FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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SD1 Attachment A1.1 – Nutrition Assessment – Proposal P1028

Infant Formula

Executive Summary

Infant formula contains macronutrients, vitamins, minerals, and other nutritive substances that are required for growth and development of infants. For each nutrient, a minimum amount is generally defined to ensure an infant receives adequate amounts. A maximum amount is also defined for some nutrients to ensure an infant does not receive unsafe amounts. In addition, permitted forms, specific components (e.g. amino acids or fatty acids), conversion factors, ratios to other nutrients, and other restrictions may also be important to consider. Where these considerations apply in the standards regulating infant formula nutrient composition varies and depends on the specific nutrient.

Nutrient composition of infant formula is prescribed in the *Australia New Zealand Food Standards Code* (the Code) Standard 2.9.1 – Infant Formula Products (and Schedule 29 in the revised Code) and in the *Codex Alimentarius* Standard 72-1981 Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (Codex STAN 72-1981). The two standards are aligned or comparable for most nutrients. However, there are some inconsistencies. New science has emerged since the standards were implemented, and some issues continue to be debated within the scientific community. Thus, the evidence base that underpins infant formula composition in Standard 2.9.1 may have changed since it was last reviewed in the late 1990s.

This assessment reviewed infant formula nutrient composition with the aim of determining whether harmonising with the compositional requirements in Codex STAN 72-1981 would pose a risk of nutritional inadequacy or harm to Australian and New Zealand (ANZ) infants. A comparative analytical approach was used in which the Codex STAN 72-1981 provision for each nutrient was assessed against a set of criteria that would indicate potential risk to infant health if Codex STAN 72-1981 for the nutrient in question was used. These assessment criteria included (where applicable):

- origin of the current standards;
- recommendations of key expert bodies
- comparison with breast milk concentrations
- estimation of intakes and comparison with ANZ Nutrient Reference Values for adequate and excess intakes
- physiological, biochemical or functional outcomes
- identification of new or emerging scientific evidence.

Compositional requirements for 33 constituents of infant formula - protein, carbohydrate, fat, vitamins (13), minerals and electrolytes (14), and nutritive substances (3) - as well as the energy content were reviewed.

For the nutrients vitamin K, thiamin, riboflavin, pantothenic acid, vitamin B12, biotin, sodium, chloride, and magnesium, Codex STAN 72-1981 (all provisions) met all of the assessment criteria. Furthermore, there was no new scientific evidence to indicate that Codex STAN 72-1981 should be amended. Therefore, it was concluded that the use of Codex STAN 72-1981 for these nutrients was unlikely to pose a risk to infant health.

For most nutrients (energy, protein, carbohydrate, fat (excluding the essential fatty acid linoleic acid, see below), vitamin A, vitamin C, vitamin D, vitamin E, vitamin B6, niacin, folate, potassium, calcium, phosphorous, zinc, iodine, copper, chromium, molybdenum, choline, carnitine, and inositol), Codex STAN 72-1981 did not meet all of the assessment criteria in relation to one or more of the factors defined in the standard (e.g. minimum and/or maximum amounts, and/or permitted form, etc.). However further examination, as detailed in this assessment report, indicated that Codex STAN 72-1981 was supported by the current scientific evidence base. Therefore, for these nutrients, it was also concluded that use of Codex STAN 72-1981 was unlikely to pose a risk to infant health.

For linoleic acid, iron, and selenium, Codex STAN 72-1981 did not meet one or more of the assessment criteria and further examination indicated that the evidence base was uncertain. For these nutrients, it was concluded that use of the Codex STAN 72-1981 amount may pose a risk to infant health. Reasons for the uncertainty are summarized below.

Conclusions: Based on recent scientific evidence and assessing against a set of specific criteria, the risk of nutrient inadequacy or harm for ANZ infants was found to be unlikely for most of the Codex STAN 72-1981 nutrient compositional requirements. However, the evidence base for several nutrients (linoleic acid, iron, and selenium) was determined to be uncertain and for these, it was concluded that use of Codex STAN 72-1981 could pose a risk to infant health.

Nutrient	Conclusion of Assessment – Uncertainties in the evidence base
Linoleic acid	• Codex STAN 72-1981 minimum amount is substantially less than the minimum amount in Standard 2.9.1 • Lack of international consensus on amount of linoleic acid in infant formula • ANZ recommendation for adequate intake (AI) is for all n-6 PUFA (not specifically for linoleic acid); Codex STAN 72-1981 does not meet recommendations made by EFSA for LA specifically • Current debate within the scientific community about infant requirements for essential fatty acids
Iron	• Codex STAN 72-1981 minimum amount is substantially less than the minimum amount in Standard 2.9.1 • Estimated intakes based on the Codex STAN 72-1981 minimum would not meet the ANZ AI for infants aged 0–<6 months and would meet approximately one quarter of the Estimated Average Requirement derived from infants aged 6–<12 months • No international consensus on the amount of iron in infant formula • Debate about the absorption of iron from infant formula compared with breast milk • Evidence that NZ infants are at risk of iron deficiency anaemia
Selenium	• Estimated intake based on the minimum amount would not meet the ANZ AI for selenium for infants aged 0–<12 months • Recent studies indicate minimum selenium in infant formula should be increased • No international consensus on the recommended minimum and maximum amounts in infant formula • Estimated intake based on the Codex STAN 72-1981 maximum amount would exceed the ANZ upper level of intake (UL) but no evidence of excess intakes or associated adverse health effects

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1 Introduction

1.1 Background

Infant formula nutrient composition has been derived from the best available scientific evidence of physiological, metabolic, and biochemical processes that underlie normal growth and development. Because infant formula is used as a sole source of nutrients for some infants, or as a supplement to breast milk and/or complementary feeding (i.e. solid foods introduced after 4–6 months), its compositional requirements are explicitly defined by regulatory authorities.

Composition comprises of all the essential nutrients in addition to some nutritive substances which may be added as optional ingredients. Essential nutrients – which are the focus of this assessment - are energy, macronutrients (protein, carbohydrate, fat as well as essential amino acids and essential fatty acids) and micronutrients (vitamins and minerals). These are generally defined in terms of minimum amounts to ensure that they are present in amounts that support normal growth and development and maximum amounts to ensure they are not present in unsafe amounts. In addition, other factors such as permitted forms, sources, conversion factors, ratios to other nutrients, and other restrictions (e.g. limits on certain fatty acids) may also be defined.

Nutrient composition is regulated by the *Australia New Zealand Food Standards Code* (the Code) Standard 2.9.1 - Infant Formula Products (Standard 2.9.1) which was developed in the late 1990s and early 2000s. In general, nutrient composition requirements for Standard 2.9.1 were based on: (1) recommendations of the Life Sciences Research Office (LSRO) which published the comprehensive review *Assessment of Nutrient Requirements for Infant Formulas* in 1998 (LSRO 1998); or (2) provisions specified by Codex Standard 72-1981 *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants* (Codex STAN 72-1981) for infant formula products as well as European regulations at that time.

At the time of developing Standard 2.9.1, Codex STAN 72-1981 had been in place since 1981 and nutrient composition provisions were, by and large, based on scientific research conducted in the 1970s. The present Codex STAN 72-1981 was developed from a full review of nutrient composition conducted in 2003 by the European Commission Scientific Committee on Food (EC SCF 2003). Some of the EC SCF recommendations were reiterated from the LSRO 1998 assessment or were based on additional scientific evidence available at the time. A subsequent report summarising the recommendations of the EC SCF (2003) review was published in 2005 by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Koletzko et al. 2005). With a few exceptions, the ESPGHAN recommendations were adopted into Codex STAN 72-1981 and EC regulations.

Despite the staggered timing of the reviews that underpin Standard 2.9.1 and Codex STAN 72-1981, the two standards are largely comparable for most nutrients. Infant formula manufactured according to both standards is composed of nutrients in amounts that are adequate and safe for infants. However, some differences exist between Standard 2.9.1 and Codex STAN 72-1981 (as well as with other international regulations), new science has emerged which may suggest more optimal compositions, and some issues continue to be debated within the scientific community. These considerations are the basis for this comparative nutritional safety assessment.

1.2 Objectives and scope

This assessment aims to evaluate the evidence base for nutrient composition of infant formula and to determine whether nutrient composition specified by Codex STAN 72-1981 would pose a risk to infants consuming such products.

For some nutrients, provisions were aligned between the two standards. In these cases, the nutrient was reviewed to assess whether new science has emerged since 2003 when Codex STAN 72-1981 was last fully reviewed. For nutrients that were not aligned, the reason for the difference was investigated.

The scope of the assessment covers energy, protein, amino acids, carbohydrates, fat, fatty acids, vitamins, minerals, and certain nutritive substances (L-carnitine, choline, and inositol). Depending on the nutrient, Standard 2.9.1 and Codex STAN 72-1981 prescribe minimum amounts, maximum amounts (mandatory or voluntary), sources, permitted forms, restrictions, ratios, and conversion factors (for example, where permitted forms require a calculation of equivalent amounts).

The scope of the nutrition assessment does not include:

- Non-essential nutritive substances that are not currently permitted in Standard 2.9.1 (but are permitted in Codex STAN 72-1981).
- Nucleotides (inosine monophosphate, adenosine monophosphate, and guanosine monophosphate) and taurine which are nutritive substances currently permitted as optional additions to infant formula but are considered non-essential in both Standard 2.9.1 and Codex STAN 72-1981, and more recently by EFSA (EFSA 2014).
- Fluoride which will be covered in subsequent assessment reports in Proposal P1028.

2 Methods

2.1 Approach

2.1.1 Assessment question and criteria for comparative analysis

This nutrition assessment was carried out as a broad comparative analysis of infant formula composition. To ensure nutrients were reviewed systematically and uniformly, a general question (see below) was addressed using defined assessment criteria.

General assessment question:

For energy and essential macronutrients, vitamin and mineral or electrolyte composition of infant formula fed as the sole source of nutrition to infants aged 0–<6months and otherwise fed to infants aged 6–<12 months: do the minimum and/or maximum amounts for these nutrients, as listed in the Codex STAN72-1981 for infant formula, pose a risk of inadequacy or harm?

For each nutrient, Codex STAN 72-1981 was evaluated against the assessment criteria defined below. Codex STAN 72-1981 was taken as the starting point since the underpinning evidence for Codex STAN 72-1981 was more recently reviewed compared to Standard 2.9.1. The criteria were used to assess the evidence base for each nutrient and determine whether there is uncertainty in the adequacy or safety of the Codex STAN 72-1981 amounts or other compositional factor.

Box 1: Criteria used to assess infant formula essential nutrient composition

For each nutrient, as applicable:

1. Is Codex STAN 72-1981 substantially different from existing Standard 2.9.1? If yes, then what is the reason for the difference and is there a history of safe use at the current Codex amounts?
2. Is the Codex amount within the range measured in mature (4 week post-partum) breast milk?
3. Is Codex STAN 72-1981 based on physiological, biochemical, or functional outcomes?
4. Are there substantial limitations or unknowns identified in the evidence base for the nutrient?
5. Do the Codex amounts (minimum and maximum) meet requirements for adequate intake (AI) or Estimated Average Requirement (EAR) and the upper level of intake (UL) as set in the ANZ Nutrient Reference Values (NHMRC and MoH 2006)? If not, does it pose a risk to infant health?

2.1.2 Information sources

The evidence base for this nutrition assessment was obtained from reports published by key review panels (Table 1) and published primary research. Primary research was mainly examined when new science published since the EC SCF 2003 review had emerged or when research pertaining to the ANZ population was identified. However, primary studies published prior to the EC SCF report were also examined where necessary.

Table 1: Key reviews of infant formula nutrient composition

Expert Panel	Year	Description	Reference
LSRO	1998	The main evidence base used for the current Standard 2.9.1.	(LSRO 1998)
ANZFA	1999	The Preliminary Assessment report for Proposal P93 *Review of Infant Formula* that describes the rationale for the current Standard 2.9.1.	(ANZFA 1999a)
EC SCF	2003	The comprehensive review of infant formula compositional requirements that underpins the current Codex STAN 72-1981	(EC SCF 2003)
ESPGHAN	2005	Mainly a summary of the EC SCF 2003 recommendations with some amendments	(Koletzko et al. 2005)
EFSA	2014	Essential composition of infant formula	(EFSA 2014)

2.1.3 Calculations

Numerous calculations were applied to address relevant criteria in the comparative analysis. These are summarised in Appendix 1, along with an example calculation and list of conversion factors. Most calculations involved conversion of values to common units to allow comparisons between nutrient amounts in standards or reports. Therefore, comparing small differences which resulted from rounding should not be over-interpreted as differences between the values being compared.

2.1.4 Reporting results

Calculated data for all nutrients are reported in the tables in Appendix 2. From these data, many nutrients were determined to meet all of the assessment criteria (Box 1). In these cases, as designated in Appendix 2 tables, no further analyses were undertaken and no further discussion of these nutrients was included in this assessment report. For nutrients that did not meet the assessment criteria (Box 1), a brief statement indicating the issue is provided in Appendix 2 tables and further explanation is provided in the relevant section of this report.

2.2 Assumptions

2.2.1 Infant age groups

Standard 2.9.1 defines infant formula product as *a product based on milk or other edible food constituents of animal or plant origin which is nutritionally adequate to serve as the principal liquid source of nourishment for infants* where an infant is defined as a person under the age of 12 months. Therefore, nutrient composition of infant formula must be suitable to meet the nutrient requirements of infants aged 0–<12 months.

Scientific literature, guidelines, and surveys are inconsistent when describing age groups within the 0–<12 month age group. Noting that most infant nutrient requirements (aside from energy) have not been set on a monthly basis, the age ranges used for this assessment (0–<6 months and 6–< 12 months) were assumed to be equivalent to age ranges used in other information sources listed in Table 2.

Table 2: Age ranges to describe infants within 0–12 months of age

This Assessment	EC SCF[1] *(months)*	AIHW Infant Feeding Survey[2] *(months)*	ANZ NRV[3]	Has also been referred to as:
"0–<6months"	1	Birth < 1	"0–6 months"	"the first 6 months of life"
	2	1.0<2		
	3	2.0<3		
	4	3.0<4		
	5	4.0<5		
	6	5.0<6		
"6–< 12 months"	7	6.0<7	"7–12 months"	"older infants"
	8	7.0<8		
	9	8.0<9		
	10	9.0<10		
	11	10.0<11		
	12	11.0<12		

[1] *EC SCF (2003)*
[2] *Australian Infant Feeding Survey (AIHW 2011); Also used in P274 minimum age labelling (FSANZ 2014)*
[3] *NHMRC and MoH (2006)*

2.2.2 Comparison with breast milk composition

Comparisons with breast milk were based on mean nutrient concentrations measured in mature breast milk from healthy mothers where mature breast milk refers to that sampled from mothers 4 weeks post-partum.

In general, minimum and maximum amounts of nutrients in infant formula were mostly derived from breast milk composition and from nutrient intakes in breastfed infants. Limitations of using breast milk as a benchmark include:

- changes in nutrient concentrations in breast milk based on maternal health, diet and nutritional status, post-partum sampling period, circadian cycle, and time of collection (fore-milk versus hind-milk)
- the difficulty in measuring breast milk intakes in breastfed infants
- differences in bioavailability and metabolism of certain nutrients in breast milk compared with the corresponding nutrients in infant formula.

Therefore, as concluded by the EC SCF (2003), breast milk composition cannot be used as the sole basis for infant formula composition and, therefore, other biochemical, metabolic, and physiological factors were also considered for this assessment.

2.2.3 Comparison with Nutrient Reference Values

The ANZ Nutrient Reference Values (NRVs) (NHMRC and MoH 2006) are recommendations for daily nutrient intakes which were derived from scientific knowledge available at the time (Table 3). An NRV to describe Adequate Intake (AI) has been set for all essential nutrients, except iron where an Estimated Average Requirement (EAR) has been set for infants aged 6-12 months. An Upper Level of Intake (UL) has not been defined for all essential nutrients generally due to insufficient evidence to support a UL for infants in the 0-<12 month age group.

Table 3: Nutrient Reference Values and their definitions

Nutrient Reference Value	Definition*
Adequate Intake (AI)	The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.
Estimated Average Requirement (EAR)	A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.
Upper Level of Intake (UL)	The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

** As defined in NHMRC and MoH (2006)*

As one of the assessment criteria, Codex STAN 72-1981 specified minimum and maximum amounts were used to estimate minimum and maximum nutrient intakes to compare with the AI, EAR, or the UL. The term AI will be used when referring to the adequacy NRVs for nutrients because only iron for older infants has an adequacy NRV expressed as an EAR.

Estimated intakes were calculated by assuming that infant formula contained energy at the midpoint, i.e. 2725 kJ/L, of the energy range content for Codex STAN 72-1981 (as evaluated in Section 3.2). For infants aged 0–<6 months, the estimated intake assumed an average daily volume of intake of 0.8 L/day (FSANZ 2007a, p.44; EFSA 2013, p.12). For infants aged 6–<12 months, the estimated nutrient intake based on the diet of a 9 month old infant was assumed where 50% of the energy intake is derived from breast milk or breast milk substitutes (FSANZ 2009) and 50% from complementary feeding. For these older infants, the estimated minimum intake was compared to 50% of the AI (or EAR) and the estimated

maximum intake was compared to 50% of the UL (if available). An example calculation has been provided in Appendix 1.

Given these assumptions, comparison with the AI or the UL approximates whether a nutrient amount in infant formula will meet an infant's requirements. An important limitation of this comparison is uncertainty in infant NRVs due to the small studies on which they were based, or methodological reasons (e.g. AIs derived from breast milk concentration or intakes in breastfed infants which are particularly difficult to measure, and ULs derived by extrapolation from older age groups).

In recognition of the uncertainty in infant NRVs, it was assumed that estimated intakes that were only marginally less than the AI (within 15%) were considered to meet the assessment criteria. Likewise, estimated intakes that only marginally exceeded the UL (within 15%) were also considered to meet the assessment criteria. Estimated intakes that did not meet the AI (or 50% of the AI for older infants), or exceeded the UL (or 50% of the UL for older infants), by an amount that was greater than 15% were considered to be substantially different and underlying factors that would explain this difference were examined.

Calculated daily intakes based on minimum and maximum amounts represented a conservative approximation (i.e. "worst-case scenario") of meeting the specified AI (or EAR) and UL because the content of each nutrient in infant formula over the total period of formula feeding is likely to be an average amount within the prescribed range.

2.2.4 Considerations regarding guidance maximum limits

Some, but not all, nutrients have maximums specified in Standard 2.9.1 and Codex STAN 72-1981 as mandatory limits. In Codex STAN 72-1981, some nutrients have voluntary maximums which are defined as Guideline Upper Levels (GULs). In the Code some nutrients have recommended maximum amounts located in the Guidelines to Standard 2.9.1. Table 4 summarises the basis for assigning maximum amounts.

Table 4: Status of maximum amounts

Maximum amount is -	Standard 2.9.1	Codex STAN 72-1981
Not specified	(not applicable)	Applies when level may need to be determined by national authorities (iron) or when amount has not been specified (indicated as "N.S" for α-linolenic acid and carnitine)
Suggested	For vitamins and minerals these are recommended quantities to be observed as maximum levels	Termed Guidance Upper Levels (GULs), set for nutrients without sufficient information for a science-based risk assessment
Mandatory	Prescribed for vitamins and minerals and electrolytes that are considered to pose significant risk to infants if consumed in excess*	Set when there is documented risk of adverse health effects according to a science-based risk assessment approach.

** Proposal P93 – Infant Formula: Preliminary Inquiry Report Explanatory Notes (ANZFA 1999). Available at: http://www.foodstandards.gov.au/code/proposals/Pages/proposalp93reviewofinfantformula/Default.aspx*

Several nutrients have defined mandatory maximums in Standard 2.9.1 but have GULs (i.e. voluntary maximums) in Codex STAN 72-1981. When this difference occurs (*viz-a-viz* vitamin B6, vitamin E, phosphorus, magnesium, iodine, copper, zinc, manganese, selenium) the evidence base used to assign mandatory maximum for Standard 2.9.1 was compared to the conclusions of the EC SCF review (2003) to determine if mandatory prescription of these

amounts still applied. Additional factors were also considered (as applicable): (1) nutrient bioavailability, processing losses and shelf-life stability; (2) total levels of a nutrient, including naturally occurring and added nutrients; (3) the inherent variability of nutrients introduced during manufacture; and (4) whether an established history of safe use was evident.

3 Nutrition Assessment

3.1 Overview of the results

Depending on the nutrient, Standard 2.9.1 and Codex STAN 72-1981 prescribe minimum amounts, maximum amounts (mandatory or voluntary), sources, permitted forms, restrictions, ratios or relative amounts, and conversion factors. Using the data in Appendix 32and a review of recent studies reported in the scientific literature, it was evident that some nutrients met all of the assessment criteria. For these nutrients, no further discussion is required because they are unlikely to pose a risk to infant health. However, for nutrients where one or more assessment criteria were not met, a more detailed evaluation was needed, as described below.

3.2 Energy

Standard 2.9.1 and Codex STAN 72-1981 provisions for energy were examined in relation to the origin of the current standards and to estimated energy requirements for infants aged 0-12 months.

3.2.1 Origin of infant formula standards for energy levels

Energy minimum and maximum for Standard 2.9.1 (2500 and 3150 kJ/L, respectively) were set according to Codex STAN 72-1981 at the time of the preceding review of Standard 2.9.1 (ANZFA 1999). The Codex STAN 72-1981 energy range has since changed to 2500–2950 kJ/L. The reported energy density of breast milk is about 2720 kJ/L (Appendix 2, Table 20) which is comparable to the midpoint of the Codex STAN 72-1981 range (2725 kJ/L).

