditary disease. Studies support that the absorption of iron cannot be down-regulated before the age of nine months with a risk for overload in those infants with sufficient iron stores but high iron intakes. Iron-replete infants might therefore be at risk for negative health consequences if given extra iron.

The evidence for risk and benefit of iron supplementation in infancy and young childhood in developing countries were reviewed by Iannotti et al. (2006). Iron doses were 10-50 mg/day. It was reported that three out of 10 studies showed a lower weight gain in the iron fortified groups and four out of 16 an increased incidence of infections. The authors concluded that supplementation may need to be targeted to iron deficient children. A study on infants in Sweden and Honduras observed negative growth consequences associated with higher iron intakes (supplementation at 1 mg/kg body weight per day vs. no supplementation), which were however small, i.e. 0.2-0.6 cm difference in length gain both in Honduras and Sweden between four and nine months of age, and in Swedish infants there was a difference in weight gain of 100-200 g and head circumference of 0.2-0.3 cm in the five month interval (Dewey et al., 2002). In this study there was an increased likelihood of diarrhoea in a sub-group of infants with adequate iron status. In a follow-up of the study by Walter et al. (1998) at 10 years of age (Lozoff et al., 2012), the group who had received high iron formula (1.95 mg/100 kcal) scored statistically significantly lower on tests for spatial memory and visual motor integration, but not on intelligence quotient, visual perception, motor coordination and arithmetic achievement. Effects were generally small. In a subgroup of children with the highest haemoglobin concentrations at six months of age, the children who had been fed the high iron formula scored statistically significantly lower in all tests (i.e. intelligence quotient, spatial memory, visual motor integration, visual perception, motor coordination and arithmetic achievement). The drop-out between infancy and the age of 10 years was over 40 %. Other studies which investigated FOF with the currently permitted maximum concentrations of iron (i.e. 2 mg/100 kcal (12-14 mg/L)) (Fuchs et al., 1993; Stevens and Nelson, 1995; Daly et al., 1996; Gill et al., 1997; Morley et al., 1999; Williams et al., 1999) did not show any adverse effects at the levels of iron intake provided by these formulae.

The Panel notes that even though some data suggest that iron supplementation in iron-replete infants may lead to impaired growth and development and an increased risk of infections, the evidence is limited and does not allow conclusions to be drawn for the establishment of maximum iron content in IF and FOF.

In a recent review of the evidence, the ESPGHAN Committee on Nutrition (Domellöf et al., 2014) considered that formula-fed infants should receive a formula with a minimum content of iron of 4 mg/L (around 0.6 mg/100 kcal) based on current fortification practices, but acknowledged that based on theoretical considerations a minimum level of iron of 2 mg/L (around 0.3 mg/100 kcal) would be sufficient. Some RCTs have investigated the impact of feeding formulae of varying iron concentrations to infants before the age of six months. In three trials performed in the UK, Sweden and Canada (Moffatt et al., 1994; Hernell and Lönnerdal, 2002; Tuthill et al., 2002) the impact of feeding IF with iron concentrations at or below currently required minimum iron concentrations in IF (i.e. 1.8-2.1 mg/L (around 0.3 mg/100 kcal)) has been investigated with respect to their effect on iron status in infants below six month of age. The studies by Tuthill et al. (2002) and by Hernell and Lönnerdal (2002) which investigated IF with iron concentrations of < 1 vs. 5 mg/L (around < 0.2 vs. 0.8 mg/100 kcal) and 2 vs. 4 mg/L (around 0.3 vs. 0.6 mg/100 kcal), respectively, did not find any differences in iron status at three and six months of age, respectively. However, study formulae in the study by Tuthill et al. (2002) were only consumed in the first three months of life which does not allow any conclusions to be drawn with respect to the effect of a formula with a similar iron content consumed for the entire period of six months. The study by Moffatt et al. (1994) which investigated the impact of a formula with an iron concentration of 1.1 vs. 12.8 mg/L (around 0.2 vs. 2 mg/100 kcal) found a statistically significant difference in iron status between the two formula groups. However, this study was conducted in a poor population with high prevalence of anaemia which could not necessarily considered representative of the current European infant population.

These datashow that an IF providing iron at an amount of 0.3 mg/100 kcal (2 mg/L) is adequate to maintain iron status within the normal range within the first six months of life and is also supported by the theoretically calculated value of 1.5 mg/day based on iron concentrations in breast milk and differences in absorption efficiency.

One RCT (Walter et al., 1998) investigated the effect on iron status of FOF containing 2.3 mg/L (0.35 mg/100 kcal) iron (n = 405) vs. a formula providing 12.7 mg/L (1.95 mg/100 kcal) iron (n = 430) which were fed for six months to six-month-old non-anaemic infants who had been partially or exclusively breast-fed. Iron status was assessed at 12 months, and at 18 months of age in those infants who were not classified as anaemic at the 12 months follow-up. Iron deficiency was defined as two out of three measures of iron status in the abnormal range (serum ferritin < 12 µg/L, erythrocyte protoporphyrin > 100 µg/L red blood cells, or mean cell volume < 70 fL). At 12 months of age 39 % of infants were classified as iron deficient in the low iron formula group vs. 20 % in the high iron formula group ($p < 0.001$). At 18 months of age, this was 35 % vs. 17 % ($p < 0.01$). There was no significant difference in the prevalence of iron deficiency anaemia between groups at both time points.

The Panel notes that consumption of FOF containing 0.35 mg/100 kcal iron led to a significantly lower iron status in infants older than six months of age than formula with higher iron content. However, the prevalence of iron deficiency was also high in the group consuming high iron formula and there were no indications of long-term adverse effects on cognitive outcomes of consumption of a formula containing 0.35 mg/100 kcal iron during the second half of infancy. The Panel considers that in the absence of data investigating the impact of varying concentrations of iron, especially in the lower range of iron concentrations in FOF, no conclusions can be drawn from this study with respect to the nutritional adequacy of a FOF containing 0.35 mg/100 kcal iron and consumed throughout the second half of the first year of life.

FOF containing the lowest currently permitted concentrations of iron (0.6 mg/100 kcal) would provide around 2.3 mg iron per day , if an average formula consumption of 600 mL/day is assumed at this age (Fantino and Gourmet, 2008). Such concentrations require that complementary foods provide around 70 % of the daily iron intake. In the survey by Lennox et al. (2013) conducted in the framework of the UK Rolling Programme complementary contributed to around 52 % of total daily iron intakes of infants between 7-9 months of age and at 10-11 months of age this was 58 % with median total iron intakes of 7.4 mg/day and 7.6 mg/day, respectively. In a study in US breast-fed infants iron intakes from complementary foods were reported to be 1.5 mg/day and 7.2 mg/day at seven and nine months, respectively in the group consuming meat and 7.2 mg/day and 8.5 mg/day at seven and nine months, respectively in the group consuming iron fortified cereal (Krebs et al., 2006). Therefore, the Panel considers it reasonable to assume that complementary foods could provide the remaining around 5.7 mg iron per day which would be needed to be supplied to infants in order to reach daily iron intakes of 8 mg/day if a FOF with a minimum content of iron of 0.6 mg/100 kcal was consumed.

6.7.6. Recommendations

The physiological changes in iron metabolism during the first year of life are considerable. At an age below six months, most infants need little dietary iron and endogenous iron compensates for low intakes.

Based on clinical data indicating that an IF providing iron at a level of 0.3 mg/100 kcal is adequate to maintain iron status within the normal range within the first four to six months of life, the Panel proposes a minimum iron content in IF of 0.3 mg/100 kcal (0.07 mg/100 kJ). This is supported by the theoretically calculated value based on iron concentrations in breast milk and assumed differences in absorption efficiency.

Based on the consideration that around 70 % of daily iron (equivalent to 5.7 mg iron per day) could be supplied by complementary foods, a minimum content of iron in FOF of 0.6 mg/100 kcal is proposed, in line with the SCF (2003a).

There is no new evidence with respect to the impact of different iron contents in IF and FOF containing ISP. Therefore, the Panel proposes to maintain the recommendations of the SCF (2003a) with respect to the minimum iron content in such formulae (i.e. 0.45 mg/100 kcal), considering a potentially lower absorption efficiency of iron from formula containing ISP.

Therefore, the Panel proposes the minimum iron content in IF and FOF as given in Table 14.

Table 14: Proposed minimum content of iron in IF and FOF.

	Minimum IF		**Minimum FOF**	
	mg/100 kcal	mg/100 kJ	mg/100 kcal	mg/100 kJ
Cow’s milk	0.30	0.07	0.60	0.14
Goat’s milk	0.30	0.07	0.60	0.14
Protein hydrolysates	0.30	0.07	0.60	0.14
ISP	0.45	0.11	0.90	0.22

If the same formula is to be used from the first months of infancy and be suitable for the whole first year of life the minimum iron content should be 0.6 mg/100 kcal (0.14 mg/100 kJ) for milk-based formulae and formulae containing protein hydrolysates and 0.9 mg/100 kcal (0.22 mg/100 kJ) for formulae containing ISP.

6.8. Zinc

6.8.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC provides for minimum and maximum zinc contents in IF and FOF, irrespective of the protein source, of 0.5 mg/100 kcal and 1.5 mg/100 kcal. Contrary to the Directive, the SCF (2003a) had advised on a higher zinc content in IF and FOF containing ISP, namely 0.75 mg/100 kcal and 2.4 mg/100 kcal, for the minimum and maximum content, respectively.

6.8.2. Zinc content of human milk

A comprehensive review of breast milk zinc concentrations which covered 63 studies globally, including 12 from European countries (Brown KH et al., 2009) reported zinc concentrations (mean ± SD) of: 4.11 ± 1.50 mg/L below 1 month (n = 74), 1.91 ± 0.53 mg/L at 1-2 months (n = 42), 0.98 ± 0.35 mg/L at 3-5 months (n = 24), and 0.77 ± 0.22 mg/L at 6-11 months (n = 24) *post partum.*

For the first four to six months of life, breast milk provides sufficient zinc for infants (Prasad, 2003).

6.8.3. Zinc requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded on levels of zinc intakes which were considered adequate for the majority of breast-fed infants in the first half year of life of 2 mg/day and of 4 mg/day for the majority of infants in the second half of the first year of life, respectively. In a more recent draft Opinion on DRVs for zinc (EFSA NDA Panel, 2014a) to be released for public consultation, the Panel proposes a PRI for infants in the second half of the first year of life of 2.9 mg/day.

6.8.4. Zinc intakes of infants

Assuming a human milk intake of 0.8 L/day and a zinc content of 4 mg/L at two weeks of life and of 1.5 mg/L at three months of life, an exclusively breast-fed infant would consume 3.2 mg zinc per day during the first month of life and 1.2 mg/day at around three months of life. Mean/median zinc intakes of mostly formula-fed infants below six months of age were reported to range from 2.1-4.7 mg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) with the two studies which investigated zinc intake from only formula-fed infants reporting intakes of around 4 mg/day. For infants aged 6 to < 12 months zinc intakes were observed in the range of 3.1-6.7 mg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.8.5. Health consequences

Zinc is involved in many aspects of cell metabolism, with several enzymes depending on zinc for catalytic activity. It plays a role in immune function, protein synthesis, wound healing, deoxyribonucleic acid (DNA) synthesis and cell division. The current understanding of zinc deficiency in humans, much of which is marginal zinc deficiency, is based on responses to zinc supplementation. Studies have shown that physical growth and cognitive performance improved following zinc supplementation in zinc-deficient children (Fischer Walker and Black, 2004). While signs of acute zinc intoxication are gastrointestinal disturbances, chronic zinc toxicity is associated with symptoms of copper deficiency. A UL for zinc of 7 mg/day for one to three-year-old children was derived by the SCF (2002c).

6.8.6. Recommendations

The Panel’s previous Opinion (EFSA NDA Panel, 2013a) only provided advice on the levels of zinc intake considered adequate for the majority of breast-fed infants (approx. 0.4 mg/100 kcal) but did not include formula-fed infants. Therefore, the conclusions on the minimum amount of zinc in IF cannot be based on the Panel’s previous considerations. Evidence supports the concept that zinc in formula can be less available than from human milk, which needs to be considered in the establishment of the minimum content of zinc in IF and FOF.