The energy content in infant formula specified in Standard 2.9.1 was derived from energy intakes estimated from breast milk composition and volume of intake measured in the 1980's and earlier. It is now acknowledged that this is a poor reference since the measured energy content of breast milk varies widely across studies and intakes were measured using differing sampling protocols at different stages of lactation (Neville 1995).

The current Codex STAN 72-1981 energy levels are based on more accurate methods of measuring energy expenditure and energy deposition in infants (EC SCF 2003). Energy expenditure (referred to as Total Energy Expenditure (TEE) or Total Daily Energy Expenditure (TDEE) is determined by the doubly-labelled water (DLW) technique, which measures the energy expended during oxidation of energy-yielding nutrients to water and carbon dioxide using isotopically-labelled water ($^{2}H_{2}O^{18}$). Energy deposition is measured by determining body protein content plus body fat content using body composition assays and converting mass to energy units using appropriate conversion factors. Based on data from these techniques, as well as on more recent data showing the energy content of breast milk is less than previously thought, the EC SCF (2003) recommended a reduction in the maximum energy density of infant formula.

3.2.2 Meeting adequate intake requirements for energy

The Estimated Energy Requirement (EER) recommended by three expert panels (Table 5) was compared to the midpoint between the minimum and maximum energy content in Codex

STAN 72-1981. EERs are usually referenced to standard growth weights for girls and boys since energy requirements and growth varies with gender and age. For simplicity, the EER values reported in Table 5 are averaged for girls and boys.

ANZ NRV recommendations (NHMRC and MoH 2006) were based on the IOM recommendations (IOM 2002) that were derived from a report on TEE and energy deposition estimates (Butte 2001) and weight-for-height data taken from growth charts published for the United States (Kuczmarski et al. 2000). The EC SCF (2003) used the same data to evaluate infants' energy requirements. As a result, EER values provided in the EC SCF and ANZ NRV recommendations are comparable, with minor differences due to rounding in calculations.

More recent estimates of EER also reported in Table 5 were derived from revised international data for energy requirements based on higher TEE measured in formula-fed infants (FAO/WHO/UNU 2004) and new international weight-for-age data (WHO 2007a). These are data that have been used in recent FSANZ dietary exposure assessments (FSANZ 2007a, p.44).

Using the midpoint of energy density prescribed in Codex STAN 72-1981 (2725 kJ/L) and the mean volume of intake (0.8 L/day), the mean energy intake for infants 0–<6 months of age was estimated to be 2180 kJ/day. This intake is less than the overall mean EER across the 0–<6 months age range determined by all three expert bodies shown in Table 5.

However, it is reasonable to expect that larger infants will consume greater amounts of formula per feed and, therefore, daily intake will be greater than that for smaller infants.

The mean energy intake for infants 6–<12 months (consuming the mean intake volume of 0.6 L/day) would be 1635 kJ/day from formula. Assuming a theoretical diet where 50% of an infant's energy is obtained from formula (or breast milk) and 50% of from complementary foods, this amount would exceed half the mean EER by 114–155 kJ/day.

Table 5: Estimated Energy Requirements (EER) for infants 0–12 months

Age (*Completed month*)	**Expert Panel Recommendations for EER [1] *(kJ/day)***		
	EC SCF 2003[2]	**ANZ NRV[2]**	**FAO 2004/WHO 2007 [3]**
1	1901	1900	2200
2	2230	2250	2484
3	2284	2300	2562
4	2207	2300	2446
5	2401	2600	2592
6	2587	2650	2698
Mean EER for 0–<6 mo	2268	2333	2497
7	2667	2650	2720
8	2828	2850	2822
9	2976	2950	2924
10	3156	3150	3026
11	3258	3250	3094
12	3369	3350	3162
Mean EER for 6–<12 mo	3042	3033	2960

[1] *Separate data for boys and girls were cited in each report but data shown are averaged for girls and boys.*
[2] *EER derived for formula-fed and breastfed infants combined. Note both EC SCF and ANZ NRV use data from the IOM (2002) report on macronutrient requirements to calculate EER. Slight differences are due to rounding and conversion of units.*
[3] *EER was derived for formula-fed infants.*

3.2.3 Conclusion – energy

In using Codex STAN 72-1981 for energy, the resulting change to energy density involves a 6.3% decrease in the maximum energy density or a 3.5% decrease in the average energy density. There are no public health indicators to suggest that this small decrease is likely to adversely affect normal growth and development. Therefore, Codex STAN 72-1981 for energy content meets all the criteria and its use is unlikely to pose a risk to infant health.

3.3 Protein

Protein in infant formula was examined in relation to protein content (quantitation, origin of current standards, comparison with breast milk and estimation of adequate intakes), and protein quality (scoring methods, minimum amino acids amounts). New science on health effects related to protein in infant formula was also reviewed.

3.3.1 Protein content

Crude protein versus true protein

Several methods can be used to measure the protein content of infant formula and breast milk (reviewed in the Nutrition Assessment on minimum amount of L-histidine in infant formula (FSANZ 2013a)). Most commonly the Kjeldahl method is used in which total nitrogen is measured and converted to protein concentration using a protein-nitrogen conversion factor (NCF, see next section). Protein concentration derived from the total nitrogen content is referred to as crude protein because it includes protein nitrogen plus nitrogen from free amino acids (FAA) and other non-protein nitrogen (NPN) sources such as urea, creatinine, and nucleotides.

The true protein amount is derived from nitrogen in the protein component only. True protein is measured by subtracting the amount of FAA and NPN nitrogen (measured by Kjeldahl after precipitation and filtration of protein) from the total nitrogen amount, then converting to protein concentration using a NCF. Spectrophotometric methods or amino acid analyses can be used to quantify true protein content (Fusch et al. 2015). The methods have various advantages or limitations (Moore et al. 2010).

Of the total nitrogen, mature breast milk contains 20–27% NPN compared with 5–16% in cow's milk-based infant formula. Additionally, a substantial proportion of NPN in both sources is urea with breast milk containing up to about 50%, and cow's milk-based formula containing 27–65%. Urea is generally considered to be non-utilisable as a nitrogen source (Donovan and Lonnerdal 1989; Dewey et al. 1996; Rudloff and Kunz 1997). The amount of FAA can also be highly variable in breast milk and infant formula (Agostoni et al. 2000; Ventura et al. 2012).

Both crude and true protein measurements have been used in numerous studies to determine breast milk protein composition (Gidrewicz and Fenton 2014). Crude protein measurements tend to over-estimate the true protein level due to the presence of NPN. Therefore, in studies measuring protein content in breast milk and infant formula, results obtained using the two alternative methods cannot be averaged. Crude protein measurements can only be compared with studies measuring protein by the same method.

Standard 2.9.1 and Codex STAN 72-1981 do not specify a method for measuring protein content for a protein source. The EC SCF recommended that crude protein be determined with the added condition that the NPN content be taken into account and that it be not higher than 15% of the total nitrogen content (EC SCF 2003, p.41). ESPGHAN (Koletzko 2005) recommended that the protein content should be based on measurement of true protein.

EFSA (2014) recommended that the protein amount should be calculated from total nitrogen (i.e. crude) protein determination. Thus, there does not seem to be a consensus on which approach should be taken in determining protein content.

Because of the highly variable composition of the NPN fraction in breast milk and infant formulas, and methodological complexities to accurately determine NPN composition, true protein determination may be impractical to be used routinely. Protein determination based on total nitrogen measurement by the Kjeldahl is the accepted industry-standardised methodology for infant formula (AOAC International 2012) and continues to be considered as a sufficiently accurate and reliable method (Purificacion et al. 2013). Therefore, it is concluded that measurement of total nitrogen for the determination of protein content is unlikely to pose a risk to infant health.

Protein – nitrogen conversion factor (NCF)

The NCF is the ratio between protein molecular weight and the nitrogen content of the protein (Maubois and Lorient 2015). NCFs are needed because protein and amino acid amounts in infant formula are determined by measuring the total nitrogen content of a protein source and using a conversion factor to calculate the protein content.

Currently Standard 2.9.1 specifies two conversion factors: 6.38 for milk proteins and 6.25 for all other protein sources. Codex STAN 72-1981 specifies 6.25 to be used, but a footnote states that other conversion factors should be used for particular products if scientifically justified.

The 6.25 NCF was derived from the average nitrogen content of mixed food proteins which is approximately 16%. Thus, 1 g of nitrogen is equivalent to about 6.25 g of crude protein. As reviewed by the EC SCF (2003), this conversion factor was considered to be appropriate to calculate amounts of crude (or total) protein and amino acids in infant formula.

The 6.38 factor was derived from the amino acid sequence of casein protein component (6.36) and whey protein component (6.41) where regardless of the relative proportions of these protein components, the NCF remains about 6.38 (Maubois and Laurient 2015).

For the calculation of protein content, use of 6.25 for cow's milk protein sources when 6.38 is more appropriate underestimates protein content by about 2%. Given the relatively large variation in NPN amounts in cow's milk, this underestimation can be considered minor for typical cows' milk-based infant formulas. If the protein source is processed and enriched with certain protein fractions, the percentage could be greater or less but there is no data published on such NCFs.

Breast milk concentrations of individual amino acids generally have been measured in g of amino acid per L of breast milk but are converted to g per 100 g of total protein using the NCF. In this calculation, the differences between using 6.38 and 6.25 are also small (see EC SCF 2003, page 55) and within the relatively high variability of measured amino acid concentrations in breast milk. For this reason, both conversion factors are acceptable. For simplicity reasons, the 6.25 factor was used in a recent FSANZ assessment on L-histidine content in breast milk (FSANZ 2013a).

As recently reviewed (Maubois and Lorient 2015), accurate values for the NCF of a protein source are based on the complete primary structure which includes the protein backbone chain and any covalently bound side groups. Compared to cow's milk protein (comprised mainly of whey and casein proteins), soy protein has a different amino acid composition and is structurally different due to side chain glycosylation. Side chain glycosylation (which adds a carbohydrate moiety to the protein backbone chain) increases protein molecular weight but

does not increase nitrogen content. Thus for soy protein, 1 g of nitrogen has been determined to be equivalent to 5.71 g of protein. Use of the 6.25 conversion factor when 5.71 is the more appropriate would lead to an overestimation of protein content by about 10%. For this reason, the appropriate NCF is 5.71 for infant formula based on soy protein. This conclusion is consistent with long-standing international recommendations (FAO 2003 and references therein).

Protein content of breast milk

Breast milk protein content is highly variable with a range of crude protein in mature breast milk reported to be 8–21 g/L (Hester et al. 2012). Therefore, protein content is difficult to match in an infant formula. The permitted range of protein content in both Standard 2.9.1 and Codex STAN 72-1981 (which are identical) is greater than that contained in breast milk. The average crude protein content of mature breast milk (>14 days post-partum) was estimated to be 13 g/L (equivalent to about 0.48 g/100 kJ) in two recent systematic reviews (Hester et al. 2012) (Gidrewicz and Fenton 2014). This value is comparable to previous estimates (EC SCF 2003 and LSRO 1998).

The Codex STAN 72-1981 and Standard 2.9.1 range for protein content is 0.45–0.7 g/100 kJ. Based on the conversion that 1 gram of protein yields 17 kJ of energy, that permitted range corresponds to 8–12% of the total energy content of infant formula. Therefore, as a percentage of energy content, protein content in mature breast milk, which is reported to be about 7%, is comparable to that of infant formula.

A primary consideration in setting the amount of protein is to ensure that the requirements for the essential amino acids are met (see Section 3.3.3). Because no protein source that is used in infant formula exactly meets infant amino acid requirements, a higher range of protein content in formula is needed to allow for this and also batch variation during production.

Origin of infant formula standards for protein content

Standard 2.9.1 prescribes minimum crude protein content to be 0.45 g/100 kJ which is aligned with Codex STAN 72-1981. The minimum protein amount in Standard 2.9.1 was based on the crude protein content of breast milk (Australia and New Zealand Food Authority 1999). Despite recommendations at the time (LSRO 1998) that the minimum protein content should be less (0.40 g/100 kJ) based on the true protein content of breast milk (i.e. excluding NPN), the value of 0.45 g/100 kJ was retained from the previous ANZ standard because it was deemed to be consistent with international regulations and, since it is higher than the LSRO recommendation, was adequate to meet infant protein requirements. The Codex STAN 72-1981 minimum protein amount has been based on similar arguments (EC SCF Food 2003)[1].

The maximum protein content in both Standard 2.9.1 and Codex STAN 72-1981 is 0.7 g/100 kJ and is less than the maximum nitrogen content that could give rise to excess renal solute loads and an increased risk of kidney damage (EC SCF 2003, p.53).

[1] The EC SCF (2003) reports the minimum protein content to be 1.8 g/100 kcal which converts to 0.43 g/100 kJ using 1 kcal = 4.18 kJ. Thus, Codex STAN 72-1981 appears to have been rounded up to 0.45 g/100 kJ.

Meeting adequate intake recommendations for protein

In infants, the minimum protein requirement is the lowest level of dietary protein intake that will balance the losses of nitrogen from the body (through protein turnover or degradation) and support tissue deposition needed for growth and development (WHO/FAO/UNU 2007).

Determining infant protein intake requirements is technically challenging and has been approached using several methods (Dewey et al. 1996; EC SCF 2003):

1. Comparison with protein intakes in fully breastfed infants where average protein concentration in breast milk is multiplied by the average intake volume

2. Factorial approach or nitrogen balance method which involves estimating the requirements for nitrogen maintenance and the amount of nitrogen needed for growth

3. Estimating the safe level for protein intake (measured mean intake + 2SD)

4. Based on individual amino acid requirements (See Section 3.3.3)

5. Experimental approaches where infants are fed test formula compared to a control formula with known nutritional composition (See Section 3.3.4)

Estimated protein requirements by the first three approaches are comparable (Dewey et al. 1996). Approach 1 was used to determine the ANZ AI for protein which is 10 g/day for infants aged 0–6 months, and 14 g/day for infants aged 7–12 months (NHMRC and MoH 2006). Approach 3 was used for the WHO/FAO/UNU recommendations for protein intakes where the estimated safe level of intake for infants (mean of values for girls and boys) at 6 and 12 months of age was reported to be 9.8 g/day and 11.2 g/day, respectively (WHO/FAO/UNU 2007).

Estimated daily protein intake based on the minimum protein content (0.45 g/100 kJ) and a mean volume of intake of 0.8 L/day for infants 0–<6 months meets the ANZ AI for protein (NHMRC and MoH 2006). Similarly, infants aged 6–<12 months consuming 0.6 L/day at the minimum protein amount will meet 50% of the ANZ AI for protein (Appendix 2).

3.3.2 Protein quality

Protein quality is assessed by the ability of a protein source to meet an infant's amino acid requirements. A number of protein scoring systems have been developed to assess protein quality (Millward 2012). Although scoring systems have not been used for infant formula in Standard 2.9.1 or Codex STAN 72-1981 (both standards regulate protein quality by mandating minimum amounts of essential amino acids, see Section 3.3.3), new research in this area has prompted renewed interest.

Since 1991, the recommended scoring method by the FAO has been the PDCAAS (Protein Digestibility Corrected Amino Acid Score which uses: (1) an animal-based assay to measure digestibility; and (2) amino acid analyses to measure the amounts of individual amino acids in a protein source. The measurement compares the tested protein to a reference protein where the PDCAAS score is then calculated as the ratio of the amount of the limiting amino acid in the tested protein divided by the amount of the corresponding amino acid in the reference protein, and multiplied by a digestibility factor.

The FAO recently recommended using the Digestible Indispensable Amino Acid Score (DIAAS) which is a modified version of the PDCAAS that allows for a more accurate estimation of protein quality (FAO 2013). The main differences from previous methods are

(1) determination of digestibility in terms of ileal digestibility (instead of faecal digestibility), (2) no truncation (PDCAAS truncates high quality protein scores to 1.00 and artificially removes the capacity of a high quality protein to balance a low quality protein if mixed in a food), and (3) use of age-relevant reference amino acid pattern (breast milk for infants 0–12 months). For low quality protein sources (usually plant-based proteins), measurement of ileal digestibility in pigs or humans, instead of faecal digestibility usually measured in rats, may have significant effects on existing digestibility data sets, but there is currently very limited data using this method.

Protein scores using the two methods have been reported (Table 6). Milk and soy proteins, common protein sources for infant formula, score highly in both systems and are considered to be high quality proteins. It is unlikely that ileal digestibility data, when available, will substantially alter the quality scores of proteins used for infant formula products.

Table 6: DIAAS and PDCAAS values for some protein sources

Protein	DIAAS Score	PDCAAS Score
Whey protein isolate[1]	1.09	1.00
Milk protein concentrate[1]	1.18	1.00
Whey protein concentrate[1]	0.973	1.00
Soy protein isolate[1]	0.90	1.00
Wheat[2]	0.40	0.42
Whole milk powder[2]	1.22	1.00

[1] *Source: (Rutherfurd et al. 2015)*
[2] *Sources: (Schaafsma 2000; FAO 2013).*

3.3.3 Minimum amounts of essential amino acids

Definition of essential amino acids

Essential amino acids are those that are not synthesised endogenously and must be obtained from the diet (NHMRC and MoH 2006). Essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; and minimum amounts have been set for these in Standard 2.9.1 and Codex STAN 72-1981. Because infants may lack sufficient capacity to synthesize cysteine (or cystine) and tyrosine from their amino acid precursors (which are methionine and phenylalanine, respectively), these amino acids are considered to be semi-essential amino acids for infants and minimum amounts are also set in Standard 2.9.1 and Codex STAN 72-1981. Other amino acids (alanine, arginine, aspartic acid, asparagine, glutamic acid, proline and serine) are non-essential because they are obtained from respective precursors.

The terms essential, semi-essential, and non-essential amino acids have been referred to as indispensable, conditionally essential, and dispensable, respectively, in some reports on infant formula composition (LSRO 1998, EFSA 2014).

Origin of infant formula standards for minimum amino acid amounts

There are numerous differences in the minimum amounts of the essential amino acids in Standard 2.9.1 and Codex STAN 72-1981 but most are small (Table 7). Standard 2.9.1 amounts are mainly based on breast milk amino acid content reported by the 1985 FAO/WHO/UNU Expert Consultation which used breast milk amino acid concentrations reported in studies conducted between 1950 and 1970 (WHO 1985). The EC SCF (2003) considered that studies cited in the 1985 WHO report (on which the preceding Codex

standard was based as well) were unreliable due to small sample sizes, early lactation or unstated sampling periods, or inaccurate methodology for amino acid analysis. Therefore, the EC SCF (2003) analysed seven studies published between 1975 and 1991 to determine average breast milk concentrations of amino acids. ESPGHAN included one additional study in their summary of the EC SCF (2003) review (Koletzko et al. 2005). The minimum amino acid requirements in Codex STAN 72-1981 were derived from the ESPGHAN analysis.

Table 7: Minimum amounts of amino acids in Standard 2.9.1 and Codex STAN 72-1981

Essential amino acid	Minimum amounts *(mg/100 kJ)*	
	Standard 2.9.1	***Codex STAN 72-1981[a]***
Histidine	10	10 (10)
Isoleucine	21	22 (22)
Leucine	42	40 (40)
Lysine	30	27 (27)
Cysteine	-	9 (9)[c]
Cysteine, cystine and methionine	19[b]	-
Methionine	-	6 (5)
Phenylalanine	-	19 (20)
Phenylalanine and tyrosine	32[d]	-
Threonine	19	18 (18)
Tryptophan	7	8 (8)
Tyrosine	-	18 (18)
Valine	25	22 (21)

[a] *Converted to mg/100 kJ from the Codex STAN 72-1981 amount (in mg/100 kcal) using 4.18 and rounding; values in parentheses are requirements in EC Directive 2006/141/EC.*
[b] *No less than 6 mg of cysteine, cystine or combined cysteine and cystine per 100 kJ. See Section 3.3.3*
[c] *Amounts of methionine and cysteine may be added together if the ratio is less than 2:1. See Section 3.3.3.*
[d] *No less than 17 mg phenylalanine per 100 kJ. See Section 3.3.3.*

Assessment of minimum amino acid amounts

FSANZ recently assessed whether the minimum L-histidine amount could be reduced from 12 mg/100 kJ (as in Standard 2.9.1 at the time) to 10 mg/100 kJ (as prescribed in Codex STAN 72-1981) (FSANZ 2013a). Using the most recent data for breast milk composition and evidence from experimental studies on infant growth and physiological outcomes, the FSANZ 2013 assessment concluded that formula containing 10 mg/100 kJ L-histidine was comparable to the average breast milk concentration and that the growth of formula-fed infants consuming a formula containing this L-histidine amount was comparable with breastfed infants.

The analysis conducted for L-histidine was extended to all essential amino acids in infant formula for this assessment. Mean breast milk amino acids concentrations (Table 8) were determined from ten studies selected on the basis of specific criteria (FSANZ 2013a). For all amino acids, concentrations were comparable if not identical to those determined by ESPGHAN (Koletzko et al. 2005) as well as those reported in a recent systematic review (Zhang et al. 2013) and other previous reports (LSRO 1998). The values were also comparable to minimum amounts specified by Codex STAN 72-1981. Minor differences are attributable to normal variation of breast milk during lactation and uncertainties in the method for determining amino acid concentration.

Seven studies (selected as detailed in FSANZ 2013a) were then reviewed to determine whether the Codex STAN 72-1981 minimum amino acid amounts would support normal

growth and development. As with the conclusion for the L-histidine assessment (FSANZ 2013a), this analysis showed that growth rates and plasma amino acid concentrations were comparable between formula-fed and breastfed infants when formula contained amino acid amounts that were comparable to the Codex STAN 72-1981 minimum amounts listed in Table 8. Therefore, for the minimum amino acid amounts, Codex STAN 72-1981 meets all the assessment criteria and use of these minimum amounts are unlikely to pose a risk to infant health. Additional considerations on sulphur-containing and aromatic amino acids are described in the subsequent sections.