As there are no reports that zinc deficiency occur in formula-fed infants at current levels of zinc intakes from formulae, the Panel proposes maintain the minimum content of zinc in IF and FOF based on milk protein or containing protein hydrolysates proposed by the SCF (2003a)

As phytic acid has been shown to reduce zinc absorption efficiency (Lönnerdal et al., 1984; Davidsson et al., 1994; Davidsson et al., 2004), the Panel proposes to also retain the minimum content of zinc in IF and FOF containing ISP established by the SCF (2003a)

Therefore, the Panel proposes a minimum zinc content in IF and FOF based on milk protein or containing protein hydrolysates of 0.5 mg/100 kcal (0.12 mg/100 kJ). For IF and FOF containing ISP a minimum content of 0.75 mg/100 kcal (0.18 mg/100 kJ) is proposed.

6.9. Copper

6.9.1. Current compositional requirements of IF and FOF

Based on the Opinion of SCF (2003a), Directive 2006/141/EC lays down a minimum content of copper in IF and FOF of 35 µg/100 kcal and maximum content of 100 µg/100 kcal.

6.9.2. Copper in human milk

Mean concentrations of copper in breast milk observed in Europe range from 329-390 µg/L (51-60 µg/100 kcal), with medians between 368 and 400 µg/L (57-62 µg/100 kcal) (Krachler et al., 1998; Rodriguez Rodriguez et al., 2002; Leotsinidis et al., 2005).

6.9.3. Copper requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded on levels of copper intakes which were considered adequate for the majority infants of 300 µg/day.

6.9.4. Copper intakes of infants

Assuming a human milk intake of 0.8 L/day and a copper content of 350 µg/L, an exclusively breast-fed infant would consume 280 µg copper per day during the first six months of life. Mean/median copper intakes of breast-fed and formula-fed infants below six months of age were 200-400 µg/day (Hilbig, 2005; Lennox et al., 2013). Median copper intakes of infants from 6 to < 12 months were 400-900 µg/day (Hilbig, 2005; Marriott et al., 2008; Lennox et al., 2013).

6.9.5. Health consequences

Copper is an essential nutrient and an indispensable cofactor of many proteins including enzymes involved in oxidative reactions, in the production of collagen and of pigment, in iron metabolism and in the function of the heart, brain and the immune system. Copper deficiency is rare in humans and occurs predominantly in premature and small for gestational age infants fed cow’s milk formulae, patients with malnutrition and patients receiving total parenteral nutrition (TPN) devoid of copper or subjects consuming high-dose zinc supplements. Signs of severe copper deficiency include anaemia, leucopenia and neutropenia. Osteoporosis has also been observed when bones are still growing (Turnlund, 2006). Copper excess is rare and results acutely in gastrointestinal symptoms and chronically in liver and kidney dysfunction. A UL for copper of 1 000 µg/day was derived by the SCF (2003c) for children aged one to three years.

6.9.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of copper considered adequate by the Panel for this age group of 300 µg/day based on copper intakes from breast milk, this converts into a required minimum copper content of formulae of 60 µg/100 kcal.

Therefore, the Panel proposes a minimum copper content in IF and FOF of 60 µg/100 kcal (14.3 µg/100 kJ).

6.10. Selenium

6.10.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC provides for minimum and maximum selenium contents in IF and FOF of 1 µg/100 kcal and 9 µg/100 kcal while the SCF (2003a) had recommended minimum and maximum contents of 3 µg/100 kcal and 9 µg/100 kcal.

6.10.2. Selenium content of human milk

A wide range of selenium concentrations in human milk have been observed, depending on the amount of selenium consumed by the mother from natural foods. Breast milk concentrations of selenium in Europe range from 3-84 µg/L (0.46-12.9 µg/100 kcal), with a mean value of 16.3 ± 4.7 µg/L (2.51 ± 0.72 µg/100 kcal) (Krachler et al., 1998; Zachara and Pilecki, 2000; Navarro-Blasco and Alvarez-Galindo, 2003; Özdemir et al., 2008).

6.10.3. Selenium requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a selenium intake of 12.5 µg/day and 15 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.10.4. Selenium intakes of infants

Assuming a human milk intake of 0.8 L/day and a selenium content of 16 µg/L, an exclusively breast-fed infant would consume 12.8 µg selenium per day during the first six months of life. Mean selenium intakes in breast-fed and formula-fed infants in the first half year of life were reported to be 15 µg/day in one study in the UK (Lennox et al., 2013). For infants in the second half year of life selenium intakes were reported in the range of 18-22 µg/day in the Netherlands and the UK (de Boer et al., 2006; Lennox et al., 2013).

6.10.5. Health consequences

Selenocysteine is an indispensable constituent of 25 different selenoproteins. Most selenoproteins are involved in redox reactions and three deiodinases convert thyroxine to triiodothyronine. Selenium deficiency, for example following long-term TPN, malabsorption syndromes or use of special diets containing insufficient selenium, leads to impaired muscle function and loss of pigment in hair and skin. Chronic selenium excess is characterised by hair loss and nail dystrophy, breath smelling of garlic, dermatitis and neurological and endocrinological symptoms (selenosis). The SCF (2000g) has set a UL for selenium of 60 µg per day for children aged one to three years.

6.10.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of selenium considered adequate by the Panel for this age group of 12.5 µg/day based on selenium intakes from breast milk, this converts into a required minimum selenium content in formula of 2.5 µg/100 kcal (rounding up to 3 µg/100 kcal)

Therefore, the Panel proposes a minimum selenium content in IF and FOF of 3 µg/100 kcal (0.72 µg/100 kcal).

6.11. Iodine

6.11.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum iodine contents in IF and FOF of 10 µg/100 kcal and 50 µg/100 kcal, respectively.

6.11.2. Iodine content of human milk

The iodine concentration of breast milk in Europe has been observed in the range of around 50-100 µg/L (8-15 µg/100 kcal) (Costeira et al., 2009; EFSA NDA Panel, 2014b).

6.11.3. Iodine requirements of infants

In its Draft Scientific Opinion on the Dietary Reference Values for iodine (EFSA NDA Panel, 2014b), the Panel proposed an AI of iodine of 70 µg/day for infants from 7-11 months of age. No AI was set for infants from birth to six months of age, where exclusive breastfeeding is assumed to provide an adequate iodine supply. In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that an iodine intake of 90 µg/day was adequate for the majority of infants. Since there is no reason to assume that infants from birth to six months of age need more iodine than infants in the second half of the first year of life, the Panel considers in line with its most recent evaluation (EFSA NDA Panel, 2014b) that an intake of iodine of 70 µg/day is adequate for the majority of infants from birth to 12 months of age.

6.11.4. Iodine intakes of infants

Assuming a human milk intake of 0.8 L/day and an iodine content of 50-100 µg/L, an exclusively breast-fed infant would consume 40-80 µg iodine per day during the first six months of life. Mean/median iodine intakes in breast-fed and formula-fed infants from birth to below six months were reported to range from around 35-94 µg/day in Germany and the UK (Hilbig, 2005; Noble and Emmett, 2006; Lennox et al., 2013). In infants from 6 to < 12 months mean/median iodine intakes were between 42 and 118 µg/day in Germany and the UK (Noble and Emmett, 2001; Hilbig, 2005; DGE, 2008; Lennox et al., 2013).

6.11.5. Health consequences

The most critical physiological role for iodine is the normal functioning of the thyroid gland. Iodine deficiency disorders (IDD) are caused by insufficient iodine intakes leading to hypothyroidism. IDD are particularly of concern in pregnancy and infancy because of the risk of developmental brain damage. Chronic iodine deficiency may also lead to compensatory thyroid hyperplasia with goitre. Chronic excessive iodine intake can also lead to goitre. A UL for iodine was set at 200 µg/day for children aged one to three years based on biochemical changes in thyroid-stimulating hormone levels (SCF, 2002a).

6.11.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as the basis the intake levels of iodine considered adequate by the Panel for this age group of 70 µg/day based on iodine intakes from breast milk, this converts into a required minimum iodine content of formula of 14 µg/100 kcal (rounded up to 15 µg/100 kcal).

Therefore, the Panel proposes a minimum iodine content in IF and FOF of 15 µg/100 kcal (3.6 µg/100 kJ).

6.12. Chromium

6.12.1. Current compositional requirement of IF and FOF

The SCF (2003a) concluded that there were no biological or nutritional data to define a minimum and maximum content of chromium in IF and FOF. No minimum and maximum chromium content in IF and FOF is specified by Directive 2006/141/EC.

6.12.2. Chromium content of human milk

In Europe, mean concentrations of chromium in mature breast milk are highly variable ranging from 0.19-10.8 µg/L (0.03-1.7 µg/100 kcal) (Kumpulainen and Vuori, 1980; Kumpulainen et al., 1980; Clemente et al., 1982; Deelstra et al., 1988; Bougle et al., 1992; Cocho et al., 1992; Aquilio et al., 1996; Wappelhorst et al., 2002).

6.12.3. Chromium requirements of infants

No AR and no PRI for chromium for the performance of physiological functions can be defined.

6.12.4. Health consequences

The case for the essentiality of dietary Cr^{3+} for humans was uncertain when the SCF considered the element twenty years ago (SCF, 1993a), then, as now, the postulation of its essentiality was almost entirely based on case reports of patients on long-term TPN who developed metabolic and neurological defects which responded to Cr^{3+}. The Panel considers that there is as yet no convincing evidence that chromium is an essential nutrient, because no specific physiological changes due to experimental chromium deficiency have been identified. No AR for the performance of essential physiological functions can be defined. Owing to limited data the SCF (2003b) was unable to set a UL. It was stated that in a number of limited studies there was no evidence of adverse effects in adults associated with supplementary intake of chromium up to a dose of 1 mg/day.

6.12.5. Recommendations

Because of the unproven essentiality of chromium together with the fact that no specific physiological function can be ascribed to chromium, the Panel considers that the addition of chromium to IF and FOF is not necessary.

6.13. Molybdenum

6.13.1. Current compositional requirements of IF and FOF

The SCF (2003a) concluded that there were no biological or nutritional data to define a minimum and maximum content of molybdenum in IF and FOF. No minimum and maximum molybdenum content in IF and FOF is specified by Directive 2006/141/EC.

6.13.2. Molybdenum content of human milk

Mean molybdenum concentrations in human milk were reported to range between 0.72 µg/L and 4 µg/L (0.11-0.62 µg/100 kcal) with a mean of around 2.5 µg/L (0.38 µg/100 kcal) (EFSA NDA Panel, 2013c).

6.13.3. Molybdenum requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a molybdenum intake of 2 µg/day and 10 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.13.4. Molybdenum intakes of infants

Assuming a human milk intake of 0.8 L/day and a molybdenum content of 2.5 µg/L, an exclusively breast-fed infant would consume 2 µg molybdenum per day during the first six months of life. No data on molybdenum intakes in infants in the first year of live living in Europe are available.

6.13.5. Health consequences

In humans, sulphite oxidase, xanthine oxidoreductase, aldehyde oxidase and mitochondrial amidoxime reducing component require molybdenum linked with a pterin (molybdopterin) as cofactor. Only one human case report of likely dietary molybdenum deficiency has been reported in an adult patient on TPN because of short-bowel syndrome (Abumrad et al., 1981). A UL for molybdenum for children 1-3 years old was set at 100 µg/day (SCF, 2000d). This UL was extrapolated from the UL for adults (600 µg/day) which was based on reproductive toxicity and adverse effects on growth in rats.

6.13.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as the basis the intake levels of molybdenum considered adequate by the Panel for this age group of 2 µg/day based on molybdenum intakes from breast milk, this converts into a required minimum molybdenum content of formula of 0.4 µg/100 kcal. Therefore, the Panel proposes a minimum molybdenum content in IF and FOF of 0.4 µg/100 kcal (0.1 µg/100 kJ).

6.14. Manganese

6.14.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum manganese contents in IF and FOF of respectively 1 µg/100 kcal and 100 µg/100 kcal.

6.14.2. Manganese content of human milk

The mean manganese concentrations of human milk of European mothers vary from 3-30 µg/L (0.46-4.6 µg/100 kcal), but most values are around 4 µg/L (0.62 µg/100 kcal) (Mullee et al., 2012).

6.14.3. Manganese requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a manganese intake of 3 µg/day and 20-500 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.14.4. Manganese intakes of infants

Assuming an average milk intake of 0.8 L/day and a manganese content of 3-30 µg/L, an exclusively breast-fed infant would consume 2.4-24 µg manganese per day in the first six months of life. Reports on median manganese intakes of infants are only available for German infants (Hilbig, 2005) and were 30 µg/day for breast-fed and formula-fed infants between birth and six months of age and 500 µg/day for infants from 6 to < 12 months of age.

6.14.5. Health consequences

Manganese is an essential dietary mineral for mammals; it is a component of metalloenzymes such as superoxide dismutase, arginase and pyruvate carboxylase, and is involved in amino acid, lipid and carbohydrate metabolism. In animals glycosyltransferases and xylosyltransferases, which are involved in proteoglycan synthesis (e.g. for bone formation), are sensitive to manganese status (Nielsen, 1999). No specific manganese deficiency syndrome has been described in humans. The symptoms of manganese toxicity can result in a permanent neurological disorder known as manganism (ATSDR, 2012). Professional exposure by inhalation is the main cause of manganism but oral exposure to manganese, especially from contaminated water sources, can also cause adverse health effects, which are similar to those observed from inhalation exposure. No UL for manganese has been set (SCF, 2000f).

6.14.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as the basis the intake levels of manganese considered adequate by the Panel for this age group of 3 µg/day based on manganese intakes from breast milk, this would convert into a required minimum manganese content in IF and FOF of 0.6 µg/day. Taking into account that manganese from formula may be absorbed less than from human milk, the Panel proposes to retain the minimum manganese content in IF and FOF of 1 µg/100 kcal as proposed by the SCF (2003a).

Therefore, the Panel proposes a minimum manganese content in IF and FOF of 1 µg/100 kcal (0.24 µg/100 kJ).

6.15. Vitamin A

6.15.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum vitamin A contents in IF and FOF of 60 µg retinol equivalents (RE)/100 kcal and 180 µg RE/100 kcal. Retinol sources authorised for use in IF and FOF are retinol and two forms of retinyl esters, i.e. retinyl palmitate and retinyl acetate. Carotenoids are not considered in contributing to vitamin A intakes in infants owing to a lack of knowledge on the bioconversion of carotenoids in infants. Therefore, they are not considered as a source of vitamin A in IF and FOF.

6.15.2. Vitamin A content of human milk

Pre-formed vitamin A concentrations in human milk in Western countries were traditionally considered to be between 450 and 600 µg RE/L (69-92 µg RE/100 kcal), whereas considerably lower values were reported in two recent studies in Europe: 80 µg RE/L (12 µg RE/100 kcal) (Tijerina-Saenz et al., 2009) and 85 µg RE/L (13 µg RE/100 kcal) (Szlagatys-Sidorkiewicz et al., 2012).

6.15.3. Vitamin A requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin A intake of 350 µg RE/day was adequate for the majority of infants. This was based on assumed mean vitamin A content in breast milk of 450 µg/L and a daily intake of 0.8 L and rounding down.

6.15.4. Vitamin A intakes of infants

Assuming a human milk intake of 0.8 L/day and a pre-formed vitamin A content of 450 µg/L, an exclusively breast-fed infant would consume 360 µg of pre-formed vitamin A per day during the first six months of life. Mean/median vitamin A intakes of mostly formula-fed infants below six months of age were reported to range from around 510-980 µg RE/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013). For infants aged 6 to < 12 months mean/median total vitamin A intakes were observed in the range of 530-1 090 µg RE/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.15.5. Health consequences

Vitamin A has several important functions, including a role in vision, maintenance of epithelial surfaces, immune competence, growth, development and reproduction (Nordic Council of Ministers, 2013). Deficiency of vitamin A leads to neonatal growth retardation and affects several functions such as vision, immunity, and reproduction. The most specific clinical consequence of severe vitamin A deficiency is xerophthalmia and deficient dark adaptation (night blindness). The deficient dark adaptation due to inadequate vitamin A intake disappears after retinol or β-carotene supplementation (Chase et al., 1971; Sauberlich et al., 1974). In a systematic review and meta-analysis of studies on children living in Asia, Africa and Latin America aged six months to five years vitamin A supplementation was associated with reductions in mortality, morbidity, and vision problems (Mayo-Wilson et al., 2011). Vitamin A deficiency in healthy exclusively breast-fed and formula-fed infants has not been observed in Europe. Children are particularly sensitive to vitamin A, with daily intakes of about 450 µg RE/kg body weight per day leading to toxicity (Bendich and Langseth, 1989; Hathcock et al., 1990; Coghlan and Cranswick, 2001; Allen and Haskell, 2002). Signs of chronic hypervitaminosis A in infants are reported as loss of appetite, dermal dryness, loss of hair, fissuring of the corners of the mouth, bone pain, hepatomegaly, increased intracranial pressure, and failure to thrive (Fomon, 1993). A UL for preformed vitamin A (retinol and retinyl esters) for children one to three years of age of 800 µg RE/day has been set based on the risk of hepatotoxicity and teratogenicity and subsequent extrapolation to children (SCF, 2002b).

There is an interaction between iron and vitamin A. Vitamin A deficiency impairs iron mobilisation and vitamin A supplementation improves haemoglobin concentrations. Iron supplementation combined with vitamin A seem to be more effective than iron alone to improve haemoglobin concentrations (Michelazzo et al., 2013). No consistent relationship between zinc and vitamin A status was established in humans (Christian and West, 1998) although zinc supplementation improved dark adaptation in zinc-deficient patients (Morrison et al., 1978).

6.15.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of vitamin A considered adequate by the Panel for this age group of 350 µg RE/day based on pre-formed vitamin A intakes from breast milk, this converts into a required minimum vitamin A content of formula of 70 µg RE/100 kcal.

Therefore, the Panel proposes a minimum vitamin A content in IF and FOF of 70 µg RE/100 kcal (16.7 µg RE/100 kJ).

The vitamin A activity in IF and FOF should be provided by retinol or retinyl esters. In view of the existing uncertainties as to the relative equivalence of β-carotene and retinol in infants, any content of carotenes should not be included in the calculation and declaration of vitamin A activity.

6.16. Vitamin D

6.16.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC lays down 1 µg/100 kcal as the minimum and 2.5 µg/100 kcal as the maximum vitamin D content in IF and 1 µg/100 kcal as the minimum and 3 µg/100 kcal as the maximum vitamin D content in FOF.

6.16.2. Vitamin D content of human milk

The mean vitamin D content of breast milk in healthy women has been reported to be in the range 0.25-2.0 µg/L (0.04-0.31 µg/100 kcal) (Dawodu and Tsang, 2012). There is general agreement that human milk does not contain sufficient vitamin D to prevent rickets, even if the mother takes vitamin D supplements (Olafsdottir et al., 2001).

6.16.3. Vitamin D requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin D intake of 10 µg/day was adequate for the majority of infants having minimal sun exposure. Vitamin D can also be synthesised in the skin under the influence of ultraviolet B light. Consequently, requirements of dietary vitamin D depend also on geographical area and lifestyle factors determining the exposure of skin to sunlight.

6.16.4. Vitamin D intakes of infants

Mean/median vitamin D intakes of formula-fed infants below six months of age were reported to be around 9-10 µg/day in formula-fed infants (Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and 3.5 µg/day in breast-fed infants (Lennox et al., 2013). For infants aged 6 to < 12 months mean/median vitamin D intakes were observed in the range of 3.6-10.4 µg/day (Noble and Emmett, 2001; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013). The Panel notes that given that vitamin D can be synthesised endogenously low vitamin D intakes do not necessarily lead to an inadequate vitamin D status.

6.16.5. Health consequences

Vitamin D plays a key role in calcium and phosphate metabolism and is essential for bone health. There is no evidence from interventional studies to support vitamin D supplementation for other health benefits (muscle strength, prevention of infectious or allergic diseases or T1DM) in infants and young children (Braegger et al., 2013). Early signs of vitamin D deficiency are subclinical and include decreased serum concentrations of calcium and phosphorus while later signs comprise inadequate skeletal mineralisation (rickets and osteomalacia), bone deformities, bone pain, and alterations in muscle metabolism and respiratory function (SCF, 1993a). Reports of clinically manifest rickets in healthy infants have become few in Europe. In its recent consensus statement the ESPGHAN Committee on Nutrition noted that reports on vitamin D intoxication were scarce and that there was no agreement on a vitamin D toxicity threshold (Braegger et al., 2013). However, a UL might be defined even in the lack of toxicity threshold data. Recent intervention studies using doses up to 25 µg vitamin D per day (plus the amount ingested via fortified IF) for up to five months after birth did not indicate that these intakes were associated with hypercalcaemia in infants and a UL of 25 µg vitamin D per day has been established by the Panel (EFSA NDA Panel, 2012b).

6.16.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of vitamin D considered adequate by the Panel for this age group of 10 µg/day based on achievable 25(OH)D vitamin serum concentrations, this converts into a minimum vitamin D content in formula of 2 µg/100 kcal.

Therefore, the Panel proposes a minimum vitamin D content in IF and FOF of 2 µg/100 kcal (0.48 µg/100 kJ).

6.17. Vitamin E

6.17.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC lays down 0.5 mg α-tocopherol equivalents (TE)/g PUFA but not less than 0.5 mg TE/100 kcal as the minimum and 5 mg α-TE/100 kcal as the maximum vitamin E concentration in IF and FOF.

6.17.2. Vitamin E content of human milk

The value traditionally used for characterising α-tocopherol content in human milk was 3.49 mg α-TE/L (0.54 mg α-TE/100 kcal) (Jansson et al., 1981). This value was closely corroborated in three-month-old infants in one recent study (3.48 mg α-TE/L (0.54 mg α-TE/100 kcal) (Antonakou et al., 2011), whereas different values have been reported in other studies, i.e. 2.32 mg α-TE/L (0.36 mg α-TE/100 kcal) (Tijerina-Saenz et al., 2009) and 1.10 mg α-TE/L (0.17 mg α-TE/100 kcal) (Szlagatys-Sidorkiewicz et al., 2012)).

6.17.3. Vitamin E requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin E intake of 3 mg α-tocopherol per day and 5 mg α-tocopherol per day was adequate for the majority of infants in the first and second half year of life, respectively.

6.17.4. Vitamin E intakes of infants

Assuming a human milk intake of 0.8 L/day and a vitamin E content of 3.5 mg α-TE/L, an exclusively breast-fed infant would consume 2.8 mg vitamin E per day during the first six months of life. Mean/median vitamin E intakes of breast-fed and formula-fed infants below six months of age were reported to be around 3.6-6.0 mg TE/day (Hilbig, 2005; Fantino and Gourmet, 2008). For infants aged 6 to < 12 months mean/median vitamin E intakes were observed in the range of 4.3-6.5 mg TE/day (DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008).

6.17.5. Health consequences

The major biological role of α-tocopherol is its antioxidant activity contributing to the prevention of propagation of free radicals in various lipid structures within the organism. RRR-α-tocopherol is the principal isomer in animal tissues and as this form is relatively unstable, more stable tocopherol esters are commonly used in the production of IF and FOF. These forms have a biological activity lower than the one of RRR-α-tocopherol. Muscle and neurological problems can be direct consequence of human vitamin E deficiency; however, they usually develop only in sick infants and young children (e.g. in those with fat malabsorption). Vitamin E appears to have very low toxicity, and amounts of 100-200 mg/day of synthetic α-tocopherols were consumed widely as supplements in adults without reported untoward effects. No adverse effects have been described from intakes provided by food sources (Nordic Council of Ministers, 2013). The SCF (2003d) did not set a UL for infants and children. For adults, a UL of 300 mg/day was derived.