Table 8: Concentrations of essential amino acids in breast milk determined by FSANZ

Amino acid	Breast milk concentration based on 10 studies[1]				Codex STAN 72-1981 Minimum
	mg/g crude protein			*mg/100 kJ*[2]	*mg/100 kJ*
	mean	*median*	*range*		
Histidine	24	23	18–40	10	10
Isoleucine	49	49	39–60	22	22
Leucine	92	92	73–112	41	40
Lysine	63	62	50–82	28	27
Cysteine	22	21	13–32	10	9
Methionine	14	14	11–17	6	6
Phenylalanine	41	37	25–70	18	19
Threonine	42	42	22–54	19	18
Tryptophan	17	17	13–29	8	8
Tyrosine	42	37	19–72	19	18
Valine	50	48	40–66	23	22

[1] *See FSANZ 2013 for study details.*
[2] *Mean concentrations in mg/g crude protein were converted to mg/100 kJ based on a minimum protein content of 0.45 g/100 kJ.*

Sulphur-containing amino acids

Methionine and cysteine are sulphur-containing amino acids (SAA) of which only methionine is an essential amino acid. Generally, cysteine is non-essential since methionine can be converted to cysteine by the liver enzyme cystathionase. However, in infants (particularly neonates or pre-term infants) the enzyme may not be fully active and, therefore, cysteine is considered to be essential. Cysteine is the sulfhydryl form of cystine and is found in breast milk and other foods; cystine is the disulfide or oxidised form of cysteine. The nutritional benefits and the food sources of cystine are identical to those for the cysteine.

Quantifying individual SAA in breast milk or infant formula is not straightforward. Except under special reaction conditions, methionine and cysteine (and cystine) degrade during amino acid analysis (Darragh and Moughan 1998; Aristoy and Toldra 2015). In addition, the abundance of SAA, especially cysteine, in dairy protein sources is low compared to other essential amino acids and this could affect quantitative analysis of SAA (FAO 1970).

SAA minimums in Std. 2.9.1 are expressed as the summed amounts of cysteine, cystine, and methionine based on (1) the EC Directive 1991/321 (which precedes the current EC Directive 2006/141), and (2) recommendations provided by the FAO/WHO/UNE (WHO 1985). Both of these recommendations were based on studies conducted from 1950 through to the1970's during which SAA were not measured individually.

Codex STAN 72-1981 minimums for SAA are not expressed as a summed amount because they were derived using a more accurate analytical methodology that quantified individual SAA. Earlier reports (FAO 1970, WHO 1985) were also not used because data was derived from a small sample size or using unstated sampling periods. However, Codex STAN 72-1981 states that the amounts of SAA may be added together if the ratio of methionine to cysteine is less than 2:1. This ratio is based on (1) the ratio measured in breast milk which is about 1:1 and less than that measured in cow's and goat's milk (about 3:1) and (2) the potential imbalance which can affect methionine conversion to cysteine and nitrogen utilization from non-essential amino acids (Garlick 2006).

For technological reasons, correct amounts of methionine and cysteine in infant formula may be determined by difference (Aristoy and Toldra 2015) where a measured methionine amount (or cysteine) is subtracted from the measured total SAA amount. The derived amount of the corresponding SAA must be within the prescribed methionine and cysteine ratio to ensure appropriate balance of SAA.

Aromatic amino acids

The three aromatic amino acids (AAA) are tyrosine, phenylalanine, and tryptophan, of which the latter two are essential. Tyrosine is semi-essential since it can be derived from phenylalanine.

As with SAA, phenylalanine and tyrosine are difficult to quantify in breast milk individually except under special reaction conditions. Standard 2.9.1 minimum for these AAA were expressed as a summed value because breast milk concentrations of phenylalanine and tyrosine had not been reported individually at the time of the previous review (ANZFA 1999). Standard 2.9.1 also specifies a minimum amount of phenylalanine to ensure essential requirements are met. Separate amounts for phenylalanine and tyrosine have been specified in Codex STAN 72-1981 because they were measured individually in breast milk using a more recent methodology (EC SCF 2003). Therefore, the two standards are effectively aligned and use of Codex STAN 72-1981 for phenylalanine and tyrosine minimum is unlikely to pose a risk to infant health.

For the minimum amount of tryptophan, the current Standard 2.9.1 and Codex STAN 72-1981 are aligned (allowing for rounding) and this amount is consistent with the estimated breast milk concentration (see Table 8). The abundance of tryptophan in cow's milk protein is approximately half the abundance in breast milk protein. Thus, tryptophan in cow's milk-based formula can be present in limiting amounts and higher protein amounts or free tryptophan are generally added to infant formula to compensate for the lower tryptophan amount (Lien 2003).

Summary: minimum amounts of essential amino acids

Based on the points discussed above, the minimum amounts of essential amino acids in Codex STAN 72-1981 were determined to meet the assessment criteria and use of these amounts is unlikely to pose a risk to infant health.

3.3.4 Health effects related to protein in infant formula

Protein amounts have been specified as a range (0.45–0.70 g/100 kJ) in Standard 2.9.1 and Codex STAN 72-1981 to allow for differences in amino acid profile between breast milk and cow's milk (or other protein sources). Because breast milk amino acid concentrations cannot be replicated in infant formula, protein amounts in infant formula are generally higher than in breast milk to ensure amino acid requirements are met. If more protein is ingested than is needed for tissue deposition or metabolism (including synthesis of non-essential amino acids

and other nitrogen-containing compounds), then the excess is degraded and the end products are excreted. In infants, protein amounts greater than 20% of energy (equivalent to 1.2 g of protein/100 kJ based on the conversion of 1 g = 17 kJ) causes excess renal solute load and can impair water balance (EFSA NDA Panel 2012).

The association between formula feeding and increased growth rates and obesity has been of significant recent interest. This interest has been largely driven by the conclusions of observational studies showing breastfed infants have a lower risk for childhood obesity compared to formula-fed infants. However, other studies suggest that higher rates of obesity or overweight observed in formula-fed infants may also be related to feeding practices, socioeconomic status or other confounding factors (Mihrshahi et al. 2011; Li et al. 2012; Colen and Ramey 2014; Lefebvre and John 2014). A recent Dutch cohort study of 3367 infants showed that infants exclusively breastfed for 3 months, 3–6 months, or more than 6 months had the same risk of obesity at 5–6 years of age compared to formula-fed infants (van der Willik et al. 2015). Similar findings were made in a large international study which concluded that breastfeeding had little impact on body mass index (BMI) measured in 6-7 year old children (Hancox et al. 2015). Reviews have now been published suggesting that the association between formula feeding and risk of childhood obesity has not been substantiated by the currently available evidence (Cope and Allison 2008; Casazza et al. 2012; Lefebvre and John 2014; Casazza et al. 2015)

Nonetheless, the link between formula-feeding and obesity risk has led to research examining the nutrient composition of infant formula as a possible cause of childhood obesity. Based on the hypothesis that excess dietary protein intake in infancy affects metabolic and endocrine responses causing early rapid growth (Koletzko et al. 2013a; Brands et al. 2014), randomized clinical trials (RCTs) and observational studies demonstrated that infant formula containing high protein was linked to increased growth (Appendix 2, Table 23). These studies have led to the proposal that protein amounts in infant formula should be lowered (Inostroza et al. 2014). A number of issues, briefly explained below, indicate that the current evidence is not sufficient to warrant a change.

Amount of protein in high protein test formula

Most of the RCT results have originated from a single large multi-centre trial (European Childhood Obesity Trial or ECOT (Koletzko 2009) in which infants were fed a high or low protein formula from 8 weeks to 12 months of age and were compared to breastfed infants. The high protein formula - which gave rise to increased growth compared to breastfed infants - contained the maximum permitted protein amount (0.7 g/100 kJ) for approximately the first 6 months of age[2] then a follow-on formula (FOF)[3] containing 1.1 g/100 kJ up to 12 months of age. This does not reflect normal formula feeding where the protein amount contained in infant formula over the course of a year would be an average between minimum and maximum amounts (0.45–0.7 g/100 kJ). As a reference, the protein amount in infant formula sold in Australia, as labelled[4], contained a range of 0.46–0.54 g/100 kJ or average of 0.50 g/100 kJ.

[2] According to the study methods (Koletzko et al 2009), FOF was started "after introduction of complementary feeding, but not before the start of the fifth month of life" and provided to infants until the age of 12 months.

[3] Minimum and maximum protein amounts for FOF under the CODEX STAN 156-1987 are 0.45 and 1.37 g/100 kJ, respectively. Codex STAN 72-1981 for FOF is currently under review. Recent recommendations indicate that the maximum amount in FOF is too high and protein minimum and maximum amounts for FOF should be the same as that for IF (Koletzko et al. 2013b).

[4] Unpublished survey conducted by FSANZ of major brands sold in AU based on 2013/14 data (see SD1, Appendix A1.1).

Protein amount as proportion of energy in test formula

There is no UL for protein, but very high protein content (around 20% as a percentage of total energy) causes increased urea production and impairs water balance (EFSA NDA Panel 2012). The EC SCF (2003) recommended that formula should not provide more than 12% protein as a percentage of energy to ensure that the potential renal solute load was not unacceptably high. Because the ECOT high and low protein test formulas were iso-energetic, the high protein test formula contained protein at 12% of energy for infants up to 6 months, then 18% for infants aged 6–12 months. The low protein test formula provided protein at 7% of energy (infants aged up to 6 months) and 9% of energy (for 6-12 months). Thus the protein amount in the ECOT high protein formula, expressed as percentage of total energy, was 50% above the recommended safe level for the 6–12 month age group. For comparative purposes, the average protein content (0.50 g/100 kJ) corresponds to approximately 8.5% of energy based on label information of infant formula sold in Australia[3].

Effects on anthropomorphic measures

The ECOT study cannot be compared with other studies which used both differing amounts and types of protein. Results obtained from the 9 papers published from the ECOT study Appendix 3, Table 23), showed that effects on anthropomorphic measurements from 6 months to 6 years of age were small and had inconsistent statistical significance. Effects on plasma insulin parameters (Socha et al. 2011) or plasma concentrations of branched chain amino acids (Kirchberg et al. 2014), and other plasma metabolites (Rzehak et al. 2013) have been investigated to determine a mechanistic explanation. However, proposed hypotheses are not supported by sufficient mechanistic evidence to explain observed growth effects.

***Effects of high protein on long ter*m outcomes**

Few long-term outcomes have been measured. One analysis (Weber et al. 2014) from the ECOT trial has shown that infants fed a high protein formula had higher BMI and weight at 6 years of age. But the effect was marginal with weight for the high protein group slightly higher than low protein group (22.47 ± 4.28 kg and 21.88 ± 3.46 kg, respectively, P=0.064). Difference in BMI was also marginal albeit significantly higher for the high protein group compared to the low protein group (16.36 ± 2.29 and 15.86 ± 1.87, respectively, P<0.009). Adjustment for confounders attenuated these differences and a significant difference in BMI distribution was also found only at the 95th percentile of protein intake. A second ECOT analysis showed no differences between feeding groups (low protein, high protein and breast fed) in neuropsychological tests administered at 8 years of age (Escribano et al. 2016).

Long term developmental outcomes of low protein intake have not been established and they may be affected when certain amino acids are present in limiting or inadequate amounts. For example, tryptophan, the precursor of the neurotransmitter serotonin and which influences sleep patterns and possibly cognitive functions, is present in low quantities in formulas based on cow's milk compared to breast milk (Lien 2003). A study measuring plasma amino acid concentrations showed that infant formula which contained protein at the lower end of the permitted range formula (0.46 g/100 kJ) and tryptophan at 8 mg/100 kJ tryptophan (i.e. met the Standard 2.9.1 and Codex STAN 72-1981 minimum amino acid amounts) led to plasma tryptophan concentrations that were marginally, but significantly, less than that measured in breastfed infants (Sandstrom et al. 2008). It is uncertain whether the difference is clinically important but indicates the need to determine whether low protein formulas support infant development as well as growth.

Subpopulation effects

Increased obesity risk associated with higher protein intakes may be more relevant to certain

subpopulations of infants aged 0–12 months. Recent work has shown increased early rapid weight gain in infants fed high protein formula (0.65 g/100 kJ) and who had mothers with high BMI at birth. This was not the case for infants whose mothers had a normal BMI or were breastfed (Inostroza et al. 2014).

Comparing different studies

Much of the current research has focused on demonstrating that low protein formula (i.e. minimum amount of less than 0.45 g/100 kJ) are safe since they promote growth that is similar to breastfed infants. A systematic review of controlled trials of lower protein- or energy-containing infant formulas for healthy, full-term infants has recently been published (Abrams et al. 2015). Based on four trials that met the inclusion criteria (which are included in the studies listed in Appendix 3), the authors concluded that infants who were fed lower protein formulas had similar growth in infancy to breastfed infants but that further research on long term growth outcomes was needed. The authors also noted the difficulty of drawing conclusions based on studies that vary by study design, duration of the intervention, number of participants, and inclusion of probiotics, different types of protein, or other nutrients.

Other assessments by expert panels

EFSA considered some of the ECOT results in 2012 and concluded that the growth differences between infants fed high and low protein infant formula were significant but small and there was no evidence that these differences persist into later childhood or are related to obesity risk in later life (EFSA 2012, p.27). EFSA reiterated this conclusion in 2013 and 2014 in its scientific reports on infant formula composition (EFSA 2013, p.15-16; EFSA 2014, p.19-20). Therefore, EFSA has not recommended that the existing minimum protein amount (0.45 g/100 kJ) in infant formula should be lowered. However, EFSA (2014) considered that there was no physiological basis for infant formula to contain protein at a maximum amount of 0.7 g/100 kJ and its recommendation was to lower the maximum to 0.6 g/ 100 kJ. This recommendation would be consistent with the current evidence reviewed above.

The evidence base for lowering the protein amounts in infant formula due to potential links to overweight or obesity is uncertain. FSANZ concludes that the current Codex STAN 72-1981 range for protein amounts (which are aligned with Standard 2.9.1) is unlikely to pose a risk to infant health.

3.3.5 Conclusion – protein

Protein content was examined in relation to quantification of protein content, meeting adequate intake requirements for total protein and essential amino acids, protein quality, and current research on health effects related to protein. Comparisons to breast milk composition and the origin of current infant formula standards were also considered.

Only total protein amount was identified for closer evaluation as a possible concern owing to research suggesting that high protein intake is associated with a higher risk of childhood obesity. However, based on a review of current research, FSANZ concludes that the protein minimum and maximum should be retained at the current amounts in Standard 2.9.1 and Codex STAN 72-1981. For other aspects pertaining to protein specified in Codex STAN 72-1981 (protein-NCF, minimum amounts of essential amino acids), Codex STAN 72-1981 was found to meet all of the assessment criteria and use of these amounts is unlikely to pose a risk to infant health.

3.4 Fat

Fat provides energy and essential fatty acids (FAs) required for cell membranes. Fatty acids

are also precursors for lipid signalling molecules. Approximately 95% of fat in milk products and vegetable oils (the main sources of fat for infant formula) is present as FAs with the remainder as glycerol, phospholipids, and sterols (Jensen et al. 1991; Greenfield and Southgate 2003).

FAs can be classified according to the number of double bonds. Saturated fatty acids (SFA) have no double bonds, monounsaturated fatty acids (MUFA) have one double bond, and polyunsaturated fatty acids (PUFA) have two or more double bonds. FAs also have variable carbon chain lengths classified as short chain (SC, chain length < 6 carbon), medium chain (chain length = 6 < 20 carbons), and long chain (LC; chain length ≥ 20 carbons)[5]. Depending on the fat source, infant formula contains a mixture of these FAs where specified amounts of certain FAs are added to match breast milk composition, achieve particular ratios, or are restricted due to their presence in certain fat sources (such as cow's milk or vegetable oils).

Provisions for fat in infant formula vary considerably between Standard 2.9.1 and Codex STAN 72-1981 and include considerations of total content, amounts of essential FAs and other lipid provisions (shown schematically in SD1, Figure 4.1).

3.4.1 Fat content

The Codex STAN 72-1981 minimum fat amount is the same as that in Standard 2.9.1, but there is a marginal difference for the maximum total fat amount, which is due to rounding. Both standards are consistent with the amount of total fat reported in breast milk (Appendix 2, Table 21).

Using the Codex STAN 72-1981 midpoint of the energy content (2725 kJ/L) and the mean intake volume of 0.8 L/day for infants 0–<6 months, the estimated minimum intake is 23 g/day which is less than the ANZ AI (31 g/day). FSANZ does not consider that the difference would pose a health risk because the ANZ AI is calculated from reported concentration at the upper range of breast milk concentrations (40 g/L) rather than the average fat content of breast milk (NHMRC and MoH 2006). The estimated intake for infants 6–<12 months based on the mean volume of intake (0.6 L/day) would give 17 g/day. This would meet 50% of the AI (16 g/day) which is sufficient for this age group which is assumed to receive 50% of its nutrient intake from infant formula and 50% of from complementary foods.

As recommended in previous reviews (LSRO 1998; ANZFA 1999; EC SCF 2003), the total fat amount has been specified at the current minimum and maximum in both standards for over 20 years. The EFSA (2014) recommendation was consistent with the Codex STAN 72-1981 provisions for total fat content. Therefore, for the minimum and maximum amounts of fat, Codex STAN 72-1981 did not meet all of the assessment criteria. However, further analysis indicated that use of these amounts would be unlikely to pose a risk to infant health.

3.4.2 Source of fat

There are no provisions that prohibit the use of a particular source of fat in Standard 2.9.1 or Codex STAN 72-1981. Typically cow's milk and vegetable oils are the main sources with supplementary long chain polyunsaturated fatty acids (LC-PUFAs) added since the FA content of cow's milk and vegetable oils are primarily medium or short chain PUFAs and saturated fatty acids (SFA) (EFSA 2014). LC-PUFAs are sourced from fish oil, egg yolk lipid or oil isolated from specific algae or fungi permitted for infant formula use in Standard 2.9.1.

[5] Across the scientific literature, there is variation in the carbon chain length that is used to define fatty acids. Consistent with Standard 2.9.1, long chain fatty acids have a chain length of $\geq$ 20 carbon units and medium chain triglycerides are defined as which contain predominantly the saturated fatty acids designated by 8:0 and 10:0.

Codex STAN 72-1981 specifies that commercially hydrogenated oils and fats should not be used. This provision is consistent with the recommendation of EFSA (2014) since partial hydrogenation can increase trans-fatty acid (TFA) content. TFA content is also restricted in both Standard 2.9.1 and Codex STAN 72-1981 by a prescribed maximum amount (See Section 3.4.5).

3.4.3 Essential fatty acids

Linoleic acid (LA) and α-linolenic acid (ALA) are essential FAs that are metabolised, respectively, to n-6 and n-3 LC-PUFAs (Koletzko et al. 2008; Agostoni 2008). Arachidonic acid (AA), an n-6 LC-PUFA, and docosahexaenoic acid (DHA), an n-3 LC-PUFA, are the biologically active LC-PUFAs which may be present in infant formula. Eicosapentaenoic acid (EPA), an n-3 LC-PUFA which may also be present in infant formula, is a precursor for cell signalling molecules called eicosanoids that control inflammatory, immunological, and other cellular responses.

Codex STAN 72-1981 expresses LA and ALA in milligram amounts per energy unit whereas Standard 2.9.1, which was based on the LSRO (1998) recommendations, expresses these amounts as a percentage of total FAs (Table 9). The two standards can be compared by converting the % of total FAs using the minimum or maximum fat amounts and assuming 95% of fat is FAs (Greenfield and Southgate 2003) (See also Appendix 1).

Table 9: Amounts of essential FAs linoleic acid (LA) and α-linolenic acid (ALA)

Type of Fat	Minimum Amounts		Maximum amounts	
	Standard 2.9.1	Codex STAN 72-1981	Standard 2.9.1	Codex STAN 72-1981
Total fat (g/100 kJ)	1.05	1.05	1.5	1.4
Total FA (g/100 kJ) [1]	1.00	1.00	1.43	1.33
LA % of total FA mg/100 kJ	9 90 [2]	-- 70	26 371 [2]	-- 330 (GUL)
ALA % of total FA mg/100 kJ	1.1 11 [2]	-- 12	4 57 [2]	-- N.S.

[1] *Calculated from minimum and maximum fat amounts, assuming FA content is 95% of total fat (Greenfield and Southgate 2003).*
[2] *Calculated from the % of total FA and Total FA (g/100 kJ) shown in table.*

LA amount

The minimum amount of LA (C18:2 n-6) in Codex STAN 72-1981 (70 mg/100 kJ) is substantially less than Standard 2.9.1 (90 mg/100 kJ) (Table 9). Both values are consistent with breast milk concentrations which range from 8-17% of total fatty acids (LSRO 1998). The large range is due to the variation in total fat content and maternal intake of LA, and difficulty in measuring FAs quantitatively (Stam et al. 2013).

Standard 2.9.1 amount for minimum LA was based on the recommendation of the LSRO (1998). The Codex STAN 72-1981 minimum is the amount specified in the preceding version of Codex STAN 72-1981 and appears to be aligned with the US and EC regulations at that time. Despite ESPGHAN (1991) and the EC SCF (2003) recommendations to increase the minimum amount to 120 mg/100 kJ, Codex STAN 72-1981 has not changed and there are no published reports to indicate why the higher value was not adopted by Codex. According to EC SCF (2003) the higher amount was recommended to enable a balance between

saturated, monounsaturated and polyunsaturated fats and maintain LA at an amount that is at least 4.5% of total energy.