6.17.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of vitamin E considered adequate by the Panel for this age group of 3 mg α-tocopherol per day based on vitamin E intakes from breast milk, this converts into a required minimum vitamin E intake of 0.6 mg α-tocopherol/100 kcal as RRR-α-tocopherol.

Therefore, the Panel proposes a minimum vitamin E content in IF and FOF of 0.6 mg α-tocopherol/100 kcal (0.14 mg/100 kJ). This figure is based on RRR-α-tocopherol activity.

6.18. Vitamin K

6.18.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC lays down 4 µg/100 kcal as the minimum and 25 µg/100 kcal as the maximum vitamin K content in IF and FOF, while the SCF (2003a) had proposed 4 µg/100 kcal as the minimum and 20 µg/100 kcal as the maximum content.

6.18.2. Vitamin K content of human milk

Mean vitamin K concentrations in human milk are around 2.5 µg/L (0.38 µg/100 kcal) but vary considerably from 0.85-9.2 µg/L (0.13-1.4 µg/100 kcal) (IoM, 2001).

6.18.3. Vitamin K requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin K intake of 5 µg/day and 8.5 µg/day was adequate for the majority of infants in the first and second half year of life respectively. These values were based on the guidance value proposed by the SCF (1993a) of 1 µg/kg body weight per day.

6.18.4. Vitamin K intakes of infants

Assuming a human milk intake of 0.8 L/day and a vitamin K content of 2.5 µg/L, an exclusively breast-fed infant would consume 2 µg vitamin K per day during the first six months of life. No information on vitamin K intakes in infants living in Europe is available.

6.18.5. Health consequences

Vitamin K is needed primarily for the synthesis of various factors and proteins involved in blood coagulation. Following the postnatal period when haemorrhagic disease of the neonate may develop, no studies have been conducted that assess any functional marker of vitamin K sufficiency or deficiency in infants and young children. The suggested associations between phylloquinone intakes and bone health or prevention of atherosclerosis are inconsistent (Nordic Council of Ministers, 2013). While low vitamin K stores at birth may predispose to haemorrhages in healthy neonates and young infants, later in life clinical consequences of vitamin K deficiency are seen almost exclusively in sick children. Natural vitamin K seem free of toxic side effects. The SCF concluded in its Opinion that there was no evidence of adverse effects associated with supplementary intakes of vitamin K in the form of phylloquinone of up to 10 mg/day for limited periods of time (SCF, 2003a).

6.18.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of vitamin K considered adequate by the Panel for this age group of 5 µg/day, this converts into a required minimum vitamin K content of formula of 1 µg/100 kcal.

Therefore, the Panel proposes a minimum vitamin K content in IF and FOF of 1 µg/100 kcal (0.24 µg/100 kJ).

6.19. Thiamin (vitamin B1)

6.19.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum thiamin contents in IF and FOF of 60 µg/100 kcal and 300 µg/100 kcal.

6.19.2. Thiamin content of human milk

The average content of thiamin in human milk is 200 µg/L (31 µg/100 kcal) (IoM, 1998) with a range of 150-330 µg/L (23-51 µg/100 kcal) (SCF, 2003a).

6.19.3. Thiamin requirements of infants

The SCF (1993a) defined the AR and PRI for thiamin for all age groups to be 72 µg/MJ (30 µg/100 kcal) and 100 µg/MJ (42 µg/100 kcal), respectively. In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a thiamin intake of 200 µg/day and 300 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.19.4. Thiamin intakes of infants

Assuming a human milk intake of 0.8 L/day and a thiamin content of 200 µg/L, an exclusively breast-fed infant would consume 160 µg thiamin per day during the first six months of life. Mean/median thiamin intakes in mostly formula-fed infants from birth to six months were reported to range from around 150-700 µg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 300-1 000 µg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.19.5. Health consequences

Thiamin in its phosphorylated forms is a coenzyme in the oxidative decarboxylation of 2-oxoacids, for example pyruvate, 2-oxoglutarate, branched-chain 2-oxoacids and in the transketolase reaction among hexose and pentose phosphates. of the rate of thiamin utilisation depends on carbohydrate intake and is related to energy intake. Thiamin deficiency as a consequence of dietary insufficiency has been shown to lead to growth restriction, recurrent infections and sudden infant death. Thiamin deficiency is rare in higher income countries but an outbreak of lactic acidosis and encephalopathy was reported in young infants who had received a formula unintentionally devoid of thiamin as the sole source of nutrition (Fattal-Valevski et al., 2005). There are no adverse effects known to be associated with excessive thiamin consumption. No UL for thiamin was set by the SCF (2001b).

6.19.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of thiamin considered adequate by the Panel for this age group of 200 µg/day based on thiamin intakes from breast milk, this converts into a required minimum thiamin content in formula of 40 µg/100 kcal.

Therefore, the Panel proposes a minimum thiamin content in IF and FOF of 40 µg/100 kcal (9.6 µg/100 kJ).

6.20. Riboflavin (vitamin B2)

6.20.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum riboflavin contents in IF and FOF of 80 µg/100 kcal and 400 µg/100 kcal.

6.20.2. Riboflavin content of human milk

The average content of riboflavin in human milk is around 350-600 µg/L (54-92 µg/100 kcal) (Picciano, 1995; IoM, 1998).

6.20.3. Riboflavin requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a riboflavin intake of 300 µg/day and 400 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.20.4. Riboflavin intakes of infants

Assuming a human milk intake of 0.8 L/day and a riboflavin content of 450 µg/L, an exclusively breast-fed infant would consume 360 µg riboflavin per day during the first six months of life. Mean/median riboflavin intakes in mostly formula-fed infants from birth to six months were reported to range from around 300-700 µg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 500-1 400 µg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.20.5. Health consequences

Riboflavin is a precursor of two flavin co-enzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD have a role in many biochemical reactions, as components of enzymes that catalyse oxidation/reduction reactions in numerous metabolic pathways. They are required for lipid degradation, synthesis of steroids and glycogen and amino acid metabolism. Flavo-enzymes are also involved in niacin synthesis from tryptophan, in the conversion of vitamin B6 into pyridoxalphosphate, and in the production of methyl-tetrahydrofolate. There is an interaction with iron metabolism. Dietary riboflavin deficiency is rare. It leads to non-specific symptoms, particularly of mucosal tissues (cheilosis, glossitis, keratitis, gastrointestinal disturbances) and in a late stadium to hypochromic anaemia. Excess riboflavin consumption has not been associated with adverse effects in humans. Therefore, no UL could be established by the SCF (2000a).

6.20.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of riboflavin considered adequate by the Panel for this age group of 300 µg/day based on riboflavin intakes from breast milk, this converts into a required minimum riboflavin content of formula of 60 µg/100 kcal.

Therefore, the Panel proposes a minimum riboflavin content in IF and FOF of 60 µg/100 kcal (14.3 µg/100 kJ).

6.21. Niacin

6.21.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC provides for minimum and maximum niacin contents in IF and FOF of 0.3 mg/100 kcal and 1.5 mg/100 kcal. The SCF (2003a) concluded in its Opinion on minimum and maximum contents in IF and FOF of 0.3 mg/100 kcal and 1.2 mg/100 kcal.

6.21.2. Niacin content of human milk

The average content of niacin in mature human milk from European mothers has been reported to be in the range of 1.8-2.2 mg/L (0.28-0.34 mg/100 kcal) (DHSS, 1977; Ford et al., 1983).

6.21.3. Niacin requirements of infants

Niacin requirements are given as niacin equivalents (NE), the sum of preformed niacin plus niacin produced from tryptophan (assuming that 60 mg tryptophan are equivalent to 1 mg NE); this definition is valid only when the diet contains both niacin and sufficient tryptophan. The niacin requirement is moreover dependent on the energy intake, with an AR and PRI for all age groups of 1.3 mg NE/MJ (0.55 mg NE/100 kcal) and 1.6 mg NE/MJ (0.67 mg NE/100 kcal), respectively.

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a niacin intake of 2 mg NE/day and 5 mg NE/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively. These values are in line with a more recent draft Opinion on DRVs for niacin endorsed by the Panel for public consultation (EFSA NDA Panel, 2013d).

6.21.4. Niacin intakes of infants

Assuming a human milk intake of 0.8 L/day and a niacin content of 2 mg/L, an exclusively breast-fed infant would consume 1.6 mg niacin per day during the first six months of life. Mean/median niacin intakes in mostly formula-fed infants from birth to six months were reported to range from around 4-10 mg NE/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 4.5-14 mg NE/day (Noble and Emmett, 2001; Hilbig, 2005; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.21.5. Health consequences

Niacin, that is both nicotinic acid and nicotinamide, is the precursor of the nicotinamide nucleotide coenzymes nicotine adenine dinucleotide (NAD) and nicotine adenine dinucleotide phosphate (NADP), which are crucial for many oxidation/reduction reactions and associated with both catabolic and anabolic processes. It can be provided in the diet and be formed in the human body from its precursor amino acid tryptophan. Long-term inadequate intake of niacin and tryptophan can lead to the development of pellagra. The profile of adverse effects after excessive intake of nicotinic acid and nicotinamide is different. For nicotinic acid the main adverse effects are flushing and hepatotoxicity. For nicotinamide no such adverse effects have been reported at intakes of several grams per day, except for hepatotoxicity in rare cases when slow-release preparations of nicotinamide were applied. The UL for children aged one to three years of nicotinic acid (2 mg/day) and nicotinamide (150 mg/day) have been derived from adult values on the basis of reference body weights (SCF, 2002d).

6.21.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of niacin considered adequate by the Panel for this age group of 2 mg/day based on niacin intakes from breast milk, this converts into a required minimum niacin concentration of 0.4 mg/100 kcal.

Therefore, the Panel proposes a minimum niacin content in IF and FOF of 0.4 mg/100 kcal (0.10 mg/100 kJ). This is preformed niacin.

6.22. Pantothenic acid

6.22.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum pantothenic acid contents in IF and FOF of 0.4 mg/100 kcal and 2 mg/100 kcal.

6.22.2. Pantothenic acid content of human milk

The average content of pantothenic acid in human milk is reported to be around 2.5 mg/L (0.38 mg/100 kcal) (EFSA NDA Panel, 2014e).

6.22.3. Pantothenic acid requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a pantothenic acid intake of 2 mg/day and 3 mg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively. These values are in line with the more recent Opinion of the Panel on DRVs for pantothenic acid (EFSA NDA Panel, 2014e).

6.22.4. Pantothenic acid intakes of infants

Assuming a human milk intake of 0.8 L/day and a pantothenic acid content of 2.5 mg/L, an exclusively breast-fed infant would consume 2 mg pantothenic acid per day during the first six months of life. No information on pantothenic acid intakes in infants living in Europe is available.

6.22.5. Health consequences

Pantothenic acid is required in the synthesis of coenzyme A (CoA) and acyl carrier proteins and thus has a central role in a wide variety of metabolic pathways. Pantothenic acid deficiency is rare because of the wide spread nature of this nutrient. Deficiency occurs only in individuals on a diet free of pantothenic acid or given pantothenic acid antagonists (EFSA NDA Panel, 2013a). Pantothenic acid has a very low toxicity and minor adverse gastrointestinal effects occurred only at very high intake levels (10-20 g/day). The SCF estimated that no UL could be established for pantothenic acid (SCF, 2002e).

6.22.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as the basis the intake levels of pantothenic acid considered adequate by the Panel for this age group of 2 mg/day based on pantothenic acid intakes from breast milk, this converts into a required minimum pantothenic acid content of formula of 0.4 mg/100 kcal.

Therefore, the Panel proposes a minimum pantothenic acid content in IF and FOF of 0.4 mg/100 kcal (0.10 mg/100 kJ).

6.23. Vitamin B6

6.23.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC provides for minimum and maximum vitamin B6 contents in IF and FOF of 35 µg/100 kcal and 175 µg/100 kcal. The SCF (2003a) concluded in its Opinion on minimum and maximum contents in IF and FOF of 35 µg/100 kcal and 165 µg/100 kcal.

6.23.2. Vitamin B6 content of human milk

The content of vitamin B6 in breast milk varies greatly and is dependent on maternal intakes. The average concentration of vitamin B6 in milk of unsupplemented well-nourished mothers is 130 µg/L (20 µg/100 kcal), reflecting a maternal vitamin B6 intake of less than 2.5 mg/day (IoM, 1998). The pyridoxine content of human milk may be marginal for infants whose mothers' vitamin B6 intake is low.

6.23.3. Vitamin B6 requirements of infants

In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin B6 intake of 100 µg/day and 400 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively. The dietary requirement for vitamin B6 varies in relation to the dietary consumption of protein (Hansen et al., 1996).

6.23.4. Vitamin B6 intakes of infants

Assuming a human milk intake of 0.8 L/day and a vitamin B6 content of 130 µg/L, an exclusively breast-fed infant would consume 104 µg vitamin B6 per day during the first six months of life. Mean/median vitamin B6 intakes in mostly formula-fed infants from birth to six months were reported to range from around 200-500 µg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 400-1 150 µg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.23.5. Health consequences

Pyridoxine and pyridoxal found in plants and animal products, respectively, are converted to pyridoxal phosphate in tissues. Pyridoxal phosphate acts as a co-enzyme in the metabolic transformation of amino acids, decarboxylation, transamination and racemization, the metabolism of lipids and nucleic acids and in glycogen metabolism. Symptomatic dietary vitamin B6 deficiency was described in infants with pyridoxine responsive convulsive seizures in the early 1950s associated with hypochromic microcytic anaemia, vomiting, diarrhoea, failure to thrive, lethargy or hyper-irritability (Borschel, 1995). It was concluded that an intake below 50 µg/day can cause vitamin B6 deficiency whilst an intake of about 70 µg/day did not. Reversible acute neuropathy and encephalopathy were observed in an infant with infantile type I hyperoxaluria at age 10 weeks under treatment with megadoses of pyridoxine (1 000 mg/day); symptoms disappeared with 400 mg/day (de Zegher et al., 1985). The UL for vitamin B6 is based on neurotoxicity which may appear in a mild form at doses of 100 mg/day in adults. By applying an uncertainty factor of 4, the UL of 25 mg for adults was derived. A UL for children 1-3 years of age of 5 mg/day has been set by extrapolation from adults (SCF, 2000b).

6.23.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of vitamin B6 considered adequate by the Panel for this age group of 100 µg/day based on vitamin B6 intakes from breast milk, this converts into a required minimum vitamin B6 content in formula of 20 µg/100 kcal.

Therefore, the Panel proposes a minimum vitamin B6 content in IF and FOF of 20 µg/100 kcal (4.8 µg/100 kJ).

6.24. Biotin

6.24.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum biotin contents in IF and FOF of 1.5 µg/100 kcal and 7.5 µg/100 kcal.

6.24.2. Biotin content of human milk

The average content of biotin in human milk is around 5 µg/L (0.8 µg/100 kcal) (EFSA NDA Panel, 2014c).

6.24.3. Biotin requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a biotin intake of 4 µg/day and 6 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively. These values are in line with the more recent Opinion of the Panel on DRVs for biotin (EFSA NDA Panel, 2014c).

6.24.4. Biotin intakes of infants

Assuming a human milk intake of 0.8 L/day and a biotin content of 5 µg/L, an exclusively breast-fed infant would consume 4 µg biotin per day during the first six months of life. No information on biotin intakes in infants from birth to six months living in Europe is available. For infants from 6 to < 12 months of age data are available from one study only (DGE, 2008), which reported median biotin intakes of around 20-23 µg/day.

6.24.5. Health consequences

Biotin is a co-factor for the enzymes acetyl-CoA carboxylase, propionyl-CoA carboxylase, β-methylcrotonyl-CoA carboxylase and pyruvate carboxylase, which play critical roles in the synthesis of fatty acids, the catabolism of branched-chain amino acids and gluconeogenesis. Dietary biotin deficiency is rare and does not occur in breast-fed infants. It is characterised by fine scaly dermatitis, hair loss, conjunctivitis, ataxia and delayed child development. Cases of biotin deficiency have been observed in patients receiving long-term TPN without biotin supplementation and in patients with biotinidase deficiency, as well as in people who had consumed large amounts of raw eggs. The SCF estimated that no UL could be established for biotin (SCF, 2001c).

6.24.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of biotin considered adequate by the Panel for this age group of 4 µg/day based on biotin intakes from breast milk, this converts into a minimum biotin content of formula of 0.8 µg/100 kcal (rounding up to 1 µg/100 kcal).

Therefore, the Panel proposes a minimum biotin content in IF and FOF of 1 µg/100 kcal (0.24 µg/100 kJ).

6.25. Folate

6.25.1. Current compositional requirements of IF and FOF

Directive 2006/141/EC provides for minimum and maximum folate contents in IF and FOF of 10 µg/100 kcal and 50 µg/100 kcal. In its Opinion, the SCF (2003a) concluded on minimum and maximum contents of folate in IF and FOF of 10 µg/100 kcal and 30 µg/100 kcal.

6.25.2. Folate content of human milk

The average content of folate in human milk was found to be around 80 µg/L (12.3 µg/100 kcal) (Houghton et al., 2009).

6.25.3. Folate requirements of infants

Because the absorption efficiency of folates varies depending on their chemical form, dietary folate equivalents (DFE) have been defined by IoM (1998) as 1 DFE = 1 µg food folate = 0.6 µg folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a folic acid supplement taken on an empty stomach. In the Panel's previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a folate intake of 65 µg DFE/day and 80 µg DFE/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.25.4. Folate intakes of infants

Assuming a human milk intake of 0.8 L/day and a folate content of 80 µg/L, an exclusively breast-fed infant would consume 64 µg folate per day during the first six months of life. None of the surveys conducted in infants from birth to below six months of life reported folate intakes as DFE. For infants from 6 to < 12 months only the German VELS study (Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern) (DGE, 2008) reported folate intakes as DFE. Median folate intakes in this study were 62 µg and 78 µg DFE per day in girls and boys respectively.

6.25.5. Health consequences

Folate is essential for one-carbon transfer reactions, including those involved in glycine/serine and homocysteine/methionine interconversion, and in purine and pyrimidine synthesis. Folate deficiency impairs *de novo* DNA synthesis and consequently cellular replication. Folate deficiency has also been associated with irritability, forgetfulness, neuropathy and depression. Poor folate status in the periconceptual period increases the risk for neural tube defects. Folate deficiency has not been reported in breast-fed infants even in mothers with low folate status. Excess dietary folate is mainly excreted in the urine. Consumption of high amounts of folic acid by subjects deficient in cobalamin increases the risk of neurological damage by masking the haematological manifestations of cobalamin deficiency. The SCF noted that in nearly all studies showing neurological damage, the folic acid dose was ≥ 5 mg folic acid/day which was taken to represent the Lowest Observed Adverse Effect Level (LOAEL). Using an uncertainty factor of 5 the UL for adults was set at 1 mg/day. The UL for folic acid for children aged one to three years of 200 µg/day was derived by extrapolation based on body weight (SCF, 2000c). A UL for food folate was not set.

6.25.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of folate considered adequate by the Panel for this age group of 65 µg DFE/day based on folate intakes from breast milk, this converts into a minimum folate content in formula of 13 µg/100 kcal (rounded up to 15 µg/100 kcal).

The Panel proposes a minimum folate content in IF and FOF of 15 µg DFE/100 kcal (3.6 µg DFE/100 kJ).

6.26. Cobalamin (vitamin B12)

6.26.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum cobalamin contents in IF and FOF of 0.1 µg/100 kcal and 0.5 µg/100 kcal.

6.26.2. Cobalamin content of human milk

The mean value in breast milk of a group of 24 healthy Californian women, most of whom had consumed supplements containing 6 µg cobalamin/day during pregnancy, was 1.2 µg/L (0.18 µg/100 kcal) (range: 0.2–5.0 µg/L (0.03-0.77 µg/100 kcal) (Lildballe et al., 2009). In a recent longitudinal study, cobalamin concentration of breast milk from 25 Danish mothers was measured at two weeks, four months and nine months of lactation (Greibe et al., 2013). Most women were taking daily multivitamins supplements, providing 1.0–4.5 µg cobalamin. Median (range) concentrations of cobalamin in hindmilk were 1.03 (0.28–2.55), 0.39 (0.19–0.94), and 0.60 (0.22–2.63) µg/L at two weeks, four months, and nine months, respectively. The respective concentrations per 100 kcal were 0.16 (0.04-0.39), 0.06 (0.03-0.14), and 0.09 (0.03-0.40) µg/100 kcal.

6.26.3. Cobalamin requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a cobalamin intake of 0.4 µg/day and 0.5 µg/day was adequate for the majority of infants in the first half and in the second half of the first year of life, respectively.

6.26.4. Cobalamin intakes of infants

Assuming a human milk intake of 0.8 L/day and a cobalamin content of 0.5 µg/L, an exclusively breast-fed infant would consume 0.4 µg cobalamin per day during the first six months of life. Mean/median cobalamin intakes in mostly formula-fed infants from birth to six months were reported to range from around 1.3-1.8 µg/day (Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 1.2-3.6 µg/day (Noble and Emmett, 2001; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.26.5. Health consequences

In humans, cobalamin is required as a coenzyme for two reactions: the isomerisation of methylmalonyl-CoA to succinyl-CoA by mitochondrial methylmalonyl-CoA mutase and the methylation of homocysteine to methionine by methionine synthase. Cobalamin deficiency is rare in infants but can occur in infants breast-fed by vegan mothers with (subclinical) cobalamin deficiency. In infants, cobalamin deficiency results in cerebral atrophy and symptoms of encephalopathy with developmental retardation. No adverse effects have been associated with excess cobalamin intake from food or supplements in healthy individuals. No UL could be established by the SCF for cobalamin (SCF, 2000e).

6.26.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake levels of cobalamin considered adequate by the Panel for this age group of 0.4 µg/day based on cobalamin intakes from breast milk, this converts into a minimum cobalamin content in formula of 0.08 µg/100 kcal (rounded up to 0.1 µg/100 kcal).

Therefore, the Panel proposes a minimum cobalamin content in IF and FOF of 0.1 µg/100 kcal (0.02 µg/100 kJ).

6.27. Vitamin C

6.27.1. Current compositional requirements of IF and FOF

Based on the Opinion of the SCF (2003a), Directive 2006/141/EC provides for minimum and maximum vitamin C contents in IF and FOF of 10 mg/100 kcal and 30 mg/100 kcal.

6.27.2. Vitamin C content of human milk

Mean vitamin C concentrations in human milk were reported to range from 35-90 mg/L (5.4-13.8 mg/100 kcal) (EFSA NDA Panel, 2013e). The amount of vitamin C excreted via breast milk depends on the vitamin C status of the mother, and the vitamin C content in human milk reflects maternal vitamin C intake more than the infant’s requirement (WHO/FAO, 2004).

6.27.3. Vitamin C requirements of infants

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a vitamin C intake of 20 mg/day was adequate for the majority of infants.

6.27.4. Vitamin C intakes of infants

Assuming a human milk intake of 0.8 L/day and a vitamin C content of 60 mg/L, an exclusively breast-fed infant would consume 48 mg vitamin C per day during the first six months of life. Mean/median vitamin C intakes in mostly formula-fed infants from birth to six months were reported to range from around 40-90 mg/day (Hilbig, 2005; Noble and Emmett, 2006; Fantino and Gourmet, 2008; Lennox et al., 2013) and in infants from 6 to < 12 months from around 33-94 mg/day (Noble and Emmett, 2001; Hilbig, 2005; de Boer et al., 2006; DGE, 2008; Fantino and Gourmet, 2008; Marriott et al., 2008; Thorsdottir et al., 2008; Lennox et al., 2013).