The Codex STAN 72-1981 maximum for LA is a GUL (330 mg/100 kJ) and was marginally increased from that recommended by EC SCF (2003) and ESPGHAN (Koletzko et al. 2005) to be consistent with established best practice for infant formula manufacturing in the United States (CCNFSDU 2006, p.11). The Standard 2.9.1 maximum (370 mg/100 kJ) is a mandatory limit but the basis for this was not found and recent recommendations (EFSA 2014) do not indicate that a mandatory limit is needed. Therefore the voluntary Codex STAN 72-1981 maximum amount for LA (which is lower than Standard 2.9.1) is unlikely to pose a risk to infant health.

ALA amount

Minimum amounts for ALA (18:3 n-3) prescribed in both standards are basically aligned (Table 9) and are approximately within reported breast milk composition ranges.

Codex STAN 72-1981 does not set a maximum amount but rather limits the amount of ALA in infant formula by imposing a maximum ratio of LA to ALA of 15:1 (see Section 3.4.3). Applying this restriction to maximum permitted amount of LA in Codex STAN 72-1981 (GUL, 330 mg/100 kJ) corresponds to a maximum amount of ALA of 22 mg/100 kJ. This amount is more restrictive than the current Standard 2.9.1 provisions for maximum ALA (57 mg/100 kJ) and therefore is unlikely to pose increased risk to infant health.

Adequate intakes for LA and ALA

Standard 2.9.1 and Codex prescribe minimum amounts for LA and ALA whereas the ANZ AI for essential FAs (g/day) was defined for dietary n-6 PUFAs (which would include LA) and n-3 PUFAs (which would include ALA) (NHMRC and MoH 2006). Therefore, the AIs cover a wider range of FAs. Because infant formula could contain a mixture of n-3 and n-6 PUFA depending on the fat source and whether preformed LC-PUFAs (AA and/or DHA) are added, comparison of the minimum LA and ALA amounts to the ANZ AIs is not straightforward.

As an indicative comparison, minimum intakes calculated from Standard 2.9.1 and Codex STAN 72-1981 minimum amounts for LA and ALA were compared to estimated intakes considered to be adequate for the majority of European infants that were recently reported by EFSA (EFSA 2013) (Table 10). An infant consuming infant formula containing the minimum LA amount in Codex STAN 72-1981 (70 mg/100 kJ) would not meet the EFSA recommendation for the 0–<6 month group but would be comparable to the recommended intake for the 6–<12 month age group. The Standard 2.9.1 minimum for LA (90 mg/100 kJ) also would not meet the EFSA AI for 0–<6 months. However, that is not the case for the 6-<12 month age group.

For ALA, both Standard 2.9.1 and Codex STAN 72-1981 are approximately aligned. Estimated intakes based on the minimum amount were less than the EFSA AI calculated for 0-<6 month age group but the differences would be within conventional rounding rules.

Amounts of LA and ALA must be balanced since the metabolic conversions of LA to AA, and ALA to DHA utilise the same enzymatic pathways. Studies suggest that excessive LA compared to the ALA amount can affect n-3 LC-PUFA (DHA and EPA) synthesis (Makrides et al. 2000a; Wood et al. 2015). The imbalance is avoided by specifying the minimum and maximum ratios of LA: ALA or with addition of DHA.

EFSA (EFSA NDA Panel 2014) used adequate intakes derived for LA and ALA (EFSA NDA Panel 2013) to recommend the minimum amounts of LA and ALA in infant formula to be 120

mg/100 kJ and 12 mg/100 kJ, respectively, reiterating the 2003 EC SCF recommendations. EFSA (2014) also recommended that preformed DHA be added to infant formula but did not recommend that a specific ratio of LA:ALA was needed (see Section 3.4.3). Therefore the higher amount of LA recommended by EFSA may be needed to ensure appropriate balance between n-6 LC-PUFA and n-3 LC-PUFA when DHA is also added.

Based on the above discussion, the evidence base for the minimum amount for LA, does not support the Codex STAN 72-1981 minimum amount but is more consistent with the current Standard 2.9.1. Therefore, FSANZ concludes that the Codex STAN 72-1981 minimum amount for LA did not meet the assessment criteria and use of this minimum could pose a risk to infant health.

The Codex STAN 72-1981 minimum for ALA, although aligned with Standard 2.9.1, also gave rise to an estimated daily intake that was lower than the requirement derived by EFSA, albeit by a marginal amount. Given the assumptions in this calculation and that, over time, infant formula is likely to contain an average amount that is greater than the minimum amount (i.e. the "worst-case" scenario used across this assessment), it can be reasonably concluded that the Codex STAN 72-1981 minimum amount for ALA is unlikely to pose a risk to infant health.

Table 10: Meeting adequate intake recommendations for LA and ALA

Fatty acid	Standard minimum[1] *(mg/100 kJ)*	Intake for 0–<6 months *(g/day)*		Intake for 6–<12 months *(g/day)*	
		Estimated from minimum [2]	EFSA AI[3]	Estimated from minimum[2]	EFSA AI[3]
LA			2.4		1.2
Standard 2.9.1	90	2.0		1.5	
Codex STAN 72-1981	70	1.5		1.1	
ALA			0.30		0.15
Standard 2.9.1	11	0.26		0.20	
Codex STAN 72-1981	12	0.26		0.20	

[1] *Standard 2.9.1 specifies minimum LA and ALA as 9% and 1.1 % of total fatty acids, respectively. Percentages were converted to mg/100 kJ using the total fat minimum (1.05 g/100 kJ), assuming 95% of fat is fatty acid (Greenfield and Southgate 2003).*

[2] *Calculated using midpoint of the energy content (2725 kJ/L) and mean volume of intake of 0.8 L/day for infants 0–<6 months and 0.6 L/day for infants 6–<12 months.*

[3] *EFSA (2013) reported adequate intakes for infants aged 0–12 months as % energy: LA = 4%, ALA = 0.5%. Conversion to g/day is shown in Appendix 1.*

Ratio of LA:ALA

LA and ALA are metabolised by common enzymic pathways to n-6 and n-3 LC-PUFAs, respectively (Gibson et al. 2011). Small ratios of LA: ALA (between 5:1 and 10:1) allows for greater endogenous synthesis of n-3 PUFA such as DHA (Makrides et al. 2000b). However, when DHA is added to formula (i.e. not relying on an infant's capacity to synthesise DHA from ALA), the LA:ALA ratio is less important as long as the balance of other LC-PUFAs are not compromised (see Section 3.4.4). For this reason, the EC SCF (2003) and EFSA (2014) considered that a specified LA:ALA ratio was needed only when formula was not supplemented with PUFAs (e.g. DHA).

The LA:ALA ratio is prescribed in both Codex STAN 72-1981 and Standard 2.9.1 to be a minimum of 5:1 and a maximum of 15:1 to permit an appropriate balance of n-6 and n-3 PUFA. The maximum ratio specified in Standard 2.9.1 was set to align with EC regulations at the time of the last review of the standard (ANZFA 1999). There is no evidence that the

LA:ALA ratio as currently specified in Standard 2.9.1 and Codex STAN 72-1981 is likely to pose a risk to infant health.

3.4.4 Addition of long chain polyunsaturated fatty acids (LC-PUFA)

Docosahexaenoic acid

Standard 2.9.1 permits the optional presence of DHA; and Codex STAN 72-1981 permits addition of DHA to infant formula as an optional ingredient.

Docosahexaenoic acid (DHA) is an n-3 LC-PUFA. DHA accumulates in the membranes of neural tissue and the retina in the first 2 years of life. DHA is present in breast milk but, depending on maternal diet and intake of the fatty acid, concentrations are highly variable ranging from about 0.1% to 1.4 % of total FAs (Brenna et al. 2007; FSANZ 2007b). DHA has been shown to be preferentially inserted into brain membranes over other LC-PUFAs (Makrides et al. 1994). Therefore, if DHA is added to infant formula, then endogenous EPA and AA also need to be present in amounts that allow balanced incorporation of these PUFAs into cells (See Section 3.4.4).

Based on a large body of observational studies on DHA supplementation in infants, there has been considerable debate about the need for a mandatory minimum of preformed DHA to support visual acuity and cognitive development in infant formula. ESPGHAN (2005) concluded that more evidence demonstrating the definitive amounts of DHA that gives rise to beneficial effects was needed and therefore, no mandatory minimum was recommended. Subsequently, several key reviews have examined the need for added DHA, with the following conclusions:

- The FSANZ (2007b) assessment of the appropriate ratio of LC-PUFAs examined supplementation of infant formula with DHA alone or in combination with AA. The assessment determined that addition of these LC-PUFA to infant formula had minimal impact on the growth and development of infants and that the only potential benefit was improvement in visual acuity, although data at the time was not sufficient to make a definitive conclusion.

- Koletzko et al. (2008) concluded that the available evidence supports the addition of DHA to infant formula at a minimum of 0.2% of total fat to support visual and cognitive development and a maximum level of 0.5% due to a lack of studies with DHA higher than this amount.

- A systematic review of 15 RCTs examining the benefit of formula supplemented with DHA alone or in combination with AA in term infants concluded that added DHA does not consistently show benefit on visual acuity, cognitive outcomes, or physical growth (Simmer 2001).

- The EFSA (2014) recommended that DHA be added to infant formula on the basis that: (1) it is an essential component of nerve and retinal cells and is involved in normal brain and visual function; (2) DHA accumulates in brain cells in the first 2 years of life; and (3) DHA measured in erythrocytes[6] of formula-fed infants more closely resembles breastfed infants when formula was supplemented with preformed DHA instead of ALA. EFSA noted that there is no convincing evidence that added DHA has benefits past infancy but that there is also a lack of studies assessing long-term developmental

[6] The fatty acid composition of erythrocyte membranes are less variable than plasma and are considered to be a better marker of dietary fatty acid status (Harris and Thomas 2010).

outcomes. On this basis it was considered prudent to include preformed DHA in infant formula in amounts similar to breast milk. Notably, inclusion of DHA without concomitant addition of AA was recently questioned (Koletzko et al. 2015).

- A recent systematic review of RCTs assessing the effects of DHA and/or AA supplemented infant formula found that none of the neurodevelopmental measures used consistently demonstrated beneficial neurocognitive outcomes (Sun et al. 2015).

On the basis of these reviews, the benefit of DHA supplementation on infant growth and development has not been established. Therefore, a mandatory minimum amount of DHA is not supported. There is no evidence that voluntary DHA addition, as currently prescribed in both Standard 2.9.1 and Codex STAN 72-1981, poses a risk to infant health.

Arachidonic acid (AA)

Arachidonic acid (AA) is an n-6 LC-PUFA derived from LA. The C20 polyunsaturated FA is present in cell membranes and is a precursor to a class of signalling compounds referred to as eicosanoids that are required for normal cell functions. AA concentrations in breast milk range from 0.24% to 1.0% of total FAs (Brenna et al. 2007) (FSANZ 2007b) and vary to a lesser extent than DHA.

Standard 2.9.1 sets a maximum amount of AA of 1% of total FAs when present in infant formula and the same maximum has been set for DHA when it is present. Codex STAN 72-1981 does not list AA as an optional ingredient but notes where DHA is added, the AA content should reach at least the same concentration as DHA. Both standards specify provisions to ensure an appropriate balance of n-6 and n-3 LC-PUFA if they are added (See Section 3.4.4). Therefore, there is a difference in how the AA restriction is expressed in the two standards but there appears to be no difference in the maximum amount of AA that could be present when applying either Standard 2.9.1 or Codex STAN 72-1981.

The EFSA (2014) considered that AA added to infant formula is unnecessary even in the presence of added DHA. The panel based their conclusion on evidence that no functional outcomes have been shown in relation to infant growth and development even when DHA supplementation of infant formula resulted in lower erythrocyte AA concentrations (suggesting DHA was preferentially incorporated into cell membranes). This conclusion was questioned in a recent opinion, which noted that clinical trials have not been conducted that demonstrate the safety of added DHA without added AA (Koletzko et al. 2015).

Ratios of DHA, AA, and LC-PUFA

To avoid imbalances of n-6 and n-3 PUFAs, Standard 2.9.1 restricts the maximum amounts and the ratio of n-6 and n-3 LC-PUFAs (Table 11). These provisions were based on the LSRO recommendations (LSRO 1998). Since then, there have been inconsistent recommendations regarding these restrictions. Although the EC SCF (2003) recommended the maximums for n-6 and n-3 LC-PUFAs (2% and 1% of total FA, respectively) which are consistent with Standard 2.9.1, these percentages were not reiterated by ESPGHAN (Koletzko et al. 2005) and were not adopted into Codex STAN 72-1981. However, EC requirements are the same as Standard 2.9.1 for maximum amounts of n-6 LC-PUFA and n-3 LC-PUFA (European Commission 2006).

If LC-PUFAs are present in infant formula, Standard 2.9.1 prescribes maximum amounts of n-6 and n-3 PUFAs and the ratio n-6:n-3 ≥ 1. The restriction is intended to apply to maximum amounts and ratios of AA and DHA. This provision resulted from a 2007 FSANZ assessment for an Application to change the Code which sought to amend the n-6:n-3 ratio from 2:1 to at least 1:1 (or n-6 ≥ n-3) (FSANZ 2007b).

If added, Codex STAN 72-1981 expresses maximum amounts for DHA and AA: the maximum limit of DHA is 0.5% of total FAs with a prescribed ratio AA ≥ DHA to avoid metabolic imbalance between n-3 LC-PUFAs and n-6 LC-PUFAs. Since AA and DHA are metabolites of n-6 LC-PUFA and n-3 LC-PUFA, respectively, the standards in this regard are effectively aligned. No restrictions on maximum amounts of n-6 or n-3 are specified.

The EFSA (2014) considered that there was no need to set a specific DHA:AA ratio and that concomitant supply of AA was also unnecessary when DHA was added. However, it has been noted that addition of DHA without AA is not supported by clinical trials and has been associated with adverse effects on growth (Koletzko et al. 2015). Therefore, in the absence of more conclusive evidence (i.e., beyond that reviewed in the previous FSANZ assessment for on PUFA in infant formula (FSANZ 2007b)) on the appropriate ratios of DHA:AA, or n-6:n-3, Codex STAN 72-1981, which is comparable to Standard 2.9.1, would be unlikely to pose a risk to infant health.

Table 11: Comparison of Standard 2.9.1 and Codex STAN 72-1981 maximum amounts and ratios for optional DHA or AA addition[1]

Standard	Maximum amounts n-6 and n-3 LC-PUFA			Maximum amounts (if present) AA and DHA		
	n-6 LC-PUFA	n-3 LC-PUFA	Ratio	AA	DHA	Ratio
	% Total FA			*% Total FA*		
Standard 2.9.1	2%	1%	n6 ≥ n3	1%	(0.5%)[2]	NS[3]
Codex STAN 72-1981	NS	NS	NS	(0.5%)[4]	0.5%	AA ≥ DHA

[1] *Values in parentheses were derived from the percentages and ratios specified in standards. Abbreviations: DHA=docosahexaenoic acid; AA=arachidonic acid; LC-PUFA= long chain polyunsaturated fatty acids; FA=fatty acids; NS=not specified*
[2] *If maximum AA (1% of total FA) is added, then maximum of 0.5% DHA can be added to meet the Standard 2.9.1 specified 2:1 ratio.*
[3] *If AA or DHA added, the restrictions specified for n-3 and n-6 LC-PUFAs apply*
[4] *If maximum 0.5% DHA is added, then AA was calculated to 0.5% since it should be present to at least the same amount to meet Codex STAN 72-1981 restriction that AA ≥DHA*.

Eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is an n-3 LC-PUFA which is a precursor to DHA and various eicosanoids (prostaglandins, thromoboxanes, and leukotrienes) involved in signalling pathways that are required for normal cell function. EPA is present in breast milk where EPA content is always less than DHA content. For this reason, both Standard 2.9.1 and Codex STAN 72-1981 specify that DHA, if added, must be present in excess of EPA (EPA<DHA). From a review of the evidence, EFSA (2014) agreed that comparison with the relative concentration in breast milk was an appropriate approach. Therefore, the Codex approach for EPA (which is aligned with Standard 2.9.1) would be unlikely to pose a risk to infant health.

Phospholipids

Codex STAN 72-1981 specifies that phospholipid (PL) amounts should be no greater than 72 mg/100 kJ whereas Standard 2.9.1 does not prescribe a maximum content amount for PLs. The EC SCF (2003) indicated that PLs can be added for technological (as an emulsifier) or nutritional (as a source of LC-PUFA) purposes. However, they specified that the PL amount should not exceed 1 g/L (equivalent to 37 mg /100 kJ based on the Codex midpoint energy content of 2725 kJ/L) regardless of the purpose of addition.

Breast milk concentrations of PLs were reported to be in the range of 0.06–2.0 g/L and were considered to be highly variable depending on the period of lactation and because of unreliable assay methods (Jensen et al. 1995, p.502). Using more reliable methodology (HPLC or NMR), more recent estimates indicate mature breast milk (i.e. >4 weeks) contains 23.8 mg total PL/100 g milk or approximately 0.24 g/L (Giuffrida et al 2013, Garcia 2012). These measurements suggest that 72 mg/100 kJ permitted in Codex STAN 72-1981 (equivalent to approximately 2 g/L using a midpoint energy content of 2725 kJ/L) may be too high.

Nutritional benefits of PLs added to infant formula have been of recent interest. Functionally, PLs are a structural component of cell membranes and are precursors to a number of molecules that are used in cellular signal transduction pathways (e.g. phosphatidylinositol 4, 5-bisphosphate). The total content of PL in cow's milk is approximately the same as that in breast milk although the concentrations of specific types of PL (e.g. phosphatidylcholine, phosphatidylethanolamine, and others) varies between species (Jensen et al. 1995, p.504). In breast milk and cow's milk, PLs are a constituent of the milk fat globule membrane (MFGM) fraction. However, infant formula lacks MFGM because this fraction is normally removed during cow's milk processing.

The potential nutritional benefits of the MFGM fraction has been examined in recent intervention trials measuring effects of infant formula supplemented with PL-containing MFGM (at concentrations equivalent to breast milk or higher) on growth or developmental outcomes (Billeaud et al. 2014; Timby et al. 2014). Although these trials suggested beneficial effects, the results are too preliminary to indicate addition of PL is nutritionally safe or beneficial (Fewtrell 2015). The EFSA (2014) considered the evidence showing the benefit of addition PL as a source of LC-PUFA instead of triacyl glycerides (LA or ALA) to be insufficient. There is no published evidence that PL addition at current permitted amount in Codex STAN 72-1981 is a problem for infants.

Given the potential bioactivity of PLs, the lack of adequate safety data, and unknown biological activity of certain types of PL in infants, FSANZ considers that the amount of PL in infant formula should not exceed the amount that normally occurs in breast or cow's milk (i.e. approximately 0.25 g/L).

3.4.5 Other lipids

Trans fatty acids (TFAs)

Both standards restrict the content of TFAs to a maximum of 4% (Standard 2.9.1) or 3% (Codex STAN 72-1981) of total FA content. Codex STAN 72-1981 also restricts the use of commercially hydrogenated oils and fats (see Section 3.4.2). The reason for the non-zero specification is to allow for TFAs that are naturally present in the milk of ruminant animals as a result of bioconversion by bacteria in the rumen. The restriction is based on reports of short- and long-term adverse health effects (EC SCF 2003; ESPGHAN 2005). Since Codex STAN 72-1981 is more restrictive than Standard 2.9.1, the Codex amount for maximum TFA is unlikely to impact adversely on infant health.

Medium chain triglycerides (MCTs)

MCTs are a mixture of triglycerides of saturated FAs, mainly caprylic acid ($C_8H_{16}O_2$; 50–80%) and capric acid ($C_{10}H_{20}O_2$; 20 – 50%) with a minor contribution of caproic ($C_6H_{12}O_2$; 1–2%) and lauric ($C_{12}H_{24}O_2$; 1–2%) FAs. They contain no less than 95 percent of saturated FAs with 8 and 10 carbon atoms (FSANZ 2006a). Historically MCTs were added to infant formula to improve absorption and digestion of fat in formula-fed infants, particularly pre-term infants with impaired fat absorption and digestion.

Standard 2.9.1 specifies that infant formula must not contain MCTs on the basis that (1) these fats are not normally present in significant amounts in breast milk; (2) the long term effects of infants consuming a relatively high amount of saturated fats are unknown; and (3) there is no convincing evidence that the inclusion of MCTs in infant formula has any benefit to infant health (ANZFA 1999; LSRO 1995). More recently, the EC SCF (2003) noted that there was no nutritional need to add MCTs to infant formula on the basis of similar arguments and, accordingly, Codex STAN 72-1981 does not specify anything about MCTs. EFSA (2014) reiterated this conclusion.

Standard 2.9.1 permits the presence of MCTs when they are used as a processing aid in preparations of fat soluble vitamins. This was a result of Application 563 *Medium Chain Triglycerides in Infant Formula* Products (FSANZ 2006a). The nutritional and safety assessment determined that MCTs used as a processing aid were not a safety risk to infants since their concentration would be increased by no more than 0.002% (w/w) of the total fat content of infant formula and therefore present in very low amounts.

Therefore the current evidence and expert recommendations support the provisions for MCTs as specified in Standard 2.9.1.

Myristic and lauric acids

Myristic (14 carbon chain) and lauric (12 carbon chain) acids are medium chain saturated FAs that have variable concentrations in breast milk (approximately 6-15% of total FAs) depending on maternal diet (Jensen et al. 1995). Standard 2.9.1 does not restrict the content of myristic and lauric acids whereas Codex STAN 72-1981 restricts these to a maximum of 20% of total FAs. This restriction appears to be retained from previous EU regulations set in the 1980s where the rational was based on the cholesterol-elevating effects observed in adults with these saturated fats (EC SCF 2003).

The EFSA (2014) considered that there was no evidence to impose restrictions on levels of myristic and lauric acids on the basis that (1) by comparison, breast milk contains higher concentrations of palmitic acid (approximately 20–25% of total FAs), which also increases cholesterol; and (2) plasma cholesterol is higher in breast-fed than in formula-fed infants but there is no evidence of long-term adverse health effects. Therefore the current evidence does not support the Codex restriction on the amount of the myristic and lauric acids.