6.27.5. Health consequences

Vitamin C (L-ascorbic acid and dehydroascorbic acid) is an enzyme cofactor for biochemical reactions catalysed by mono-oxygenases, dioxygenases and mixed function oxygenases. Vitamin C plays an important role in the biosynthesis of collagen, is essential for the synthesis of carnitine and catecholamines, and is also involved in the metabolism of cholesterol to bile acids. Vitamin C in aqueous solution readily scavenges reactive oxygen and nitrogen species, as well as singlet oxygen and hypochlorite, and is part of the antioxidant network of the body (EFSA NDA Panel, 2013e). Frank vitamin C deficiency leads to scurvy but has been observed only after the sixth month of life in infants fed a diet consisting of cow’s milk with no fruits and vegetables. Vitamin C is of low acute toxicity and available data on adverse effects are limited. No UL has been set by the Panel but available human data suggest that supplemental daily doses of vitamin C up to about 1 g in addition to normal dietary intakes in adults are not associated with adverse effects (EFSA, 2004).

6.27.6. Recommendations

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as the basis the intake levels of vitamin C considered adequate by the Panel for this age group of

20 mg/day, based on three times the amount known to prevent scurvy, this converts into a minimum vitamin C content of formula of 4 mg/100 kcal.

Therefore, the Panel proposes a minimum vitamin C content in IF and FOF of 4 mg/100 kcal (0.96 mg/100 kJ).

7. Maximum content of micronutrients in IF and FOF

As the different protein and fat sources used in the manufacture of formula and the water used to reconstitute powdered formula contribute to the total nutrient content of a formula in varying amounts, maximum contents of nutrients have been established in order to ensure the safe use of formula while limiting technological manipulations of the natural nutrient content of food constituents used in the production of formulae.

From a nutritional point of view, the minimum contents proposed by the Panel cover the nutritional needs of virtually all healthy infants born at term and there is no need to exceed these amounts in formulae, as nutrients which are not used or stored have to be excreted and this may put a burden on the infant's metabolism. Therefore, the Panel emphasises that maximum amounts should not be interpreted as target values but rather as upper limits of a range which should not be exceeded.

Specifications for the currently permitted maximum amounts of micronutrients in formulae were generally calculated as three to five times the minimum amounts established at the time, took into account established history of apparent safe use (Codex Stan 72-1981, Codex Stan 156-1987, the Directive 2006/141/EC, and the SCF (2003a)), and were not based on scientific evidence for adverse effects owing to the lack of such evidence for most nutrients.

The Panel notes that there are no reports on any adverse effects associated with the use of formulae complying with the current specifications as laid down in Directive 2006/141/EC, although there are no studies available which were designed to investigate the short or long-term health consequences of consumption of formulae containing the currently permitted maximum amounts of micronutrients in IF or FOF.

The Panel acknowledges that the scientific data available to derive ULs for infants remain scarce for most micronutrients and only for vitamin D a UL for infants could be set (EFSA NDA Panel, 2012b). For magnesium, zinc, selenium, iodine, molybdenum, vitamin A, niacin, vitamin B6 and folic acid, ULs for children aged one to three years have been established (SCF, 2000b, 2000c, 2000d, 2000g, 2001a, 2002b, 2002d, 2002a, 2002c). Assuming an energy intake from formula of 500 kcal/day (average of the AR for energy of boys and girls aged three to four months), regular consumption of a formula by an infant containing the currently permitted maximum amounts of zinc, iodine, vitamin A and folate (if the whole amount is provided in the form of folic acid) would imply that the ULs would be exceeded for these nutrients. Assuming an energy intake from formula of 700 kcal/day (highest observed mean energy intakes in infants below six months of age), intakes of selenium would also exceed the UL. The Panel acknowledges that the ULs used in this estimation were those derived for young children and there is considerable uncertainty with respect to the extrapolation to infants.

8. Specifications for other ingredients in IF and FOF

8.1. Choline

Choline is currently mandatory in IF and Directive 2006/141/EC provides for minimum and maximum choline contents of 7 mg/100 kcal and 50 mg/100 kcal, respectively. In its Opinion, the SCF (2003a) concluded on minimum and maximum contents of choline in IF of 7 mg/100 kcal and 30 mg/100 kcal.

In human milk, choline is found as free choline, phosphocholine, glycerophosphocholine, sphingomyelin, and phosphatidylcholine all of which are available to the nursing infant. Total choline concentrations in human milk are influenced by maternal choline intake, length of lactation and genetic polymorphisms. The concentration of total choline in mature milk is 160-210 mg/L (25-32 mg/100 kcal) (Holmes-McNary et al., 1996; Holmes et al., 1996). In one study (reported in two publications) mean choline levels were found to range between 144 and 170 mg/L (22-26 mg/100 kcal) and to increase from 70 mg/L (11 mg/100 kcal) in colostrum to 151 mg/L (23 mg/100 kcal) (range 57-318 mg/L (9-49 mg/100 kcal)) in mature milk (Ilcol et al., 2005; Allen, 2012). In a second study (Fischer et al., 2010) comparing breast milk concentrations in unsupplemented and supplemented mothers, the average choline content in breast milk amounted to 125 and 149 mg/L (19 and 23 mg/100 kcal), respectively. The average total choline concentration in mature human milk can be taken to be around 160 mg/L (20 mg/100 kcal).

Choline is predominantly provided via the diet, but the human body can also form choline *de novo* via two pathways that both lead to phosphatidylcholine. The extent to which choline is a required dietary constituent under normal circumstances is not clear. However, in conditions of increased need or of impaired synthesis, choline is considered to be conditionally indispensable. Choline has a number of important functions: it is a precursor of phospholipids, platelet activating factor, betaine and the neurotransmitter acetylcholine and it is involved in the metabolism and transport of lipids. Dietary deficiency of choline in adults has been reported to cause liver steatosis (Buchman et al., 1995) and liver (Zeisel et al., 1991) and muscle damage (Fischer et al., 2007). Choline was not considered in the derivation of ULs in the EU. . A UL for choline of 1 g/day for children aged one to three years has been set by IoM (1998). The UL was extrapolated from the UL of adults based on relative body weights. The UL for adults was based on a case report of hypotension and several studies involving cholinergic effects and fishy body odour after oral administration of large doses of choline. No UL for infants was set owing to the lack of data and concerns about the infant’s ability to handle excess amounts.

In the Panel’s previous Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union (EFSA NDA Panel, 2013a), the Panel concluded that a choline intake of 130 mg/day was adequate for the majority of infants below six months of age and of 150 mg/day for infants from 6 to < 12 months.

Assuming an average energy intake of an infant below six months of age of 500 kcal/day and taking as a basis the intake level of choline considered adequate by the Panel for this age group of 130 mg/day based on choline intakes from breast milk, this converts into a minimum choline content in formula of 26 mg/100 kcal (rounded down to 25 mg/100 kcal). Therefore, the Panel proposes a minimum choline content in IF of 25 mg/100 kcal (6.0 mg/100 kJ) and. The addition of choline to FOF is not necessary.

8.2. There are no reports on adverse effects occurring with the current specifications of choline in IF. Inositol

Inositol is currently mandatory in IF and Directive 2006/141/EC provides for minimum and maximum inositol contents of 4 mg/100 kcal and 40 mg/100 kcal, respectively, in line with the SCF (2003a).

In addition to sugars such as lactose, glucose, galactose and mannose, human milk also contains sugar alcohols/polyols, in particular inositol, mostly as myo-inositol, either free or in phosporylated forms. The myo-inositol concentration has been found to be higher in colostrum and reaches a relatively stable concentration of around 130-325 mg/L (20-50 mg/100 kcal) in mature human milk (Cavalli et al., 2006; Jóźwwik et al., 2013). Inositol plays a role in many important biological functions including the regulation of cell osmolality, processes of cell signalling, as structural components of the developing neural system and in the production of the phospholipids for pulmonary surfactant. Endogenous *de-novo* synthesis of inositol appears to be efficient in newborn infants. Together with inositol provided by human milk, this makes it unlikely that healthy, term, breast-fed infants could become depleted of inositol (Brown LD et al., 2009). However, it is not known if endogenous inositol synthesis is sufficient in the absence of dietary inositol.

Considering that it is unknown if endogenous synthesis of inositol in newborns is sufficient in the absence of dietary inositol, the Panel proposes to maintain the minimum inositol content in IF of 4 mg/100 kcal (0.96 mg/100 kJ) proposed in 2003 by the SCF. The Panel considers that the addition of inositol to FOF is not necessary, as the supply from complementary food is sufficient in older infants.

There are no reports on adverse effects occurring with the current specifications of inositol in IF.

8.3. Taurine

The addition of taurine to IF and FOF is currently permitted by Directive 2006/141/EC on a voluntary basis up to a maximum of 12 mg/100 kcal. Taurine concentrations in term milk have been found to be around 4.7 mg/100 kcal (Zhang et al., 2013) with highest observed taurine concentrations of around 12 mg/100 kcal (Rassin et al., 1978).

It has been suggested that taurine plays a role in intestinal fat absorption, hepatic function, and auditory and visual development in pre-term or low-birth-weight infants. However, clinical data on long-term effects on neurological development in these infants are lacking (Verner et al., 2007) as is evidence that the addition of taurine to IF has any clinical benefits for term infants.

Taking into account the lack of convincing evidence for a benefit of the addition of taurine to IF and/or FOF, the Panel considers that the addition of taurine to IF or FOF is not necessary.

There are no reports on adverse effects occurring with the current specifications of taurine in formula.

8.4. L-Carnitine

The addition of L-carnitine at an amount of at least 1.2 mg/100 kcal is currently mandatory for IF containing ISP or protein hydrolysates but not for IF based on cow’s or goat’s milk protein, as the latter were considered to provide L-carnitine naturally from the respective milk source.

L-Carnitine is considered an indispensable nutrient for newborn infants because of a temporarily insufficient synthesising capacity. In studies investigating L-carnitine concentrations in milk from different species, mean total carnitine concentrations have been reported to be in the range of 0.9-1.6 mg/100 kcal in human milk (Sandor et al., 1982; Penn et al., 1987; Ferreira, 2003), 4.1-6.7 mg/100 kcal in cow’s milk (Sandor et al., 1982; Penn et al., 1987; Ferreira, 2003) and 3.2-4.4 mg/100 kcal in goat’s milk (Sandor et al., 1982; Penn et al., 1987). The natural carnitine content of animal milks may be decreased by dilution or fractionation of the milk source when cow’s and goat’s milk proteins are used in the manufacture of IF. Therefore, a minimum L-carnitine content should be also set for IF based on milk protein.

The Panel notes that no new data have become available since the Opinion of the SCF (2003a) which would indicate that a minimum content of L-carnitine of 1.2 mg/100 kcal in IF is insufficient to ensure adequate growth and development of infants. The Panel proposes a minimum L-carnitine content in IF of 1.2 mg/100 kcal (0.3 mg/100 kJ), irrespective of the protein source used, which is an amount similar to the content of L-carnitine in human milk. The Panel considers that the addition of L-carnitine to FOF is not necessary, as the supply from complementary food and from endogenous synthesis is sufficient in older infants.

8.5. Nucleotides and nucleosides

The addition of nucleotides to IF and FOF is currently permitted by Directive 2006/141/EC on a voluntary basis up to a maximum of 5 mg/100 kcal. If added, the maximum nucleotide content is regulated to be: cytidine 5’-monophosphate (CMP) 2.5 mg/100 kcal, uridine 5’-monophosphate (UMP) 1.75 mg/100 kcal, adenosine 5’-monophosphate (AMP) 1.50 mg/100 kcal, guanosine 5’-monophosphate (GMP) 0.50 mg/100 kcal, inosine 5’-monophosphate (IMP) 1.00 mg/100 kcal.

Nucleotides and nucleosides are dispensable nutrients synthesised *de novo* in human metabolism. Nucleotides are structural components of ribonucleic acid (RNA) and DNA. Nucleotides, such as ATP, transfer chemical energy. Other nucleotides are involved in the synthesis of proteins, lipids and carbohydrates (e.g. NAD, FAD) (SCF, 2003a).

Human milk contains free nucleosides, free nucleotides, RNA and DNA. The concentrations of "total potentially available nucleotides", defined by some authors as the sum of free nucleosides, free nucleotides, nucleotide-containing adducts (such as NAD and uridinediphosphate (UDP) glucose), and nucleotide polymers were reported to be around 10.5-11.0 mg/100 kcal in milk from Asian, American and European mothers. The major sources were nucleotide polymers, primarily RNA, (around 43-48 %), free nucleotides (around 36-40 %) and free nucleosides (around 6.5-8 %) (Leach et al., 1995; Tressler et al., 2003). Average concentrations of nucleotides in human milk were observed to be in the range of around 0.7-4.5 mg/100 kcal for CMP, 0.3-2.3 mg/100 kcal for UMP, 0.05-1.9 mg/100 kcal for GMP, 0.2-1.7 mg/100 kcal and 0-1.4 mg/100 kcal for IMP (Gil and Sanchez-Medina, 1982; Janas and Picciano, 1982; Leach et al., 1995; Thorell et al., 1996; Tressler et al., 2003; Liao et al., 2011) with four studies out of six reporting IMP concentrations below the limit of detection.

It should be noted that the presence of nucleotides and nucleosides in human milk does not necessarily indicate a specific benefit for the infants as they may also be by-products of milk formation that reflect metabolic activity of the mammary gland tissue, shedding of somatic cells and occurrence of microorganisms, without having a specific function for the infant (SCF, 2003a).

Nucleotides have been studied in healthy term infants with respect to their effect on clinically relevant outcomes such as antibody titres after vaccination (Pickering et al., 1998; Yau et al., 2003; Schaller et al., 2004), the incidence or severity of infections (Carver et al., 1991; Brunser et al., 1994; Yau et al., 2003), the incidence of diarrhoea (Brunser et al., 1994; Yau et al., 2003; Singhal et al., 2008) and growth (Carver et al., 1991; Pickering et al., 1998; Lasekan et al., 1999; Yau et al., 2003; Schaller et al., 2004; Singhal et al., 2010). The Panel notes that even if effects on some of the antibody titres measured in the studies were observed, these effects were not consistent and that no effects of nucleotides on the incidence or severity of infections, on the incidence of diarrhoea, and on growth were observed.

Taking into account the lack of convincing evidence for a benefit of the addition of nucleotides to IF and/or FOF, the Panel considers that the addition of nucleotides to IF or FOF is not necessary.

There are no reports on adverse effects occurring with the current specifications of nucleotides in formula.

8.6. "Probiotics" and "synbiotics"

The addition of live microorganisms to IF and FOF was not mentioned in the corresponding Directives except for production of acidified IF and FOF, for which the use of non-pathogenic L (+)-lactic acid producing bacterial cultures is permitted (Regulation (EC) No 1333/2008). However, according to Directive 2006/141/EC, IF and FOF may contain other food ingredients than those listed in Annex I and II, provided those ingredients have been shown to be suitable for infants through a systematic review of the available data or, when necessary, by appropriate studies (Article 5 and 6).

IF and FOF with added live bacteria, claiming to confer health benefits (known as "probiotics"), have been introduced into the EU market. Several bacterial strains included in IF and FOF have been evaluated with regard to safety and potential beneficial health effects to date. These include, *Bifidobacterium animalis* subsp. *lactis* CNCMI-3446 (also named *B. bifidum* and *B. lactis* Bb12), alone or in combination with either *Streptococcus thermophilus* or with both *S. thermophilus* and *Lactobacillus helveticus*, *L. johnsonii* La1, *B. longum* BL999 plus *L. rhamnosus* LPR, *L. rhamnosus* GG, *L. reuteri* ATCC 55730, *L. salivarius* CECT5713 and *L. fermentum* CECT5716 (Braegger et al., 2011; Gil-Campos et al., 2012; Maldonado et al., 2012; Mugambi et al., 2012).

The health outcomes evaluated in RCTs include growth, gastrointestinal infections/diarrhoea, respiratory tract infections/symptoms, colic/irritability, allergic manifestations, stool frequency and consistency, and antibody production (Braegger et al., 2011; Gil-Campos et al., 2012; Holscher et al., 2012; Mugambi et al., 2012; Szajewska and Chmielewska, 2013).

The most recent systematic reviews (Mugambi et al., 2012; Szajewska and Chmielewska, 2013) concluded that many of the studies conducted with IF and FOF supplemented with "probiotic" bacteria did not exert significant physiological or health effects compared to non-supplemented formulae. These reviews also revealed uncertainty regarding the beneficial effects reported for formula supplemented with some of the strains through lack of consistency across studies, to methodological limitations and to the existence of data from single studies only (Braegger et al., 2011; Szajewska and Chmielewska, 2013). Such uncertainty applies to the effects of IF supplemented with *B. animalis* subsp. *lactis* CNCMI-3446 (alone or in combination) on diarrhoea, which were not consistent across studies with substantial methodological limitations and also applies to the effects of other strains evaluated only in single studies such as *L. johnsonii* La1 and *L. salivarius* CECT5713 on diarrhoea, *L. salivarius* CECT 5713 on respiratory tract infections, and *L. rhamnosus* LGG on defecation frequency and stool consistency (Braegger et al., 2011).

A couple of recent studies have evaluated the effects of the strain *L. fermentum* CECT 5716, isolated from human milk, added to GOS containing IF compared to the same IF without the bacterium. The first study was designed to evaluate the safety and tolerance of the IF supplemented with *L. fermentum* CECT 5716 in infants of one to six months of age and, as secondary outcomes, also evaluated infections, reporting reductions in the incidence rate of gastrointestinal infections, but not of respiratory or total infections (Gil-Campos et al., 2012). The second study was conducted on healthy six-month-old infants and reported reductions in the incidence rate of gastrointestinal and upper respiratory tract infections, but not on lower respiratory tract infections, otitis, urinary tract infections or febrile episodes, between the ages of 6 and 12 months in the *L. fermentum* CECT 5716 group compared to the control group (Maldonado et al., 2012). The Panel notes that the two studies were not consistent regarding the effects on respiratory tract infections and that one of the studies on gastrointestinal infections was not designed for this purpose but for evaluating safety. Another recent single study reported that *B. animalis* subsp. *lactis* CNCMI-3446 added to formula containing partially hydrolysed whey protein increased anti-poliovirus-specific immunoglobulin A (IgA) concentration ($p < 0.05$) but not anti-rotavirus-specific IgA, following immunisation, and only in a subgroup of caesarean delivered infants (Holscher et al., 2012).

IF and FOF containing "probiotics" have also been studied in relation to any potential untoward effects, such as delayed growth, diarrhoea and allergic reactions (Braegger et al., 2011; Gil-Campos et al., 2012; Maldonado et al., 2012; Azad et al., 2013). It has been generally concluded that currently evaluated "probiotic"-supplemented IF do not raise safety concerns with regard to growth or other adverse effects, although in many studies adverse events were inconsistently reported (Azad et al., 2013) and further evaluations of safety in long-term studies are needed (Braegger et al., 2011).

A few studies have been conducted with IF or FOF supplemented with combinations of "probiotics" and "prebiotics" (named "synbiotics") on growth, infections, asthma/wheezing, crying and stool frequency/constipation (reviewed by Braegger et al., 2011; and Azad et al., 2013). The synbiotics evaluated in RCTs in infants include: *B. longum* BL999 plus GOS/FOS, *B. longum* BL999 plus *L. rhamnosus* LPR plus GOS/FOS, *B. longum* BL999 plus *Lactobacillus paracasei* ST11 plus GOS/FOS, *L. paracasei* subsp. *paracasei* plus *B. animalis* subsp. *lactis* plus GOS and *Bifidobacterium breve* M-16V plus GOS/FOS. The evidence on the effectiveness is very limited and only data from single studies are available. These have reported effects on increased stool frequency for three of the synbiotics tested (*B. longum* BL999 plus GOS/FOS, *B. longum* BL999 plus *L. rhamnosus* LPR plus GOS/FOS and *L. paracasei* subsp. *paracasei* plus *B. animalis* subsp. *lactis* plus GOS) (reviewed by Braegger et al., 2011) and on parents-reported asthma-like symptoms, but not on total serum IgE and specific IgE against aeroallergens (van der Aa et al., 2011 reviewed by Azad et al., 2013).

Safety of "synbiotics" added to IF and FOF has also been evaluated concluding that they do not raise concerns with regard to growth or other adverse effects, although evidence is limited (Braegger et al., 2011).

The Panel notes that evidence available on beneficial effects of IF or FOF supplemented with "probiotics" and "synbiotics" on infant health mainly comes from single studies and studies with methodological limitations or it is inconsistent across the few studies that are comparable. Therefore, the Panel considers that there is insufficient information to draw conclusions on beneficial effects on infant health of "probiotics" added to IF and FOF and even less in the case of synbiotics. There is no evidence to raise concerns about the safety of the tested "probiotics" or "synbiotics".

Taking into account the lack of convincing evidence for a benefit of the addition of "probiotics" or "synbiotics" to IF and/or FOF, the Panel considers that the addition of "probiotics" and/or "synbiotics" to IF or FOF is not necessary.

9. Use of formulae by young children

The Panel was also asked to advise the Commission with respect to the appropriate age range of use and the essential composition of so-called "growing-up milks" or young-child formulae.

In its previous Opinion (EFSA NDA Panel, 2013a) the Panel considered that, despite the fact that an adequate amount of energy and nutrients can be supplied by a balanced and varied diet, intakes of ALA, DHA, iron, vitamin D and iodine in some infants and young children living in Europe are low and some sub-groups in this population may be at risk of inadequacy.

In the same Opinion the Panel noted that formulae, including young-child formulae, are one of several means to increase intakes of these critical nutrients in infants and young children living in Europe with inadequate or at risk of inadequate status of these nutrients. However, other means, such as fortified cow's milk, fortified cereals and cereal-based foods, supplements or the early introduction of meat and fish into complementary feeding and their continued regular consumption, are efficient alternatives to increase intakes of these nutrients. The selection of the appropriate form and vehicle through which these nutrients are provided in the diet will depend on national dietary habits, health authorities, the regulatory context and caregivers' preference.

The Panel concluded that no unique role of young-child formulae with respect to the provision of critical nutrients in the diet of infants and young children living in Europe can be identified, so that they cannot be considered as a necessity to satisfy the nutritional requirements of young children when compared to other foods that may be included in the normal diet of young children. The median content of ALA, DHA (if added), iron, vitamin D and iodine in currently marketed young-child formulae is within the range of permitted concentrations in FOF and, except for iron, also in IF. The Panel notes that formula consumed during the first year of life can continue to be used by young children. Therefore, the Panel does not consider it necessary to propose specific compositional criteria for formulae consumed after one year of age.

10. Recommendations for further research

The Panel emphasises:

- the necessity to generate reliable analytical data on the amino acid pattern of human milk protein at different stages of lactation;

- the necessity for appropriate studies to fill the gaps in the knowledge of protein requirements of infants in the second half of the first year of life;

- the lack of human studies evaluating the safety and adequacy of most IF and FOF presently on the market containing protein hydrolysates;

- the necessity to generate data on the decrease of bioavailability of certain amino acids through different methods of processing;

- the lack of non-digestible oligosaccharides that mimic those present in human milk and appropriate human studies evaluating the safety and potential health benefits of non-digestible oligosaccharides and bacteria whose growth in the infant's gut is promoted by breast-milk.

CONCLUSIONS

The Panel concludes that:

- There is consensus that breast milk is the preferred food for all healthy infants and provides an adequate supply of all nutrients to support healthy growth and development (with the exception of vitamin K during the first weeks of life and of vitamin D).

- All formulae intended for infants must be safe and suitable in meeting the nutritional requirements and to promote growth and development of infants born at term when used as a sole source of nutrition during the first months of life and when used as the principal liquid element in a progressively diversified diet after the introduction of appropriate complementary feeding. Nutrients and substances should be added to formulae for infants only in amounts that serve a nutritional or other health benefit.