Monounsaturated fatty acids

Only erucic acid, which is a 22-carbon chain monounsaturated FA, has been restricted in infant formula due to its presence in relatively high concentrations in vegetable oils and potential adverse health effects (FSANZ 2003). Both Standard 2.9.1 and Codex STAN 72-1981 specify erucic acid content to be no more than 1% of total FA content. Although EFSA (2014) concluded that infant formula made from vegetable oils containing erucic is safe from a toxicological point of view, there is no additional indication that the restriction for erucic acid should be removed.

3.4.6 Conclusion – fat

Fat composition of infant formula includes consideration of total content, and amounts of essential lipids linoleic acid (LA) and α-linolenic acid (ALA) as well as numerous provisions for other lipids. There is considerable variation between Standard 2.9.1 and Codex STAN 72-1981, however, most of the differences are minor. Codex STAN 72-1981 did not meet some of the assessment criteria (e.g. estimated total fat intake based on the minimum amount did not meet the ANZ AI) but based on current evidence, these would be unlikely to pose risk to

infant health. Suggestions for a mandatory minimum content of DHA were found to be based on mixed and inconclusive studies on infant development.

The evidence base for the minimum amount of LA did not support the Codex STAN 72-1981 amount but was more consistent with the current Standard 2.9.1 provision for this nutrient. Therefore, FSANZ concludes that the Codex STAN 72-1981 minimum LA amount did not meet the assessment criteria and further analysis indicated that use of this minimum could pose a risk to infant health.

3.5 Carbohydrates

Infant formula provisions for carbohydrate were examined in relation total carbohydrate content and restrictions for certain types of carbohydrates.

3.5.1 Carbohydrate content

Carbohydrate content is not specified in Standard 2.9.1. Instead, the amount of carbohydrate is derived from the difference between the total energy amount and the energy equivalents for the amounts of protein and fat content present. The determination of carbohydrate content by this procedure was consistent with Codex regulations at the time of the preceding review of Standard 2.9 1 (ANZFA 1999).

Codex has now prescribed minimum and maximum carbohydrate amounts based on a similar difference calculation (EC SCF 2003, p.85). Using minimum and maximum amounts of energy, fat and protein, the calculated carbohydrate content in Codex STAN 72-1981 ranged from 36–53% of energy or 2.1–3.1 g/100 kJ, which was comparable to the amount specified by Codex STAN 72-1981 (2.2–3.3 g/100 kJ). Calculated carbohydrate amounts based on current Standard 2.9.1 provisions for energy, fat, and protein were also comparable to the Codex STAN 72-1981 amount (33-53% energy or 1.9–3.1 g/100 kJ) (Table 12). Small differences between the two standards are due to the slightly higher maximum fat content in Standard 2.9.1 and to rounding in calculations. Therefore, the two standards can be considered to be effectively aligned for minimum and maximum carbohydrate amounts.

Table 12: Calculated carbohydrate amount based on fat and protein content in Standard 2.9.1 and Codex STAN 72-1981

Macronutrient		**Carbohydrate amounts**[1]							
		Standard 2.9.1				***Codex STAN 72-1981***			
Fat	g/100 kJ **% energy**	1.05 (min) **38.9**		1.5 (max) **55.5**		1.05 (min) **38.9**		1.4 (max) **51.8**	
Protein Intact cow's milk protein	g/100 kJ **% energy**	0.45 (min) **7.7**	0.7 (max) **11.9**	0.45 (min) **7.7**	0.7 (max) **11.9**	0.45 (min) **7.7**	0.7 (max) **11.9**	0.45 (min) **7.7**	0.7 (max) **11.9**
Calculated Carbohydrate Amount	g/100kJ **% energy**	3.1 **53.4**	2.9 **49.2**	2.2 **36.8**	1.9 **32.6**	3.1 **53.4**	2.9 **49.2**	2.4 **40.5**	2.1 **36.3**

[1] *Amounts as a percentage of energy (in bold red) were calculated using the conversion factors: 1 g fat = 37 kJ, 1 g carbohydrate = 17 kJ, 1 g protein = 17 kJ (Standard 1.2.8). % energy values shown above differ slightly from those in EC SCF (2003) due to rounding in the conversion from kcal to kJ. Abbreviations: Min = minimum, max = maximum.*

The average carbohydrate content in formula in Codex STAN 72-1981 is within the range of carbohydrate concentration measured in breast milk. An infant consuming infant formula containing the Codex STAN 72-1981 minimum amount equates to an intake of 60 g/day based on the midpoint of the energy content (2725 kJ/L) and mean volume of intake (0.8

L/day), and 45 g/day for 6–<12 months consuming a mean volume of 0.6 L/day. Intake based on the minimum carbohydrate amount would meet the ANZ AI (NHMRC and MoH 2006) for infants 0-<6 months (60 g/day) but is slightly less that the 50% of the AI for infants 6-<12 months (48 g/day). However, given the approximations in deriving this estimate, use of the Codex STAN 72-1981 minimum carbohydrate amount is unlikely to pose a risk to infant health.

3.5.2 Types of carbohydrates

Neither Standard 2.9.1 nor Codex STAN 72-1981 set mandatory requirements for types of carbohydrate. However, certain types of carbohydrate are restricted in Codex STAN 72-1981 by defining the preferred source or setting guidance limits:

- Lactose and glucose polymers are the preferred source in formulas based on cow's milk protein.
- Pre-cooked and/or gelatinised starches gluten-free by nature can be added up to a maximum of 30% of total carbohydrates and up to 2 g/100 mL
- Addition of sucrose and fructose should be avoided

According to the EC SCF (2003), the basis for the restrictions is:

- potential benefits of lactose on gut physiology, gut microflora, stool consistency
- potential adverse effects of glucose on increased osmolality
- potential adverse effects of sucrose and fructose for infants with hereditary fructose intolerance.

In the EU, only lactose, maltose, sucrose, glucose, malto-dextrins, glucose syrup or dried glucose syrup, pre-cooked starch or gelatinised starch (naturally free of gluten) are permitted types of carbohydrate types. Lactose content is specified as being a minimum of 50% of the total carbohydrate content. In addition, the guidance limits for lactose, glucose polymers and pre-cooked gelatine have been adopted as mandatory requirement.

Digestible carbohydrates

Digestible carbohydrates are starch or sugars that are digested and absorbed in the small intestine to be utilised as an energy source. Digestible carbohydrates have also been termed "available carbohydrate" to distinguish these carbohydrates from so-called non-digestible (or unavailable) carbohydrate (see below) which are generally metabolised through fermentation in the large bowel (FAO 1998).

Lactose, glucose, sucrose, and certain starches are the main digestible carbohydrates in infant formula. Breast milk contains approximately 60 g/L of lactose over the whole course of lactation (Mitoulas et al. 2002). Infant formula based on cow's milk also contains lactose as the main carbohydrate (approximately 50 g/L lactose). Sucrose and fructose and starch are not present in breast milk and are mainly used in non-cow's milk based formula products or formulas containing protein hydrolysates which may have a bitter taste.

As recently reviewed (Stephen et al. 2012), there are limited studies in infants aged 0–<12 months that have examined the relationship between digestible carbohydrates in infant formula and increased risk of health outcomes such as obesity, diabetes, and dental caries. As a result, there does not appear to be a strong evidence base for the mandatory restrictions adopted by EU.

EFSA (2014) agreed with Codex STAN 72-1981 guidance limits with regard to lactose, glucose, sucrose, fructose, and starches and indicated that substituting lactose with other

carbohydrates is permitted for special types of formula (e.g. low lactose). Therefore, Codex STAN 72-1981 requirements for digestible carbohydrate type are in line with recent expert considerations and are unlikely to pose a risk to infant health.

Non-digestible carbohydrates

Non-digestible carbohydrates in infant formula are oligosaccharides which are not absorbed in the small intestine and enter the large intestine where they may have beneficial effects on the growth of certain bacteria. Approximately 200 human milk oligosaccharides have been identified (Ninonuevo et al. 2006) with a combined concentration of 10-15 g/L compared to 0.05–0.08 g/L in bovine milk.

The use of so-called 'pre-biotic' carbohydrates in infant formula is an area of current research (Donovan et al. 2012; Jacobi and Odle 2012). Codex has no special provisions for addition of oligosaccharides to infant formula other than general provisions permitting optional ingredients provided that their safety and suitability are scientifically demonstrated. The outcome of a recent FSANZ assessment for the addition of inulin-type fructans and galacto-oligosaccharides (FOS and GOS) to infant formula products led to permissions to add these oligosaccharides up to defined maximum amounts (FSANZ 2013b). Evaluation of the science around addition of other oligosaccharides to IF is not within the scope of this assessment.

3.5.3 Conclusion – carbohydrate

Minimum and maximum amounts of carbohydrate in Codex STAN 72-1981 are effectively aligned with Standard 2.9.1. Indeed, these limits are simply the result of numerical calculations relating to the mandatory limits on energy, protein and fat, and so are unnecessary. Codex STAN 72-1981 includes some guidance limits on types of digestible carbohydrate which is consistent with current expert opinion. However there does not appear to be any physiological evidence to indicate that these voluntary limits should be set as mandatory restrictions.

3.6 Vitamins

Provisions for vitamins include minimum amounts, maximum amounts (either as a mandatory or voluntary limit), and in some cases, permitted forms and conversion factors. Calculated data comparing Codex STAN 72-1981 and Standard 2.9.1 to breast milk concentrations and estimated intakes are reported in Appendix 2 with main outcomes summarised in Table 13.

For some vitamins (vitamin K, thiamin, riboflavin, pantothenic acid, vitamin B12, and biotin), Codex STAN 72-1981 met all of the assessment criteria, i.e. amounts were comparable to Standard 2.9.1 amounts and were within the range of breast milk concentrations, estimated minimum and maximum intakes were appropriate to meet AI or did not exceed UL, there was an extended history of safe use, and there was no scientific evidence that the amount of these vitamins should be changed. For these vitamins, therefore, no further analysis was conducted.

For the remaining vitamins (vitamin A, vitamin D, vitamin E, vitamin C, niacin, vitamin B6 and, folate), some provisions met some of the assessment criteria but issues were identified with aspects of Codex STAN 72-1981 and these are explained in the subsequent sections.

Table 13: Outcomes of the comparative analysis: vitamins[1]

Vitamin	Provision(s) that met assessment criteria	Further analysis described in Section 3.6
Vitamin A	Minimum amount	Permitted forms and calculation, maximum amounts
Vitamin D	Maximum amount	Minimum amount and permitted forms
Vitamin E	Permitted forms	Minimum and maximum amounts, ratios to PUFA
Vitamin K	Minimum and maximum amounts, permitted forms	No further analysis
Vitamin C	Minimum amount, permitted forms	Maximum amount
Thiamin	Minimum and maximum amounts, permitted forms	No further analysis
Riboflavin	Minimum and maximum amounts, permitted forms	No further analysis
Niacin	None	Minimum and maximum amount and permitted forms
Vitamin B6	Minimum amount, permitted forms	Basis for maximum as a guidance level
Folate	None	Minimum and maximum amounts
Pantothenic acid	Minimum and maximum amounts, permitted forms	No further analysis
Vitamin B12	Minimum and maximum amounts, permitted forms	No further analysis
Biotin	Minimum and maximum amounts, permitted forms	No further analysis

[1] *Vitamins shaded in grey met all the assessment criteria and are not discussed further in Section 3.6. See Appendix 2 for numerical data.*

3.6.1 Vitamin A

Permitted forms and calculation of vitamin A content

There are two types of dietary sources of vitamin A: (1) preformed vitamin A which are retinyl esters, retinoic acid, and retinol and are obtained from animal food sources; and (2) provitamin A carotenoids (e.g. β-carotene) which are obtained from plant food sources. The amount of vitamin A in a food is calculated as retinol equivalents (RE) using the internationally accepted conversion:

1 µg RE = 3.33 IU Vitamin A = 1 µg all-trans retinol

The permitted forms of vitamin A in Standard 2.9.1 are specified as retinol, retinyl acetate, retinyl palmitate, and retinyl propionate, and β-carotene. Although other carotenoids have provitamin A activity, only β-carotene can be added to infant formula. Since several groups have noted that uncertainties exist regarding the bioavailability of β-carotene in infants (IOM 2001; EC SCF 2003), Codex STAN 72-1981 specifies that only preformed vitamin A is counted in the calculation of vitamin A activity of formula (using the conversion shown above).

Standard 2.9.1 permits β-carotene as a source of vitamin A but does not specify the conversion factors for calculating RE from β-carotene in infant formula. Since the current vitamin A requirements in Standard 2.9.1 appear to pre-date the previous review (ANZFA

1999) it seems that the intention was to allow β-carotene to contribute to the vitamin A content of infant formula.

The Codex STAN 72-1981 specification to calculate vitamin A content solely from preformed vitamin A can be regarded as more prescriptive than the current Standard 2.9.1 and, therefore, is unlikely to pose a risk to infant health.

Minimum

For infants aged 0–<6 months, the AI (250 µg/day) relates to preformed vitamin A only (i.e. as retinyl esters) and does not include β-carotene. The AI for infants aged 6–<12 months (430 µg/day) was calculated from vitamin A intake from breast milk plus vitamin A from complementary foods (i.e. as retinol equivalents) which includes some carotenes.

For most of the vitamins, comparing the estimated intake from the Codex STAN 72-1981 minimum amount to the AI (see Appendix 2) relates only to the contribution from infant formula in the calculations (see example calculation in Appendix 1). However, since the basis for the vitamin A AI is different for the older infants (NHMRC and MoH 2006), the assumption normally used in this assessment to estimate intake for infants aged 6–<12 months (i.e. receive 50% of their nutrient intake from formula and 50% from complementary foods) was not used. Instead, the estimated minimum intake was compared to the AI value that was reported to be from breast milk intake alone (186 µg/day) (NHMRC and MoH 2006).

Therefore, for both age groups, the minimum Codex STAN 72-1981 amount (which is aligned with Standard 2.9.1) was determined to meet the AI and therefore, is unlikely to pose a risk to infant health.

Maximum

Standard 2.9.1 and Codex STAN 72-1981 are aligned for the maximum amount of vitamin A (43 µg RE/100 kJ). Based on the midpoint of the Codex STAN 72-1981 energy content (2725 kJ/L) and mean volume of intake (0.8 L/day), the maximum amount would yield an intake of 937 µg/day for infants aged 0–<6 months. Infants aged 6-<12 months would have an estimated intake of 703 µg/day based on a mean volume of 0.6 L/day. The UL for preformed vitamin A is 600 µg/day foods (IOM 2001; NHMRC and MoH 2006), which equates to 300 µg /day from infant formula for infant 6–<12 months who obtain 50% of nutrients from formula and 50% from complementary foods. Therefore, based on the midpoint energy content, the maximum estimated intake from formula for both age groups potentially exceeds the UL.

The NHMRC and MoH UL was derived from the lowest-observed-adverse-effect-level (LOAEL) determined to be approximately 6000 µg/day based on symptoms of vitamin A toxicity (bulging fontanels) occurring in four infants receiving doses of 5500–60,000 µg/day of vitamin A from drops or chicken livers in addition to their usual intake for 1–3 months. An uncertainty factor of 10 was applied to account for the uncertainty in deriving a NOAEL from a LOAEL determined from a small number of subjects with a non-severe and reversible adverse effect, and variability in the presentation of other symptoms (IOM 2001). For these reasons, the UL can be considered to be conservative.

Vitamin A concentrations in breast milk are highly variable depending on the mother's diet. The EC SCF (2003) reported preformed vitamin A concentration in a range of 150–1100 µg/L across international populations. In a multinational study by Canfield et al (2003), breast milk concentrations (expressed as retinol) ranged from 298–464 µg/L. Breast milk from Australian mothers was at the lower end of this range (310 µg/L) as measured in singleton infants (mean age of 121 days) whose mothers consumed at least 3 serves of fruit and vegetables per day.

A study that measured vitamin A concentrations in infant formulas sold in Europe (and therefore formulated according to Codex STAN 72-1981) showed that vitamin A was in the range of 27–36 µg/100 kJ. The authors indicated that the vitamin A concentrations were not associated with observed adverse effects (MacLean et al. 2010). Based on this measured concentration range, which is probably comparable to formula sold in ANZ, intakes from formula would range from 589–785 µg/day and 441–589 µg /day for 0–<6 and 6–<12 months of age, respectively. Although these intake ranges still exceed the UL set by the NHMRC and MoH, given the conservative basis for the UL, they are unlikely to pose a risk to infant health.

International recommendations for the maximum vitamin A amount have been in place since at least 1998 (LSRO 1998). The EC SCF report (2003) does not comment on the potential exceedance of the UL for vitamin A. Therefore, the maximum vitamin A amount in Codex STAN 72-1981 (which is the same as Standard 2.9.1) gives rise to an estimated intake that potentially exceeds the UL. However, the estimate is based on the maximum amount and represents an amount that is unlikely to occur continuously over the period of formula feeding. Aside from the potential exceedance of the UL, there is no additional evidence that supports lowering the maximum from the current Standard 2.9.1 and Codex STAN 72-1981 amount. The vitamin A maximum of 43 µg RE/100 kJ is unlikely to pose a risk to infant health.

3.6.2 Vitamin D

Minimum

Standard 2.9.1 and Codex STAN 72-1981 are aligned for the minimum amount of vitamin D (0.25 µg/100 kJ). Both standards are unchanged from long-standing international recommendations which have resulted in the elimination of nutritional rickets in formula-fed infants (LSRO 1998).

Measured breast milk concentrations of vitamin D are highly variable and generally very low (<1 µg/L) even when sourced from vitamin D-replete mothers (Munns et al. 2006). It is generally recognised that exclusively breastfed infants with minimal exposure to sunlight do not obtain adequate vitamin D (NHMRC and MoH 2006). In Australia, vitamin D deficiency rickets in infants is rare, although a higher incidence of vitamin D-deficiency rickets has been measured in high risk children (such as those of mothers with dark skin or those that wear veils) (NHMRC 2013). Several ANZ studies have demonstrated that breastfed infants are at greater risk of vitamin D deficiency than formula-fed infants as measured by serum concentration of 25-hydroxy vitamin D (25OHD), the accepted biomarker of vitamin D status (Thomson et al. 2004; Grant et al. 2009; Munns et al. 2012; Wall et al. 2013). Therefore breast milk is not a reliable comparison for vitamin D content in infant formula.

Based on the midpoint of the Codex STAN 72-1981 energy range (2725 kJ/L) and a mean intake volume of 0.8 L/day, the minimum vitamin D amount corresponds to 5.5 µg/day for infants aged 0–<6 months. For infants 6–<12 months consuming 0.6 L/day, the estimated intake would be 4.1 µg/day. These amounts would exceed the ANZ AI (5.0 µg/day) (NHMRC and MoH, 2006) for younger infants and 50% (2.5 µg/day) of the AI for infants aged 6–<12 months old.

International expert panels have recently updated recommended intakes for vitamin D for infants 0–6 months old to 10 µg/day (IOM 2011; Braegger et al. 2013; EFSA 2013), assuming minimal sun exposure. To meet this recommendation, the minimum vitamin D amount in infant formula would need to be increased to 0.45 µg/100 kJ which is almost double the existing minimum. Infant formula sold in Australia was found to contain 9–13 µg/L vitamin D (equates to 0.33–0.48 µg/100 kJ), giving rise to an intake of 7.2–10.4 µg/day

(Paxton et al. 2013). As there were no blood measurements, the additional contribution of sunlight to vitamin D status of these infants is unknown.

Therefore, for the minimum vitamin D amount, Codex STAN 72-1981 (and Standard 2.9.1) did not meet the assessment criteria due to new recommended intakes. However, based on the considerations above, FSANZ concludes that the current minimum for vitamin D specified by both standards would be unlikely to pose a risk to infant health.

Permitted forms

Permitted forms of vitamin D in Standard 2.9.1 are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) whereas Codex STAN 72-1981 lists only the D_3 form. The EC SCF (2003) recommended vitamin D_3 because the bioavailability of vitamin D_2 (which is the plant-based form of vitamin D) in infants is unknown. FSANZ recently considered the evidence for the equivalence of vitamin D_2 and vitamin D_3 in relation to fortification of breakfast cereals and concluded that both forms are equally effective in raising the serum 25OHD concentration up to intake amounts of 25 µg/day when present in foods (FSANZ 2015). Although none of the studies in the FSANZ analysis were conducted in infants, a recent study showed serum 25OHD did not differ between breastfed infants receiving supplemental vitamin D_2 or vitamin D_3 (10 µg/day) for 1–4 months (Gallo et al. 2013). This study suggests the use of vitamin D_2 is unlikely to pose a risk to infant health.

3.6.3 Vitamin E

Minimum

The minimum amount of vitamin E[7] in Standard 2.9.1 is 0.11 mg/100 kJ which is nearly the same as that in Codex STAN 72-1981 (0.12 mg α-TE/100 kJ). The Codex STAN 72-1981 minimum corresponds to 3.3 mg/L based on the midpoint energy content of infant formula (2725 kJ/L) and is within the range of breast milk concentration of 2–5 mg/L (EC SCF 2003).

EFSA (2014) recommended minimum vitamin E amount that is marginally higher than the Codex minimum (0.14 mg α-TE/100 kJ as RRR-α-tocopherol).

For infants aged 0–<6 months, the Codex STAN 72-1981 minimum amount corresponds to a daily intake of 2.6 µg/day based on the mean volume of intake (0.8 L/day) and is less than the ANZ AI of 4 mg/day (as α-TE), but is closer to the recent EFSA recommendation of 3 mg α-TE /day (EFSA 2013). For infants 6–<12 months, the Codex STAN 72-1981 minimum amount corresponds to an intake of 2 mg/day which also does not meet the AI where nutrient intake from formula is assumed to meet 50% of the AI (i.e. 2.5 mg/day).

There is no evidence that ANZ infants have inadequate vitamin E status. The minimum amount is unchanged from LSRO (1998) recommendations indicating there is an extended period of safe use with this amount. Therefore, despite not meeting the vitamin E AI, use of the Codex minimum (0.12 mg/100 kJ) would be unlikely to pose a risk to infant health.

[7] Where vitamin E is assumed to be defined in terms of α-tocopherol equivalents (α-TE) and 100% active. The naturally occurring d-α-tocopherol (or RRR-α-tocopherol) is considered to be the only form that contributes towards meeting the vitamin E requirements because the other naturally occurring forms are "recognized poorly by the α-tocopherol transfer protein" in the liver (IOM, 2000). Synthetic forms are expressed as α-TE but these forms have a lower biologically activity than d-α-tocopherol.

Ratios of minimum vitamin E amount to dietary PUFA

Double bonds in dietary PUFA (e.g. LA and ALA) and LC-PUFA (e.g. AA, EPA, DHA) can be oxidised to the corresponding hydroperoxides, which can react with other substances in infant formula and with an infant's blood components or tissues. Vitamin E prevents oxidation of PUFA and LC-PUFA in formulas, but since vitamin E is consumed in the process, it needs to be present in amounts that can safely meet vitamin E requirements and allow for losses due to oxidation (Arab-Tehrany et al. 2012). Thus, the minimum vitamin E amount may be increased depending on the number of double bonds contained in the dietary fatty acid supply, particularly if formula is supplemented with optional LC-PUFA (see Section 3.4.4).

Standard 2.9.1 specifies that infant formula to contain a minimum of 0.5 mg vitamin E/g PUFA whereas Codex STAN 72-1981 expresses this as 0.5 mg α-TE/g PUFA.[8] Codex STAN 72-1981 also specifies the following minimum vitamin E minimum in relation to the number of FA double bonds (EC SCF 2003):

- 0.5 mg α-TE/g linoleic acid (18:2 n-6);
- 0.75 mg α-TE/g α-linolenic acid (18:3 n-3);
- 1.0 mg α-TE/g arachidonic acid (20:4 n-6);
- 1.25 mg α-TE/g eicosapentaenoic acid (20:5 n-3);
- 1.5 mg α-TE/g docosahexaenoic acid (22:6 n-3).

These ratios were calculated on the basis of the vitamin E content of 0.5 mg α-TE /g linoleic acid where linoleic acid contains two carbon-carbon double bonds (Aggett et al. 1998). The fatty acid content of the diet, and therefore these ratios, were not included when setting nutrient reference values for vitamin E in ANZ (NHMRC and MoH 2006).

Appendix 4 (Tables 26.1-26.4) show the calculated vitamin E amount that would be needed to compensate for the potential losses due to PUFA oxidation under the Standard 2.9.1 conversion and the Codex conversion. Several scenarios were applied: (1) vitamin E amounts calculated for typical vegetable oils used in IF without addition of DHA (Table 26.1); (2) vitamin E amounts calculated for typical vegetable oils plus addition of DHA, AA and EPA (according to Codex STAN 72-1981 restrictions) (Table 26.2); (3) vitamin E amounts calculated for infant formula containing minimum amounts of LA and ALA with and without addition of DHA, AA, EPA; and (4) vitamin E amounts calculated for a commercial brand of infant formula which provided sufficient level information (i.e. amounts of individual PUFAs) to carry out the conversion.

In all scenarios, there was a very small difference between using the Standard 2.9.1 conversion for α-TE /PUFA amount compared to the Codex STAN 72-1981 conversion in the amount of vitamin E needed to compensate for PUFA oxidation. The Codex conversion generally gave higher vitamin E amounts with the greatest difference calculated to be 0.04 mg/100 kJ (see Table 26.2; maximum fat content using soybean oil as source with maximum addition of DHA). Given that the prescribe range of vitamin E is 0.012–1.2 mg/100 kJ in Codex STAN 72-1981, it is unlikely that the that application of the Codex STAN 72-1981 conversion factors for vitamin E would provide any effect in terms of risk to infant health.

A recent German study measured the amount of vitamin E (as α-TE) in commercially available infant formulas that was either supplemented or not with LC-PUFA and compared the results with vitamin E amounts in breast milk (Stimming et al. 2014). All formulas were found to contain at least the minimum amount of 0.5 mg α-TE/g PUFA as specified in Codex

[8] SD1 Section 7.2.3 discusses dietary equivalencies for naturally occurring tocopherols and synthetic homologues that are specified in Standard 2.9.1 and Codex STAN 72-1981

STAN 72-1981 although PUFA-supplemented formula was found to be consistently lower in vitamin E (total and α-TE) than unsupplemented formula or breast milk, thus suggesting that increasing the vitamin E amount in PUFA-supplemented infant formula could be needed.

Therefore, Standard 2.9.1 and Codex STAN 72-1981 differ substantially for the ratio of minimum vitamin E to PUFA. However, calculations based on several scenarios indicate that application of the Codex STAN 72-1981 conversions for vitamin E in relation to the number of FA double bonds makes only a marginal difference in the amount of vitamin E needed to be present compared to application of the Standard 2.9.1 conversion. Therefore use of the Codex standard to calculate vitamin E in relation to FA double bonds is unlikely to pose a risk to infant health but there is limited evidence to indicate that different factors depending on the number of PUFA double bonds is warranted.

Maximum

The maximum amount for vitamin E is aligned for Codex STAN 72-1981 and Standard 2.9.1 and is based on the recommendations of the LSRO (1998). The Codex STAN 72-1981 amount, however, is a guidance limit whereas Standard 2.9.1 has set a mandatory maximum amount. No basis for this difference could be determined. Based on the long history of safe use for this amount and no new evidence of vitamin E toxicity in infants, it is reasonable to conclude that having a guidance limit rather than a mandatory maximum for vitamin E would be unlikely to pose a risk to infant health.

3.6.4 Vitamin C

Maximum

The maximum amount of vitamin C specified by Standard 2.9.1 (5.4 mg/100 kJ) is lower than that specified by Codex STAN 72-1981 (17 mg/100 kJ). The higher amount was set in Codex STAN 72-1981 to allow for degradation of vitamin C when formula is reconstituted with water at high temperature (CCNFSDU 2006).

A survey of over 27,000 samples of infant formula (liquid and powder) sold in Europe showed a wide range of means for vitamin C content (3.6-9.3 mg/100 kJ) (Maclean et al. 2010). The range of means + 2SD (4.3-17.2 mg/100 kJ) indicated that a few samples exceeded the Codex STAN 72-1981 maximum amount. Vitamin C in liquid infant formula is chemically labile with 30–50% reported to be lost over the shelf life. Vitamin C in powdered infant formula is more stable but also degrades when exposed to air.

Infants aged 0–<6 months consuming 0.8 L/day of infant formula containing the Codex STAN 72-1981 maximum amount (17 mg/100 kJ) and the midpoint of the Codex STAN 72-1981 energy content (2725 kJ/L) gave an estimated intake of 371 mg/day. For infants aged 6–<12 months consuming a mean volume of 0.6 L/day, the estimated vitamin C intake would be 278 mg/day.

No ANZ UL has been set for vitamin C for infants aged 0–12 months. The IOM recommended a UL of 400 mg/day for children 1–3 years of age (IOM 2000). Thus, the estimated intake from the Codex STAN 72-1981 maximum could exceed the IOM recommendation when adjusted for the lower body weight of infants aged 0–<12 months. Additionally the EC SCF (2003) and ESPGHAN (Koletzko et al. 2005) also recommended a maximum amount (7.2 mg/100 kJ) based on the reported NOAEL for vitamin C in an adult male and extrapolating to a body mass equivalent intake for infants. None of these recommendations for maximum amounts account for the rapid degradation of vitamin C in liquid or powder infant formula.

There is no evidence that formula-fed infants consume unsafe amounts of vitamin C. Therefore, the Codex STAN 72-1981 maximum for vitamin C did not meet the assessment criteria but further analysis indicated that use of this maximum would be unlikely to pose a risk to infant health

3.6.5 Niacin (preformed)

Maximum amount and permitted forms

The two forms of niacin present in foods – nicotinic acid and nicotinamide – are referred to as preformed niacin. Nicotinic acid and nicotinamide are both precursors for nicotinamide nucleotides which are dehydrogenase cofactors involved with energy metabolism. In humans, nicotinamide can also be synthesised (i.e. not preformed niacin) from tryptophan. Niacin requirements therefore are sometimes expressed as niacin equivalents (NE) which take into account the two forms of niacin and conversion of tryptophan to niacin.

Standard 2.9.1 only permits the use of niacinamide (nicotinamide) whereas Codex STAN 72-1981 lists both nicotinic acid and nicotinamide. The adverse effects associated with excessive intake of nicotinic acid and nicotinamide are different. The LSRO report (1998) reported vascular dilation or flushing, hyperuricemia, hepatic and ocular abnormalities, and occasional hyperglycaemia that occur with high doses of nicotinic acid (amounts not given) but not with niacinamide. On this basis they recommended that risks to the health and safety of infants from nicotinic acid should be assessed before use in infant formula. The EC SCF (2003) and the EFSA (2014) reiterated this evidence and recommended that only nicotinamide be used in infant formula. Despite these recommendations, the basis for the permitted use of nicotinic acid in Codex STAN 72-1981 could not be identified, and permission to use this form probably extends back to an earlier EC SCF report (Scientific Committee for Food 1983). EC regulations also permit both nicotinic acid and nicotinamide (European Commission 2006).

There are no ULs for preformed niacin, niacinamide, or nicotinic acid for infants. However, the EC SCF has defined ULs for 1–3 year-old children as 2 mg/day and 150 mg/day for nicotinic acid and nicotinamide, respectively (EC SCF 2002). These were derived from the adult ULs by extrapolating on a body weight basis, where the adult UL for nicotinic acid (10 mg/day) was based on occasional flushing (a minor health effect possibly related to transient hypotensive episodes in elderly) occurring with 30 mg per day, and applying an uncertainty factor of 3. Nicotinamide does not produce the adverse flushing response observed with nicotinic acid and, therefore, is less toxic and the UL is higher. Extrapolating on a body weight basis, the ULs for infants aged 0–<12 months would likely be less than the amounts set for 1–3 year olds.

The maximum amount of preformed niacin (as nicotinamide only) in Standard 2.9.1 (480 µg/100 kJ) is substantially higher than Codex STAN 72-1981 (360 µg/100 kJ, as nicotinamide or nicotinic acid). Using the midpoint of the energy content (2725 kJ/L) and the mean volumes of intakes, the Codex STAN 72-1981 maximum amount corresponds to an estimated intake of 7.8 mg/day for infants aged 0–<6 months and 5.9 mg/day for infants 6–<12 months. Neither of these estimated intakes approaches the 150 mg/day nicotinamide UL for 1–3 year olds. However, estimated intakes for both age groups would exceed the UL for nicotinic acid if that form was used.

The no observed adverse effect level (NOAEL) for nicotinic acid from a 28-day oral study in rats was determined to be 50 mg/kg/day (INCHEM 2005). An uncertainty factor (UF) of 100 was used to calculate the estimated dose of low concern (EDLC) for humans of 0.5 mg/kg/day. Using the average of reference weights for boys and girls aged 0–<6 months (6.0 kg) and 6–<12 months (9.0 kg) (NHMRC and MoH 2006) the EDLC corresponds to 3.0

mg/day and 4.5 mg/day, respectively. Thus, estimated intakes from the Codex STAN 72-1981 maximum also exceed an EDLC derived for infants.

In summary, the GUL in Codex STAN 72-1981 is lower than in Standard 2.9.1 and would be unlikely to pose a risk to infant health. However, Codex STAN 72-1981 lists the use of the nicotinic acid which does not have the same safety profile as that of nicotinamide and nicotinic acid intakes estimated from the Codex STAN 72-1981 maximum exceed recommendations for maximum intake amounts (i.e. UL or EDLC). There is no evidence to indicate that infant formula containing nicotinic acid has caused adverse effects in infants and this could imply either a history of safe use or that this form is not used. But given that nicotinamide is less toxic than nicotinic acid and serves the same biological function, it is preferable to use nicotinamide rather than nicotinic acid in infant formula. Therefore, the use of nicotinic acid as a form of niacin in infant formula as per Codex STAN 72-1981 does not meet the assessment criteria and it is concluded that use of this form may pose a risk to infant health

Minimum

The Standard 2.9.1 minimum niacin amount (130 µg/100 kJ) is almost twice than that set in Codex STAN 72-1981 (70 µg/100 kJ). The amount listed in Standard 2.9.1 originates from the LSRO (1998) recommendation which was based on niacin intakes in breastfed infants and includes the tryptophan contribution. The Codex STAN 72-1981 minimum amount appears to be based on the breast milk concentration of niacin, but excludes the contribution from tryptophan.

As noted by the NHMRC and MoH (2006), breast milk tryptophan concentration can be high but only 1/60th of the amount of the dietary intake of tryptophan has been estimated to contribute to the *de novo* synthesis of niacin. Because it is assumed that amino acids consumed by infants are fully utilised for protein synthesis, the ANZ AI also excludes the contribution from tryptophan. Therefore, the estimated intakes calculated from the Codex STAN 72-1981 minimum can be compared to the ANZ AI.

An 0–<6 month old infant consuming 0.8 L/day of infant formula containing the Codex STAN 72-1981 minimum of 70 µg/100 kJ and midpoint of the Codex STAN 72-1981 energy amount (2725 kJ/L) would have an estimated intake of 1.5 mg/day of niacin which approximately meets the ANZ AI of 2 mg/day, noting that the AI was rounded up from 1.8 mg/day (NHMRC and MoH 2006). For infants 6–<12 months consuming 0.6 L/day, the estimated intake would be 1.1 mg/day which is less than 50% of the niacin requirement (4 mg/day) for this age group (assuming a theoretical diet where 50% of the nutrient requirement is met from formula and 50% from complementary foods).

EFSA (2014) recommended that infant formula contain 100 µg/100 kJ as the minimum amount of preformed niacin, based on the upper range of niacin in breast milk. There is no evidence that infants consuming formula based on the Codex STAN 72-1981 minimum amount do not meet their niacin requirement. Therefore, for the minimum amount of niacin, Codex STAN 72-1981 did not meet the assessment criteria but further analysis indicated that use of this minimum amount would be unlikely to pose a risk to infant health.

3.6.6 Vitamin B_6 (Pyridoxine)

Minimum and ratio of vitamin B_6 to protein

After rounding, the minimum amount of vitamin B_6 is aligned in both standards (9 µg/100 kJ and 8.5 µg/100 kJ for Standard 2.9.1 and Codex STAN 72-1981, respectively). Vitamin B_6 is a cofactor in the enzymatic transformations of amino acids and in lipid and glucose

metabolism. In order to prevent vitamin B6 deficiency associated with high protein intakes, previous standards (National Food Authority (NFA) 1995) prescribed a relative concentration of 15 µg of vitamin B_6/g of protein when protein exceeds 0.6 g/100 kJ. Using the maximum protein amount (0.7 g/100 kJ) and the minimum vitamin B6 amount, the calculated relative concentration, based on the Codex mid-point of energy, is 13 µg of vitamin B_6/g of protein, i.e. close to the value prescribed in previous standards. The relative concentration is no longer specified in Standard 2.9.1 or Codex STAN 72-1981, probably because protein amounts in infant formula have decreased since the original work indicating high protein-induced B6 deficiency was a potential risk to infants.

Maximum

The Codex STAN 72-1981 maximum vitamin B6 amount (45 µg/100 kJ) is higher than the Standard 2.9.1 maximum (36 µg/100 kJ). The Codex STAN 72-1981 maximum was based on a reasonable margin of safety calculated as five times the minimum amount (8.5 µg/100 kJ) (Koletzko et al. 2005) whereas Standard 2.9.1 was based on LSRO (1998) recommendations which were derived from the 90th percentile of formula sold in the USA. The maximum amount is a GUL in Codex STAN 72-1981 but a mandatory amount in Standard 2.9.1. There is no evidence indicating excessive vitamin B6 intakes in formula-fed infants. Therefore, Codex STAN 72-1981 for vitamin B6 would be unlikely to pose a risk to infant health.

3.6.7 Folate

Folate terminology and forms

Folate terminology and forms were defined in a previous FSANZ assessment (FSANZ 2006b p.6) and are summarised below.

> **Folate** is a water-soluble B-group vitamin. The term folate is used generically to refer to the various forms of the vitamin, both naturally-occurring and synthetic, and its active derivatives.
>
> **Folic acid**, or pteroylmono-glutamic acid (PGA), is the most common synthetic form of folate and is the form used in fortification and in infant formula. Folic acid is rarely found occurring naturally in foods.
>
> **5-methyl tetrahydrofolate (5-methyl-THF)** is the principal form of folate that circulates in the blood. 5-methyl-THF can be synthesised and added to food as a fortificant, however, this form of folate is less stable in the final product than synthetic folic acid.

Upon absorption, folic acid is converted to the circulating and biologically active form folate (5-methyl-THF) which is the form present in breast milk. Folic acid is essentially 100% bioavailable whereas folate from foods (and presumably breast milk, although this has not been explicitly determined) is 50-60% bioavailable (NHMRC and MoH 2006). Thus, the term dietary folate equivalents (DFE)[9] have been used to correct for the varying bioavailability such that:

1 µg DFE = 1 µg food folate = 1 µg folate in breast milk = 0.6 µg folic acid as fortified foods

[9] SD1 Section 7.2.2 discusses the use of DFE to define folate amounts in infant formula.

Minimum

The minimum amount of folate in Standard 2.9.1 is 2 µg/100 kJ. Codex STAN 72-1981 differs as it lists the minimum amount of folic acid of 2.5 µg/100 kJ. The Standard 2.9.1 amount was derived from the minimum amount that was required to meet the RDI in place when the standard was developed. The RDI of 50 µg/day was defined in terms of **total** folate (Truswell 1990, p.104). Codex STAN 72-1981 was based on the recommendation of the LSRO (1998) and is specified as folic acid (EC SCF 2003; p.121). As a result, the difference between the two standards for the minimum amount may be related to the prescribed form.

For the purposes of comparing the Codex STAN 72-1981 folic acid minimum amount to breast milk folate concentration and the folate AI (which is based on breast milk content), the Codex minimum folic acid was multiplied by 1.67 (based on the conversion above) which allows for the difference on bioavailability between folic acid and folate.

The concentration of breast milk folate is highly variable and ranges from 24 to141 µg/L. Using the midpoint of the energy content (2725 kJ/L), the Codex STAN 72-1981 minimum (2.5 µg/100 kJ folic acid) corresponds to a folate concentration 114 µg folate/L and that for Standard 2.9.1 (2 µg/100 kJ of folate) corresponds to a folate concentration of 55 µg/L.

For infants aged 0–<6 months, the Codex STAN 72-1981 minimum folic acid amount at the midpoint energy amount (2725 kJ/L) and the mean intake volume (0.8 L/day) corresponds to an estimated folate intake of 91 µg/day. The ANZ AI for folate (which is expressed as dietary folate equivalents) for this age group is 65 µg DFE/day. Therefore, estimated intakes using the Codex minimum amount is sufficient to meet the folate AI.

For infants aged 6–<12 months, the Codex STAN 72-1981 minimum at the midpoint of the energy (2725 kJ/L) and the mean volume of intake (0.6 L/day) corresponds to an estimated folate intake of 68 µg/day. The ANZ AI for folate for this age group is 80 µg DFE/day. Assuming infants in this age range receive 50% of their nutrient intake from formula, and 50% from complementary foods, this intake would meet the AI from formula of 40 µg DFE/day.

Based above arguments the Codex STAN 72-1981 minimum folic acid amount did not meet the assessment criteria because differences in bioavailability of folic acid and folate precluded direct comparison to Standard 2.9.1, breast milk, and the ANZ AI. However, using a conversion that allowed for these bioavailability differences indicated that the use of the Codex minimum is unlikely to pose a risk to infant health.

Maximum

The EC ECF (2003) and ESPGHAN (Koletzko et al. 2005) recommended the maximum folic acid amount to be 7 µg/100 kJ which is approximately aligned with the current Standard 2.9.1 (8 µg/100 kJ). The higher amount in Codex STAN 72-1981 (12 µg/100 kJ) appears to have been set to allow for technological issues relating to the stability of nutrients over the shelf life of the product and other variables which reduce the actual amount in infant formula (CCNFSDU 2006).

The maximum amount in Codex STAN 72-1981 amount is a GUL whereas it is a mandatory maximum in Standard 2.9.1. No basis for the mandatory maximum was provided in the previous review of the infant formula standard (ANZFA 1999). The ANZ UL applies only to folic acid from fortified food or supplemental intake, although no folic acid UL is listed for infants and the document states “Not possible to establish for supplemental folic acid. Source of intake should be milk, formula and food only.” (NHMRC and MoH 2006).

Excessive folate intakes can mask vitamin B12 deficiency particularly in the elderly but this is not considered to be a problem in formula-fed infants (MacLean et al. 2010). The Standard 2.9.1 and Codex STAN 72-1981 maximum amounts (which are effectively aligned) have been in place since 2002 and no recent evidence (i.e. published since the last reviews of the two standards) indicating infants are at risk of excessive folate intakes was identified. Therefore, use of the Codex STAN 72-1981 GUL for folate is unlikely to pose a risk to infant health.

3.6.8 Conclusion - vitamins

Table 14 summarises results of the comparative analysis for vitamins in infant formula. For some vitamins (vitamin K, thiamin, riboflavin, pantothenic acid, vitamin B12, and biotin), no specific issues (i.e. where the Codex STAN 72-1981 amount did not meet assessment criteria) were identified (represented by ✓ Table 14) and it was concluded that use of Codex STAN 72-1981 for these vitamins would be unlikely to pose risk to infant health.