- The minimum content of a nutrient in formula proposed in this Opinion is derived from the intake levels the Panel had considered adequate for the majority of infants in the first half of the first year of life in its previous Opinion and an average amount of formula consumed during this period (500 kcal/day). From a nutritional point of view, the minimum contents proposed by the Panel cover the nutritional needs of virtually all healthy infants born at term and there is no need to exceed these amounts in formulae, as nutrients which are not used or stored have to be excreted and this may put a burden on the infant's metabolism and/or physiological functions.

- Specifications for the currently permitted maximum amounts of micronutrients in formulae were generally calculated as three to five times the minimum amounts established at the time and took into account established history of apparent safe use (Codex Stan 72-1981, Codex Stan 156-1987, the Directive 2006/141/EC, and SCF (2003a)) and were not based on scientific evidence for adverse effects owing to the lack of such evidence for most nutrients. It is emphasised that maximum amounts should not be interpreted as target values but rather as upper limits of a range which should not be exceeded.

- There are no reports on any adverse effects associated with the use of formulae complying with the current specifications for micronutrients as laid down in Directive 2006/141/EC, although there are no studies available which were designed to investigate the short or long-term health consequences of consumption of formulae containing the currently permitted maximum amounts of micronutrients in IF or FOF. Assuming an energy intake from formula of 500 kcal/day (average of the AR for energy of boys and girls aged three to four months), regular consumption of a formula by an infant containing the currently permitted maximum amounts of zinc, iodine, vitamin A and folate (if the whole amount is provided in the form of folic acid) would imply that the ULs would be exceeded for these nutrients. Assuming an energy intake from formula of 700 kcal/day (highest observed mean energy intakes in infants below six months of age), intakes of selenium would also exceed the UL. The Panel acknowledges that the ULs used in this estimation were those derived for young children and there is uncertainty with respect to the extrapolation to infants.

- Cow's milk, goat's milk and ISP are safe and suitable protein sources for use in IF and FOF based on intact protein. The use of other protein sources in IF and FOF and/or the introduction of new technologies need clinical evaluation and their safety and suitability should be established in the target population prior to their general use in IF and FOF.

- Formulae containing protein hydrolysates are insufficiently characterised by the declared protein content even if they fulfil regulatory criteria concerning amino acid patterns and contents; therefore the safety and suitability of each specific IF or FOF containing protein hydrolysates has to be established by clinical evaluation.

- The use of a default conversion factor of 6.25 to calculate the protein content from the total nitrogen content is proposed, irrespective of the protein source.

- IF and FOF should provide on an energy basis indispensable and conditionally indispensable amino acids in amounts at least equal to the reference protein (i.e. breast milk), irrespective of the protein source and the following reference pattern is proposed:

Amino acid	**mg/100 g protein**	**mg/100 kcal**	**mg/100 kJ**
Cysteine	2.1	38	9
Histidine	2.2	40	10
Isoleucine	5.0	90	22
Leucine	9.2	166	40
Lysine	6.3	113	27
Methionine	1.3	23	5
Phenylalanine	4.6	83	20
Threonine	4.3	77	18
Tryptophan	1.8	32	8
Tyrosine	4.2	76	18
Valine	4.9	88	21

- The sum of methionine and cysteine and the sum of tyrosine and phenylalanine in IF may be used for calculation purposes. If the ratio between methionine to cysteine and the ratio between tyrosine and phenylalanine, respectively, exceeds two, this must be justified by clinical evaluation. For FOF no restrictions with respect amino acid ratios need to apply, owing to the fact that complementary foods will contribute to amino acid intakes and the metabolism of older infants is more mature with respect to the capacity to convert methionine to cysteine and phenylalanine to tyrosine.

- The following composition of IF and FOF based on intact cow's and goat's milk protein is proposed:

		Range			Range	
	Unit	**IF**	**FOF**	**Unit**	**IF**	**FOF**
Energy	kcal/100 mL	60-70		kJ/100 mL	250-293	
Protein	g/100 kcal	1.8-2.5		g/100 kJ	0.43-0.60	
Total fat	g/100 kcal	4.4-6.0		g/100 kJ	1.1-1.4	
LA	mg/100 kcal	500-1 200		mg/100 kJ	120-300	
ALA	mg/100 kcal	50-100		mg/100 kJ	12-24	
DHA	mg/100 kcal	20-50		mg/100 kJ	4.8-12	
TFA	FA%	≤ 3		FA%	≤ 3	
Carbohydrates	g/100 kcal	9-14		g/100 kJ	2.2-3.3	
Lactose, unless	g/100 kcal	≥ 4.5		g/100 kJ	≥ 1.1	
"lactose-free"	g/100 kcal	≤ 0.01		g/100 kJ	≤ 0.0024	
Sucrose, fructose and sugars from honey	% of total CHO	0	≤ 20	% of total CHO	0	≤ 20
Glucose	g/100 kcal	0	0	g/100 kJ	0	0
Starches		≤ 2 g/100 mL, not more than 30 % of total CHO				
		Minimum			**Minimum**	
	Unit	**IF**	**FOF**	**Unit**	**IF**	**FOF**
Calcium	mg/100 kcal	50		mg/100 kJ	12	
Phosphorus	mg/100 kcal	25		mg/100 kJ	6	
Magnesium	mg/100 kcal	5		mg/100 kJ	1.2	
Sodium	mg/100 kcal	25		mg/100 kJ	6	
Chloride	mg/100 kcal	60		mg/100 kJ	14.3	
Potassium	mg/100 kcal	80		mg/100 kJ	19.1	
Iron	mg/100 kcal	0.3	0.6	mg/100 kJ	0.07	0.14
Zinc	mg/100 kcal	0.5		mg/100 kJ	0.12	
Copper	µg/100 kcal	60		µg/100 kJ	14.3	
Selenium	µg/100 kcal	3		µg/100 kJ	0.72	
Iodine	µg/100 kcal	15		µg/100 kJ	3.6	
Molybdenum	µg/100 kcal	0.4		µg/100 kJ	0.1	
Manganese	µg/100 kcal	1		µg/100 kJ	0.24	
Vitamin A(a)	µg/100 kcal	70		µg/100 kJ	16.7	
Vitamin D	µg/100 kcal	2		µg/100 kJ	0.48	
Vitamin E(b)	mg α-TE/100 kcal	0.6		mg α-TE/100 kJ	0.14	
Vitamin K	µg/100 kcal	1		µg/100 kJ	0.24	
Thiamin	µg/100 kcal	40		µg/100 kJ	9.6	
Riboflavin	µg/100 kcal	60		µg/100 kJ	14.3	
Niacin(c)	mg/100 kcal	0.4		mg/100 kJ	0.10	
Pantothenic acid	mg/100 kcal	0.4		mg/100 kJ	0.10	
Vitamin B6	µg/100 kcal	20		µg/100 kJ	4.8	
Biotin	µg/100 kcal	1		µg/100 kJ	0.24	
Folate	µg DFE/100 kcal	15		µg DFE/100 kJ	3.6	
Cobalamin	µg/100 kcal	0.1		µg/100 kJ	0.02	
Vitamin C	mg/100 kcal	4		mg/100 kJ	0.96	
Choline	mg/100 kcal	25	NN	mg/100 kcal	6	NN
L-Carnitine	mg/100 kcal	1.2	NN	mg/100 kJ	0.3	NN
Inositol	mg/100 kcal	4	NN	mg/100 kJ	0.96	NN

(a) pre-formed vitamin A, (b) RRR-α-tocopherol activity, (c) pre-formed niacin

Abbreviations: NN: not necessary; CHO: carbohydrates.

- For IF and FOF containing hydrolysed protein the same requirements as for formula based on intact cow's and goat's milk protein should apply, except for the minimum protein content which cannot be proposed and the adequacy of the protein content of a specific IF or FOF containing hydrolysed proteins needs to be established based on clinical evaluation. The maximum protein content should, however, not exceed 2.8 g/100 kcal (0.67 g/100 kJ).

- Also for IF and FOF containing ISP the same requirements as for formula based on intact cow's and goat's milk protein should apply, except for a minimum and maximum protein content of 2.25-2.8 g/100 kcal (0.54-0.67 g/100 kJ), a minimum phosphorus content of 30 mg/100 kcal (7.2 mg/100 kJ), a minimum iron content of 0.45 mg/100 kcal (0.11 mg/100 kJ) for IF and 0.90 mg/100 kcal (0.22 mg/100 kJ) for FOF and a minimum zinc content of 0.75 mg/100 kcal (0.18 mg/100 kJ). There should be no requirement for a minimum lactose content in IF and FOF containing ISP.

- The minimum content of nutrients in IF and FOF proposed by the Panel is identical with the exception of iron. If the same formula is to be used from the first months of infancy and be suitable for the whole first year of life the minimum iron content should be 0.6 mg/100 kcal (0.14 mg/100 kJ) for formulae based on intact cow's and goat's milk protein and formulae containing protein hydrolysates and 0.9 mg/100 kcal (0.22 mg/100 kJ) for formulae containing ISP.

- There is no necessity to add ARA, EPA, chromium, taurine, nucleotides, non-digestible oligosaccharides, "probiotics" or "synbiotics" to IF and FOF. There is also no necessity to use PL as a source of LCPUFA instead of TAG in IF and FOF or to use TAG with palmitic acid predominantly esterified in the *sn*-2 position in IF and FOF instead of TAG from other fat sources. For FOF, contrary to IF, the addition of L-carnitine, inositol and choline is not necessary.

- It is not necessary to propose specific compositional criteria for formula consumed after one year of age, as formulae consumed during the first year of life can continue to be used by young children.

DOCUMENTATION PROVIDED TO EFSA

Evidence report related to an extensive literature search and review as preparatory work for the evaluation of the essential composition of infant and follow-on formulae and growing-up milks provided by Pallas Health Research and Consultancy following a procurement procedure.

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ABBREVIATIONS

AAP	American Academy of Pediatrics
AI	Adequate Intake
ALA	alpha-linolenic acid
AMP	adenosine 5’-monophosphate
AR	Average Requirement
ARA	arachidonic acid
ATP	adenosine triphosphate
BMD	bone mineral density
BMI	body mass index
CD	celiac disease
CLA	conjugated-linoleic acid
CMP	cytidine 5’-monophosphate
CoA	coenzyme A
DFE	dietary folate equivalent
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DPA	docosapentaenoic acid
DRV	Dietary Reference Value
E%	percentages of the total energy intakes
EPA	eicosapentaenoic acid
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
FA%	percentage of total fatty acids
FAD	flavin adenine dinucleotide
FADS	fatty acid desaturase
FMN	flavin mononucleotide
FOF	follow-on formulae

FOS	fructo-oligosaccharides
GMP	guanosine 5’-monophosphate
GOS	galacto-oligosaccharides
IDD	iodine deficiency disorders
IF	infant formula
Ig	immunoglobulin
IGF	insulin-like growth factor
IGFBP	IGF-binding protein
IMP	inosine 5’-monophosphate
ISP	isolated soy protein
LA	linoleic acid
LC	long-chain
LOAEL	Lowest Observed Adverse Effect Level
MCFA	medium-chain fatty acid
MUFA	monounsaturated fatty acid
NAD	nicotine adenine dinucleotide
NE	niacin equivalents
NPN	non-protein nitrogen
PL	phospholipid
PRI	Population Reference Intake
PUFA	polyunsaturated fatty acid
RCT	randomised controlled trial
RI	Reference Intake range for macronutrients
RNA	ribonucleic acid
SCF	Scientific Committee on Food
SCFA	short-chain fatty acid
SD	standard deviation
SFA	saturated fatty acid

SNP	single-nucleotide polymorphism
T1DM	type 1 diabetes mellitus
TAG	triacylglycerol
TE	tocopherol equivalents
TFA	*trans*-fatty acids
ToR	Terms of Reference
TPN	total parenteral nutrition
UDP	uridinediphosphate
UL	Tolerable Upper Intake Level
UMP	uridine 5’-monophosphate
VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern
WHO	World Health Organization