Minor issues were identified for the remainder of the vitamins (vitamin A, vitamin D, vitamin E, vitamin C, niacin (preformed), vitamin B6, and folate; represented by ✗ in Table 14). In these cases, further analysis also indicated that use of Codex STAN 72-1981 for these vitamins would be unlikely to pose risk to infant health.

Maximum amounts for vitamins in both Codex STAN 72-1981 and Standard 2.9.1 are designated as either GULs (not mandatory) or specified maximums (mandatory). In both standards, mandatory maximums are set for vitamins A and D as these are the only vitamins for which there is a known intake above which adverse effects are a possibility. In both standards, maximum amounts for most vitamins (vitamin E, vitamin K, thiamin, riboflavin, niacin, vitamin B12, pantothenic acid, folic acid, vitamin C, and biotin) are designated as GULs. Only vitamin B6 and vitamin E are designated as mandatory maximums in Standard 2.9.1 but are GULs in Codex STAN 72-1981. No evidence was identified to indicate that a mandatory amount was needed for these vitamins. Therefore, based on an extended history of safe use, it was concluded that Codex STAN 72-1981 voluntary maximums for vitamin B6 and vitamin E, would be unlikely to pose a risk to infant health.

In general, maximum amounts for water soluble vitamins are higher in Codex STAN 72-1981 compared to Standard 2.9.1. The increased amounts reflect significant losses that are recognised to occur with processing or during shelf life of infant formula products (MacLean et al. 2010). No evidence of adverse health effects associated with high intakes of water soluble vitamins in infants consuming formula based on Codex STAN 72-1981 has been reported.

Table 14: Summary of comparative analysis: vitamins[1]

Vitamin	Codex STAN 72-1981					Further analysis	Conclusion
	Minimum			Maximum			
	Is it aligned with Std 2.9.1*?*	*Does it compare with breast milk?*	*Does intake from min meet AI?*[2]	*Is it aligned with* Std 2.9.1*?*	*Is intake from max less than UL?*[2]	*Issue examined:*	*Use of Codex STAN 72-1981 is:*
Vitamin A	✓	✓	✓	✓	×	Calculation of RE; Codex max potentially exceeds UL	Unlikely to pose a risk to infant health
Vitamin D	✓	×	×	✓	✓	Current debate about AI	Unlikely to pose a risk to infant health
Vitamin E	✓	✓	×	×	#	Codex min meeting the AI, Ratios of min to dietary PUFA, Codex max is a GUL	Unlikely to pose a risk to infant health
Vitamin K	✓	×	✓	✓	#	No issues; breast milk contains negligible amounts	Unlikely to pose a risk to infant health
Vitamin C	✓	✓	✓	×	#	Codex max is higher than Std 2.9.1	Unlikely to pose a risk to infant health
Thiamin	✓	✓	✓	✓	#	No issues	Unlikely to pose a risk to infant health
Riboflavin	✓	✓	✓	✓	#	No issues	Unlikely to pose a risk to infant health
Niacin	×	✓	✓	✓	#	Codex min is greater than Std 2.9.1; permitted forms are different	Unlikely to pose a risk to infant health
Vitamin B6	✓	✓	✓	×	#	Codex max is GUL; ratio of vitamin B6 to protein amount	Unlikely to pose a risk to infant health
Folate	×	✓	×	×	#	Calculation of folic acid to compare with breast milk, Codex max higher than Std 2.9.1	Unlikely to pose a risk to infant health
Pantothenic acid	✓	✓	✓	✓	#	No issues	Unlikely to pose a risk to infant health
Vitamin B12	✓	✓	✓	✓	#	No issues	Unlikely to pose a risk to infant health
Biotin	✓	✓	✓	✓	#	No issues	Unlikely to pose a risk to infant health

[1] ✓= *Codex amount meets this criteria;* × *= Codex amount does not meet this criteria (see text Section 3.7); # = no UL has been defined. Abbreviations: Std.2.9.1 = Standard 2.9.1; min = minimum amount; max = maximum amount; est = estimated; AI = adequate intake; UL = upper level of intake; GUL = guidance upper level; BM = breast milk; RE = retinol equivalents; PUFA = polyunsaturated FA.*

[2] *See Section 2.2.3 for assumptions that were applied in this comparison*

3.7 Minerals and Electrolytes

Infant formula provisions for minerals and electrolytes include minimum amounts, maximum amounts (either as a mandatory or voluntary limit), and ratios to other nutrients in the cases of zinc, calcium and phosphorus. Calculated results comparing Codex STAN 72-1981 and Standard 2.9.1 to breast milk concentrations and estimated daily intakes are reported in Appendix 2 and outcomes summarised in Table 15.

For sodium, chloride, and magnesium, Codex STAN 72-1981 was found to meet all the assessment criteria: the specified amounts were comparable to the amounts in Standard 2.9.1 and were within the range of breast milk concentrations, estimated minimum and maximum intakes were appropriate to meet AI or did not exceed the UL, there was an extended history of safe use, and there was no evidence that the Codex STAN 72-1981 provisions for these nutrients should be changed. For these nutrients, no further analysis was conducted.

For potassium, calcium, phosphorus, iron, zinc, iodine, copper, manganese, selenium, chromium, and molybdenum, Codex STAN 72-1981 was found to meet some of the assessment criteria but issues were identified with certain aspects that were examined further, as described in the subsequent sections.

Table 15: Outcomes of the comparative analysis: minerals and electrolytes [1]

Mineral or Electrolyte	Provision(s) that met assessment criteria	Further analysis described in Section 3.7
Sodium	Minimum and maximum amounts	No further analysis
Chloride	Minimum and maximum amounts	No further analysis
Potassium	Maximum amount	Minimum amount
Calcium	Minimum and maximum amounts	Calcium:phosphorus ratio
Phosphorus	Maximum amount	Minimum amount, Calcium:phosphorus ratio
Magnesium	Minimum and maximum amounts	No further analysis
Iron	None	Minimum and maximum amounts
Zinc	Minimum amount	Maximum amounts, ratio of zinc to other nutrients
Iodine	None	Minimum and maximum amounts
Copper	None	Minimum and maximum amounts
Manganese	Minimum amount	Maximum amount
Selenium	None	Minimum and maximum amounts
Chromium	Minimum amount	Maximum amount
Molybdenum	Minimum amount	Maximum amount

[1] *Nutrients shaded in grey met all the assessment criteria and are not discussed further in Section 3.7.*

Infant formula is typically sold as a powder and mixed with potable tap water. The quantity of minerals contained in reticulated water supplies is generally low. Specific mention is made where this may not be the case and it would make a difference to conclusions. Minimums and maximums refer to the product as sold and do not include minerals derived from water.

3.7.1 Potassium

Minimum

The minimum potassium amount in Standard 2.9.1 (20 mg/100 kJ) is greater than the Codex STAN 72-1981 amount (14 mg/100 kJ). Standard 2.9.1 was set to align with the former Codex requirements (ANZFA 1999a) whereas the Codex STAN 72-1981 amount was based on the LSRO report (1998). The LSRO used the mean breast milk concentration minus one standard deviation calculated from several studies[10] to give 400 mg/L and a recommended minimum of 14 mg/100 kJ. The LSRO used the mean minus one standard deviation value because it represented a history of use for a large population in which deficiency had not been reported.

For infants 0–<6 months, the estimated intake from the Codex STAN 72-1981 minimum is 305 mg/day assuming the midpoint energy content (2725 kJ/L) and mean volume of intake (0.8 L/day). This intake does not meet the ANZ AI of 400 mg/day for infants aged 0–6 months (NHMRC and MoH 2006).

For infants aged 6–<12 months, the ANZ AI is 700 mg/day. The estimated intake is 229 mg/day based on a mean intake volume of 0.6 L/day. Assuming infants in this age range receive 50% of their nutrient intake from formula, and 50% from complementary foods, this intake also would not meet the AI from formula of 350 mg/day.

The ANZ AI for potassium was derived from the average breast milk concentration of potassium (500 mg/L) measured in similar studies to that used by the LSRO[11] (IOM 2005), and using mean intake volumes of 0.78 L/day and 0.6 L/day for the 0–6 and 7–12 month age groups, respectively (NHMRC and MoH 2006).

EFSA (2014) recommended the minimum potassium amount (19.1 mg/100 kJ) by calculating the amount need to meet the AI of 400 mg/day.

Therefore, the Codex STAN 72-1981 minimum does not meet the AI because it is derived using different methodology from Standard 2.9.1. The Codex STAN 72-1981 minimum was based on breast milk concentration minus one standard deviation whereas the ANZ AI and the EFSA (2014) minimum amount are based on the mean breast milk concentration. There is no evidence of potassium deficiency in the infant population in countries which have adopted the lower Codex STAN 72-1981 minimum amount. Therefore, in the absence of studies suggesting the lower amount in Codex STAN 72-1981 leads to adverse health effects, it can be concluded that the Codex STAN 72-1981 minimum for potassium is unlikely to pose a risk to infant health.

3.7.2 Phosphorus

Minimum

Standard 2.9.1 and Codex STAN 72-1981 are aligned for the minimum phosphorus amount (6 mg/100 kJ) and the amount is within the concentration range in breast milk. The estimated intake based on the Codex STAN 72-1981 minimum is 131 mg/day and 98 mg/day for infants aged 0–<6 months and 6-<12 months, respectively. The estimated intake is less than 50% of the AI for the 6–<12 month old age group (138 mg/day, assuming infants in this age range receive 50% of their nutrient intake from formula, and 50% from complementary foods).

[10] Studies were Dewey 1984, Dewey and Lonnerdal, 1983, Lemons 1982, and Picciano 1981. Mean = 500 mg/L.
[11] Studies were Dewey and Lonnerdal, 1983, Lemons 1982, Keenan 1982, Picciano 1981, and Gross 1980. Mean = 500 mg/L.

Because this is an overly conservative estimate of intake, there is no evidence of phosphorus deficiency in infants, and the minimum amount has been set at this amount at least since the previous ANZ standard for infant formula, it is concluded that the Codex STAN 72-1981 minimum (which is the same as Standard 2.9.1) would be unlikely to pose a risk to infant health.

Maximum

Standard 2.9.1 and Codex STAN 72-1981 are aligned for the maximum phosphorus amount (25 and 24 mg/100 kJ, respectively) but the amount is mandatory in Standard 2.9.1 and a GUL in Codex STAN 72-1981.

Excess phosphorus intake may induce hypocalcaemia which can also be caused by low calcium intake and low vitamin D status (Greer 1989). Previous recommendations indicate that the maximum phosphorus amount should be 17 mg/100 kJ (LSRO 1998, EC SCF 2003). The higher amount that is currently set in both standards is to allow for the relative unavailability of phosphorus from soy-based formulas which includes phosphorus in the form of phytate.

Hypocalcaemia due to excess phosphorus in formula-fed infants is prevented by limiting the calcium phosphorus ratio (see section 3.7.3). There have been several clinical reports of hypocalcaemia in formula-fed early neonates (<14 days) but in these studies, the hypocalcaemia was considered to be associated with vitamin D deficiency resulting from low maternal vitamin D status (Thomas et al. 2012; Do et al. 2014; Cho et al. 2015). There is no recent evidence indicating that older infants (>14 days) consuming infant formula develop hypercalcemia due to excess phosphorus intakes.

No additional studies were found suggesting adverse effects linked to high phosphorus intakes in formula-fed infants. Therefore, the Codex STAN 72-1981 GUL for the maximum phosphorus amount did not meet the assessment criteria but further analysis indicated that use of this maximum as a voluntary amount would be unlikely to pose a risk to infant health.

3.7.3 Calcium and Phosphorus Ratio

Standard 2.9.1 and Codex STAN 72-1981 are aligned for the minimum and maximum amounts for calcium and phosphorus. A weight per weight ratio of calcium to phosphorus (Ca:P ratio) is also prescribed in both Standard 2.9.1 and Codex STAN 72-1981 to prevent hypocalcaemia which could occur if formula contained the minimum calcium content (12 mg/100 kJ) and the maximum phosphorus content (24 mg/100 kJ), which would give a Ca:P ratio of 0.5.

The minimum Codex STAN 72-1981 Ca:P ratio is 1:1 (EC SCF 2003) whereas Standard 2.9.1 is 1.2:1 based on former Codex requirements. Expert reports (Scientific Committee for Food 1983; LSRO 1998; EC SCF 2003; Koletzko et al. 2005) do not indicate a rationale for this shift and it is therefore assumed that the Codex STAN 72-1981 ratio is rounded down from previous expert recommendations.

The maximum Ca:P ratio is 2 in both Standard 2.9.1 and Codex STAN 72-1981 and is based on the Ca:P ratio measured in breast milk. There is no scientific evidence to indicate that the maximum Ca:P ratio is inappropriate.

Therefore Codex STAN 72-1981 for the minimum Ca:P ratio is marginally lower than that prescribed in Standard 2.9.1 but further analysis indicated that it would be unlikely to pose a risk to infant health.

3.7.4 Iron

Minimum

The Codex STAN 72-1981 minimum amount for iron (0.1 mg/100 kJ) is substantially lower than the Standard 2.9.1 minimum (0.2 mg/100 kJ). Iron requirements in infancy continue to be of some concern since iron deficiency is the most common micronutrient deficiency worldwide (Domellof et al. 2014).

Origin of infant formula standards for iron

The Standard 2.9.1 iron minimum was based on evidence showing:

- iron-fortified infant formula in the US is associated with declining prevalence of anaemia
- no side effects at this amount from constipation or impaired Zn/Cu absorption
- mild iron deficiency also may result in adverse effects such as impaired cognitive development
- amount of iron absorbed from commercially iron-fortified cereal is low and the amount of iron-fortified cereals consumed by infants is small (ANZFA 1999).

The EC SCF (2003) recommended the minimum iron amount 0.07 mg/100 kJ for cow's milk formula and this was increased to 0.1 mg/100 kJ in Codex STAN 72-1981 to allow for the lower bioavailability of iron in soy-based formula (EC SCF 2003 p.135). The recommendation was reiterated by ESPGHAN (Koletzko et al. 2005) which also reported on relatively new evidence that iron absorption from formula is comparable to breast milk and potential risks linked to excess iron. Although the evidence showed that adverse effects (lower length gain, higher prevalence of diarrhoea and upper respiratory tract infection) were only associated with supplemental iron and not iron-fortified infant formula, ESPGHAN concluded that iron content should be kept as low as possible as long as iron deficiency is prevented. Based on a recent systematic review (Domellof et al. 2013), a subsequent ESPGHAN report recommended that infant formula should contain iron in the range of 4–8 mg/L of iron (Domellof et al. 2014). This range is equivalent to 0.15–0.30 mg/100 kJ based on the midpoint energy content of 2725 kJ/L.

The American Academy of Pediatrics (AAP) advises substantially higher iron content for infant formula: 10–12 mg/L, which corresponds to about 0.40 mg/100 kJ (Baker and Greer 2010). The AAP recommendation was mainly based on a RCT showing that breastfed infants provided with supplemental iron (7.5 mg/day) had higher haemoglobin concentrations with no adverse effects compared to infants who consumed formula that was not supplemented with iron. However, others proposed that these results indicate an adverse effect because it reflects the infants' incapacity to down-regulate iron absorption when exposed to surplus iron (Lonnerdal and Hernell 2010; Hernell and Lonnerdal 2011).

In line with the EC SCF (2003), EFSA (2014) recommended that minimum amount of iron to be 0.07 mg/100 kJ based on clinical data that this amount would maintain iron status within the normal range within the first four to six months of life and on breast milk iron concentration (allowing for differences in absorption efficiency). EFSA also recommended the higher minimum amount specified in Codex STAN 72-1981 (0.1 mg/100 kJ) for use for soy-based formula.

Meeting intake recommendations for iron

The ANZ AI of 0.2 mg/day for infants aged 0–<6 months was calculated after excluding three low quality studies of the iron content of breast milk that had been used by the IOM in their

calculations (IOM 2001; NHMRC 2005). However it is specifically stated that this AI is for breastfed infants only and that the bioavailability of iron in infant formula is 10–20% that of breast milk (NHMRC and MoH 2006). This would suggest that the iron intake of formula-fed younger infants should be 2–4 mg/day.

For infants aged 0–<6 month, the estimated intake based on the Codex STAN 72-1981 minimum is 2.3 mg/day using the midpoint energy content (2725 kJ/L) and a mean intake volume (0.8 L /day). This is substantially lower than the range of 4–8 mg/day cited by Domellof et al (2013) as safe and effective. Using the minimum amount in Standard 2.9.1, the estimated iron intake would be 4.6 mg/day.

For infants 6–<12 months, an EAR of 7 mg/day was derived by modelling iron requirements, i.e. estimating the requirements for absorbed iron at the 50th percentile with use of an upper limit of 10% iron absorption, and rounding (NHMRC and MoH, 2006). The EAR equates to 3.5 mg/day as the minimum intake from infant formula, assuming 50% of iron intake is from formula and 50% from complementary foods. This may not include an allowance for differences in iron bioavailability from infant formula compared to breast milk, which is not known.

An older infant consuming infant formula (0.6 L/day) containing the Codex STAN 72-1981 minimum amount would receive about 1.6 mg/day, or approximately one-quarter of the EAR. EFSA (2014) considered that it could be assumed that complementary foods would provide the remaining iron (which they estimated to be about 5.7 mg/day) necessary to reach daily iron intakes of 8 mg/day.

New evidence

Iron deficiency anaemia (IDA) is the most well described adverse health outcome of iron deficiency (ID) and there are numerous biochemical markers used to characterise iron status and IDA (Domellof et al. 2013). Several recent studies have shown that normal birthweight, term infants aged 6-24 months consuming iron-fortified formula have a lower risk of ID or IDA compared to breastfed (consuming the more bioavailable iron in breast milk) or infants fed non-fortified formula (Soh et al. 2002; Soh et al. 2004; Grant et al. 2007; Thorisdottir et al. 2012; Brunt et al. 2012; Thorisdottir et al. 2013). An Australian survey was conducted in older children aged 1–4 years and found that the prevalence of anaemia was very low and did not exceed the prevalence expected for non-iron deficiency anaemia (Mackerras et al. 2004)

Studies on iron intakes are difficult to interpret for formula-fed infants. A study of infants in Adelaide showed that formula-fed infants at 9 months of age had a mean iron intake of 11.9 mg/day (SD 3.9, N=220) compared to 6.3 mg/day for breast fed infants (Conn et al. 2009). Recently published results from the InFANT trial showed similar iron intakes where formula-fed 9-month old infants had mean iron intake of 11.4 mg/day (SD 3.8, N= 10). The trials reported prevalence of inadequate iron intake in infants to be 9% (Conn et al. 2009) and 32.6% (Atkins et al. 2016). A recent NZ survey found that 41% of infants aged 6–11 months did not meet the EAR for iron and this was associated with an increased risk of ID (relative risk = 18.45, 95% CI 3.24, 100.00) (Wall et al. 2009). However prevalence of inadequate iron intakes or risk of ID in all of the above studies was reported for breastfed and formula-fed infants collectively and therefore the risk of inadequate iron intake for the formula-fed cohorts cannot be determined.

Overall, there is no international consensus on the minimum amount of iron in infant formula. Formula-fed infants have a lower risk of ID or IDA than breastfed infants and there is evidence for inadequate iron status in some population groups of older infants. The Codex STAN 72-1981 minimum is lower than Standard 2.9.1 so presumably the risk of ID or IDA would increase using the Codex STAN 72-1981 amount. Without information showing that

the bioavailability of iron in infant formula is better than has been previously assumed, the Codex STAN 72-1981 minimum amount for iron did not meet the assessment criteria and further analysis indicated that it could pose a risk to infant health.

Maximum

Standard 2.9.1 specifies a maximum iron amount of 0.5 mg/100 kJ. A maximum amount of 0.4 mg/100 kJ was advised by the EC SCF (2003) and ESPGHAN (Koletzko et al. 2005) although this was not adopted into Codex STAN 72-1981 and the maximum was left to national authorities to establish. Thus, the EC has set the maximum iron amount to be 0.3 mg/100 kJ (European Commission 2006). The EFSA (2014) considered that the maximum iron content in infant formula could not be established due to insufficient evidence.

Infants consuming formula containing the maximum iron amount (0.5 mg/100 kJ as specified in Standard 2.9.1) at a midpoint energy level (2725 kJ/L) and a mean volume of intake would receive an estimated 11 and 8.2 mg/day for ages 0–<6 months and 6–<12 months, respectively. These intakes are below the UL (20 mg/day, or 10 mg/day for infants 6–<12 months receiving 50% of their intake from formula, and 50% from complementary foods) (NHMRC and MoH 2006).

The lack of a maximum amount for iron in Codex STAN 72-1981 means that the assessment criteria have not been met and therefore, use of Codex STAN 72-1981 could pose a risk to infant health. The maximum specified by Standard 2.9.1 is higher than international recommendations and estimated intakes based on this amount would not exceed the UL. Therefore, the current maximum in Standard 2.9.1 is unlikely to pose a risk to infant health.

3.7.5 Zinc

Maximum

The maximum zinc amount is a mandatory limit in Standard 2.9.1 (0.43 mg/100 kJ) and as a guidance limit in Codex STAN 72-1981 (0.36 mg/100 kJ). The Standard 2.9.1 maximum is higher to allow for lower zinc absorption from soy-based formulas due to the presence of the mineral-chelating phytochemical phytic acid (ANZFA 1999a; Agostoni et al. 2006). The Codex STAN 72-1981 amount is based on the recommendation of ESPGHAN (Koletzko et al. 2005) which was reduced from the ES SCF (2003) recommendation of 0.57 mg/100 kJ on the basis that high intakes may interfere with the absorption of other micronutrients (e.g. copper).

The Codex STAN 72-1981 maximum zinc amount equates to 7.8 mg/day, and 5.9 mg/day for infants aged 0–<6 months and 6–<12 months, respectively, using the midpoint of the energy content (2725 kJ/L) and mean daily intake volumes. These amounts exceed the UL of 4 mg/day (NHMRC and MoH 2006) for ages 0–<6 months, or 2 mg/day for ages 6–<12 month old assuming 50% of zinc intake would be consumed from formula and 50% from complementary foods.

High intakes of zinc can inhibit copper absorption (Hambidge and Krebs 1989). The ANZ UL is based on three studies which used plasma copper concentrations as a measure of adverse outcomes (NHMRC and MoH 2006). In two of these studies, consumption of infant formula containing zinc at 5.8 mg/L (which was converted to a NOAEL of 4.5 mg/day based on the intake volume of 0.78 L/day), had no effect on plasma copper concentration. In the third study, administration of zinc to 6–12 month old infants at 10 mg/day reduced plasma copper concentrations but this study contained a number of limitations. Firstly, the study was designed to examine the effect of daily zinc supplementation on the incidence and severity of diarrhoea in infants in Indian villages; that is, it was conducted in an unhealthy population not

representative of the general population. Additionally, the study cohort had elevated plasma copper concentrations in both test and placebo groups prior to the commencement of the study. At the conclusion of the study, a relatively modest difference in plasma copper concentrations occurred between the test and placebo groups (~15 µg /mL) with copper concentrations still elevated in both groups. Despite these limitations, the study was included in the derivation of the UL and thus the UL is overly conservative (FSANZ 2011, p.102-104).

The Codex STAN 72-1981 maximum zinc amount is lower than Standard 2.9.1 and would be unlikely to pose a risk to infant health. No evidence was identified to support the mandatory maximum amount currently set in Standard 2.9.1. Therefore, adopting a GUL would be unlikely to pose a risk to infant health.

Ratio of zinc to other micronutrients

Because high zinc intakes can impact on copper bioavailability, Standard 2.9.1 specifies that the zinc to copper (Zn:Cu) ratio must not exceed 15:1. Codex STAN 72-1981 does not specify a Zn:Cu ratio. Furthermore, the EC SCF (2003), ESPGHAN (Koletzko et al. 2005), and EFSA (2014) do not make any recommendations for a Zn:Cu ratio. The Zn:Cu ratio in human milk is about 10:1 (Lonnerdal 1989).

Standard 2.9.1 is based on the LSRO (1998) which commented on the importance of a Zn:Cu ratio from an extreme case of zinc-induced copper deficiency in an infant who received oral zinc supplementation of 16–24 mg/day from the age of 0–6 months (i.e. much greater than zinc intake from formula estimated from the Codex STAN 72-1981 maximum amount, see preceding section). The LSRO report also inferred that the Zn:Cu ratio should not exceed 20:1 based on estimated zinc and copper intakes in breastfed infants (Hambidge and Krebs 1989) although no specific recommendation was made. There are no further studies indicating the appropriate Zn:Cu ratio in infant formula. Using the midpoint of the Codex STAN 72-1981 minimum and maximum amounts for zinc and copper, the Zn:Cu ratio would be about 12:1. Therefore, it is concluded that the lack of a specification for the Zn:Cu ratio in Codex STAN 72-1981 is unlikely to pose a risk to infant health.

Excess zinc may also interfere with calcium and iron absorption (Jovani et al. 2001) although neither Codex STAN 72-1981 nor Standard 2.9.1 prescribes a specific ratio for these micronutrients. It is likely that the minimum amounts of iron and calcium more than compensate for the potential loss of availability due to excess zinc intake (LSRO 1998). Since the time of the reviews and studies cited above were published, no further evidence of zinc-induced micronutrient deficiencies occurring in healthy term formula-fed infants has emerged.

3.7.6 Iodine

Minimum

The minimum iodine amount in Codex STAN 72-1981 is 2.5 µg /100 kJ which is double the minimum specified in Standard 2.9.1 (1.2 µg/100 kJ). The Standard 2.9.1 minimum was based on the previous ANZ standard and the minimum amount specified by Codex STAN 72-1981 at that time (ANZFA 1999a). The Codex STAN 72-1981 amount (based on the EC SCF 2003) was derived from the average breast milk iodine concentration (BMIC) (Atkinson et al. 1995) and the amount needed to meet iodine reference intakes as set by different government and expert bodies.

BMIC is highly variable and depends on dietary iodine intake. A more recent estimate of the mean BMIC from an iodine sufficient population is 146 µg/L (Zimmermann 2009) which, based on the mean energy content of breast milk (2720 kJ/L) (Nommsen et al. 1991; Hester

et al. 2012) converts to 5.4 µg/100 kJ. Therefore, minimum iodine amount specified by Codex STAN 72-1981 is half that of breast milk from an iodine sufficient population.

Meeting the AI

The Codex STAN 72-1981 iodine minimum equates to 68 µg/L based on the midpoint energy content of 2725 kJ/L, giving an estimated intake of 55 µg/day using the mean intake volume (0.8 L/day) for 0–<6month old infants. This does not meet the AI of 90 µg /day (NHMRC & MoH, 2006) for this age group.

For infants 6–<12 months, the iodine AI is 110 µg/day. The estimated iodine intake from the Codex STAN 72-1981 minimum amount for this age group would be 41 µg/day based on the mean intake volume (0.6 L/day). Assuming infants in this age group would obtain 50% of their iodine requirements from complementary foods and 50% from formula, estimated iodine intake from formula also does not meet the AI.

The ANZ AI is based on the recommendation of the FAO/WHO report issued in 2001 which assumed that that BMIC in an iodine sufficient population was 115 µg/L and on a New Zealand study showing a similar BMIC in breast milk (Johnson et al. 1990). Noting that this concentration is less than the mean BMIC in sufficient mothers reported in the Zimmerman (2009) study (146 µg/L), the minimum iodine amount would need to be increased to about 4 µg/100 kJ to meet the ANZ AI. The EFSA NDA Panel recommended a similar higher minimum (3.6 µg/100 kJ) in their recent review of infant formula composition (EFSA 2014).

The iodine content of potable water varies across ANZ from about 10–50 µg/L (FSANZ 2008) and potentially contributes to iodine intakes in formula-fed infants. For infants 0-<6 months, this would lead to an additional 8–40 µg/day iodine intake, depending on where the infant lives. Added to the minimum amount of iodine from infant formula (which is unlikely to be the norm), the total amount could provide sufficient iodine intake.

Recent experimental studies on infant iodine status

Median urinary iodine concentration (MUIC) is the main indicator for the iodine status of populations (as reviewed by WHO 2007b). MUIC measures urinary iodine excretion using a single urine sample (the spot test) or multiple samples (24-hour test). Despite considerable shortcomings in the spot test due to differences in daily iodine intake and urine volume, this method using casual samples has been used most often for infant studies. The WHO (2007) indicated that population MUIC greater than 100 µg/L for children < 2 years old defines adequate iodine status for the population.

A limited number of studies have compared BMIC and infant MUIC in formula-fed and breastfed infants (Table 16). Moreover, mandatory use of iodised salt in bread was introduced in 2009 to address iodine deficiency in ANZ (FSANZ 2008) and there are some studies comparing pre- and post-fortification time periods. Because BMIC and MUIC vary with post-partum sampling time and geographical location, studies in Table 16 are not comparable to each other.

Prior to mandatory fortification, NZ formula-fed infants (N = 230) had better iodine status than their breastfed counterparts (MUIC of 99 versus 44 µg/L; infants and toddlers sampled at 6–24 months, (Skeaff et al. 2005). The breastfeeding women in this study had a mean BMIC of 22 µg/L whereas formula concentrations of iodine were presumably higher since it would have contained iodine in the range of 34–273 µg/L (based on the midpoint of the energy content) to be compliant with Standard 2.9.1.

The BMIC in post-partum NZ mothers consuming iodine supplements in the pre-fortification period was examined by Mulrine et al (2010). The BMIC of unsupplemented mothers was lower than supplemented mothers but none of the supplemented groups achieved a BMIC that was considered to be iodine sufficient (100–200 µg/L to maintain infants in a positive iodine balance (Zimmerman 2009). Infant MUIC measured at 6 months in all treatment groups indicated that NZ infants were iodine deficient. Another small NZ study measured a higher BMIC in post-fortification breast milk but the increase was not statistically different from pre-fortification breast milk (Brough et al. 2013). The study also did not include MUIC measurement.

Table 16: Studies comparing BMIC and MUIC in breastfed and formula-fed infants[1]

Study and Location	Endpoint	Sample time	Treatment group[2]	Concentration[3] *µg/L*
Skeaff (2005) South Island, NZ	BMIC	6–24 mo	All mothers	22 (N = 39, 95% CI 18–26)
	Infant MUIC	6–24 mo	FF	99 (N = 51, IQ range 6.7–16.7)
			BF	44 (N = 43, IQ range 23–82)
Mulrine (2010)[4] Dunedin, NZ	BMIC	0–6 mo	Unsup	25-43
			Sup 75 µg/day pp	29-50
			Sup 150 µg/day	44-78
	Infant MUIC	6 mo	Unsup	47
			Sup 75 µg/day pp	50
			Sup 150 µg/day pp	66
Brough (2013) Palmerston North, NZ	BMIC	>3 weeks pp	Pre-fortification	55 (±48, N=32)
			Post-fortification	63 (±63, N = 36)
Huynh (2015) Adelaide, Australia	BMIC	At birth	Pre-fortification	103 (median, N=291)
		At birth	Post-fortification	187 (median, N=653)
		3 mo pp	Post-fortification	126 (median, N=543)
	Infant MUIC	3 mo pp	FF, Post-fortification	198
			BF, Post-fortification	195
Andersson (2010) Switzerland	BMIC	6 mo	All mothers	50.6 (N=149)
	Infant MUIC	6 mo	FF, Iodine = 46 µg/L	105 (N=311)
			BF	82 (N=196)
Gordon (2014) Boston USA	BMIC	Not reported	All mothers	59.7
	Infant MUIC	2 mo	FF, Iodine = 158.9 µg/L	182.5 (N=44)
			BF	203.5 (N=39)

[1] *Abbreviations: BMIC = mean breast milk iodine concentration (unless otherwise indicated); MUIC = median urinary iodine concentration; IQ = interquartile; CI = confidence interval; pp = post-partum; mo = months; Unsup = unsupplemented; sup = supplemented, FF = formula-fed, BF = breastfed.*

[2] *Iodine supplementation studies refer to maternal supplementation*

[3] *Concentrations are mean values, as reported, unless otherwise indicated.*

[4] *This study does not compare BF with FF but was included because it provides data for NZ infants pre-fortification.*

Studies from mothers and infants in Adelaide, Australia pre- and post-fortification have been recently published (Huynh 2015). Unlike the NZ data, BMIC sampled at birth was significantly higher post-fortification compared to pre-fortification and was within the range considered to

be sufficient (BMIC >100 µg/L) for samples obtained at birth and at 3 months post-partum. Although MUIC measured in infants pre-fortification was not available, MUIC at 3 months post-partum was the same in breastfed and formula-fed infants (195 µg/L and 198 µg/L respectively, total N = 628) and all infants were in the iodine sufficient range. The iodine concentration of formula was not reported but presumably contained iodine in the range of 34–273 µg/L (based on the midpoint of the energy content) to be consistent with Standard 2.9.1. This study suggests that ANZ infants who are formula-fed receive adequate intakes of iodine despite the minimum amount not meeting the AI.

Other international studies are consistent with the above ANZ studies. A Swiss study (Andersson et al. 2010) also reported lower MUIC in breastfed infants compared to formula-fed infants and this was associated with BMIC that was in the deficient range. MUIC measured in a small sample of infants from Boston (Gordon et al. 2014) was well above the MUIC threshold of 100 µg/L in both formula-fed and breastfed infants indicating iodine sufficiency in both groups.

Current formula sold in ANZ may contain iodine in an amount that is consistent with Codex STAN 72-1981. Raising the minimum iodine content to the Codex STAN 72-1981 minimum amount of 2.5 µg/100 kJ may increase iodine intakes in formula-fed infants who are more likely to meet the AI.

Based on the above arguments the Codex STAN 72-1981 minimum iodine amount did not meet the assessment criteria but further assessment indicated the use of this minimum is unlikely to pose a risk to infant health.

Maximum

The maximum iodine amount in Codex STAN 72-1981 (14 µg/100 kJ) is listed as a GUL and is higher than the mandatory maximum in Standard 2.9.1 (10 µg/100 kJ).

Standard 2.9.1 is based on the LSRO recommendation which considered the maximum amount prescribed in the US at that time (18 µg/100 kJ) was too high. The LSRO recommended the maximum amount of 8.4 µg/100 kJ, based on the 75th percentile amount of iodine in infant formulas sold within the US. The Standard 2.9.1 maximum appears to be rounded up from this value. Because excess iodine can inhibit thyroxine synthesis, the EC SCF recommended a maximum (14 µg/100 kJ as in Codex STAN 72-1981) that was reduced from upper limits of 24 and 18 µg/100 kJ set by international expert panels (EC SCF 2003, p.151).

The maximum iodine amount is difficult to set due to the large variability in the iodine content of cow's milk which depends on season, and hygienic and agricultural techniques (EC SCF 2003). There is no ANZ UL for iodine in infants (NHMRC and MoH 2006) or the US (IOM). Using the Codex STAN 72-1981 maximum amount, the estimated intake for infants 0–<6 months (based on the midpoint energy content of 2725 kJ/L and mean volume of intake of 0.8 L/day) would be 305 µg/day, and 229 µg/day for infants 6–<12 months.

Probable safe upper limits for iodine intake have been suggested to be 150 and 140 µg/kg bw/day for infants aged 0–6 months and 7–12 months, respectively (FAO/WHO 2001). Using international weight-for-age data (WHO 2007a) and the Codex STAN 72-1981 maximum iodine amount, estimated maximum iodine intakes for infants aged 0–<12 months would be well under these upper limits (Table 17).

Therefore, for the maximum amount of iodine, Codex STAN 72-1981 did not meet the assessment criteria but further analysis indicated that use of this voluntary maximum would be unlikely to pose a risk to infant health.

Table 17: Comparison of estimated maximum iodine intake to probable safe upper limits

Age	Weight-for-age[1]	Estimated iodine intake from Codex STAN 72-1981 maximum [2]		Probably safe upper limit[3]
months	*kg*	*µg/day*	*µg/kg bw/day*	*µg/kg bw/day*
Birth < 1	4.4	305	69	150
1.0<2	5.4	305	56	150
2.0<3	6.2	305	49	150
3.0<4	6.7	305	46	150
4.0<5	7.2	305	42	150
5.0<6	7.6	305	40	150
6.0<7	8.0	229	29	140
7.0<8	8.3	229	28	140
8.0<9	8.6	229	27	140
9.0<10	8.9	229	26	140
10.0<11	9.1	229	25	140
11.0<12	9.3	229	25	140

[1] WHO (2007a) Child Health Standards http://www.who.int/childgrowth/standards/en/
[2] See Appendix 1 for details of calculations and Appendix 2, Table 22
[3] FAO/WHO (2001) http://www.fao.org/docrep/004/y2809e/y2809e0i.htm#bm18.6

3.7.7 Copper

Minimum

Standard 2.9.1 specifies a minimum amount of copper of 14 µg/100 kJ which is higher than the Codex STAN 72-1981 minimum of 8.5 µg/100 kJ. The Codex STAN 72-1981 value is based on the mean breast milk concentration but references for this value were not provided (EC SCF 2003).

The minimum Codex STAN 72-1981 amount corresponds 232 µg/L based on the midpoint of energy content (2725 kJ/L). This amount is comparable to breast milk copper concentration reported as 200–400 µg/L (Casey et al. 1995), 350 µg/L (Yamawaki et al. 2005), and 150–200 µg/L (Lonnerdal 2008).

An infant consuming infant formula containing the Codex STAN 72-1981 minimum amount would provide an estimated 186 µg/day for infants 0–<6 months of age which does not meet the ANZ AI for copper (200 µg/day) (NHMRC and MoH 2006). However, in combination with copper intake from potable tap water, estimated to be 0.35 mg/L (FSANZ 2011), the total daily intake for infants 0–<6 months is estimated to be 465 µg/day. Thus the total intake is likely to meet the ANZ AI. For infants aged 6–<12 months, estimated copper intakes based on the Codex STAN 72-1981 minimum (139 µg/day) would also meet 50% of the AI of 110 µg/day, assuming 50% of nutrient intakes for this age group would be consumed from formula and 50% from complementary feeding.

Therefore, for the minimum amount of copper, Codex STAN 72-1981 did not meet the assessment criteria but further analysis indicated that use of this minimum would be unlikely to pose a risk to infant health

Maximum

Standard 2.9.1 specifies a mandatory maximum amount for copper (43 µg/100 kJ) whereas Codex STAN 72-1981 is lower and is a GUL (29 µg/100 kJ). The basis for these values is unclear in the relevant reports (ANZFA 1999a; EC SCF 2003; Koletzko et al. 2005).

For infants 0–<6 months old, infant formula containing Codex STAN 72-1981 maximum copper amount at the midpoint energy content (2725 kJ/L) and mean volume of intake (0.8 L/day) gives rise to an estimated intake of 632 µg/day. In combination with copper from tap water (see preceding section) the estimated total intake would be 912 µg/day. Using the Standard 2.9.1 maximum, estimated intake would be 937 µg /day or 1192 µg /day when copper from tap water is included.

For infants 6–< 12 months old, intakes based on the Codex STAN 72-1981 maximum for infants and the mean intake volume (0.6 L/day), would be 474 µg /day from infant formula, or 684 µg /day with copper from tap water included.

There is no UL for copper for infants aged 0–12 months. The copper UL for children and adolescents is 1 mg/day based on extrapolation of adult value (10 mg/day) on the basis of relative body weight and rounded down (NHMRC and MoH, 2006). Using the Standard 2.9.1 maximum copper amount, infants 0–<6 months could exceed this upper limit.

Despite being a voluntary limit, the Codex STAN 72-1981 maximum amount is substantially less than Standard 2.9.1. Therefore, excessive or high copper intake could be less likely using the Codex STAN 72-1981 maximum. Copper toxicity is not known to occur in full-term breastfed or formula-fed infants (Lonnerdal 1989) and no recent studies were identified that suggested potential adverse effects linked to high copper intakes in formula-fed infants.

Therefore, the Codex STAN 72-1981 GUL for copper did not meet the assessment criteria but further analysis indicated that use of this amount as a GUL would be unlikely to pose a risk to infant health. See Section 3.7.5 for a discussion of the zinc to copper ratio.

3.7.8 Manganese

Maximum

Both Standard 2.9.1 and Codex STAN 72-1981 specify the maximum manganese amount to be 24 µg/100 kJ, which is mandatory in the case of Standard 2.9.1 and a GUL in Codex STAN 72-1981.This amount was based on the LSRO recommendation (1998) which cited evidence that manganese intakes in formula-fed infants were higher than breastfed infants, although evidence of adverse effects was not cited. The LSRO also concluded that this maximum was significantly below the estimated adult LOAEL and much higher than the amount likely to be present in cow's milk-based formulas.

For infants 0–<6 months, the maximum manganese amount at the midpoint energy content (2725 kJ/L) and a mean volume of intake (0.8 L/day) gives an estimated intake of 523 µg/day. For infants 6–<12 months the estimated intake would be 392 µg/day.

There is no ANZ UL for manganese for any age groups although the ANZ NRV (NHMRC and MoH 2006) indicated that manganese is neurotoxic at high concentrations upon inhalation and that intake beyond that normally present in food and beverages could represent a health risk (NHMRC and MoH 2006). Drinking water has also been suggested as a potential source of high manganese exposure (Ljung and Vahter 2007). Australian and New Zealand NZ drinking water guidelines (MoH 2005; NHMRC 2011) have set maximum levels for manganese in drinking water at 0.5 mg/L and 0.4 mg/L, respectively. The typical

concentration of manganese in tap water is less than 10 µg /L (NHMRC 2011). This would add 8 µg/day (for infants 0–<6 months) and 6 µg/day for 6–<12 months to the daily intake of a formula-fed infant. Both are small contributions in relation to estimated manganese intakes from formula alone.

No additional studies were found suggesting adverse effects linked to manganese intakes in formula-fed infants. Therefore, the Codex STAN 72-1981 GUL for the maximum manganese amount did not meet the assessment criteria but further analysis indicated that use of this voluntary maximum would be unlikely to pose a risk to infant health.

3.7.9 Selenium

Minimum

Standard 2.9.1 and Codex STAN 72-1981 are nearly aligned for the minimum selenium amount (0.25 µg/100 kJ and 0.24 µg/100 kJ, respectively. Standard 2.9.1 was based on the LSRO (1998) recommendations. The EC SCF recommended a higher minimum selenium content of 0.72 µg/100 kJ based on the amount of selenium to achieve the AI of 15 µg/day established by the IOM (2000) as well as the mean breast milk selenium concentration (EC SCF 2003). However, the Codex STAN 72-1981 minimum amount appears to be taken from the ESPGHAN (Koletzko et al. 2005) recommendation (0.24 µg/100 kJ) which cited highly variable breast milk concentrations and reference intakes for selenium as the basis for this conclusion.

The minimum Codex STAN 72-1981 amount converts to 6.5 µg/L based on the midpoint energy content of 2725 kJ/L. This is considerably less than breast milk selenium concentrations reported in experimental studies and the reports of expert bodies (Table 18). Breast milk selenium concentration is variable depending on geography (i.e. soil selenium levels). Consequently, breast milk selenium concentration from ANZ mothers was generally lower than the concentration measured in North American and other international studies.

Table 18: Summary of reported selenium (Se) breast milk concentrations and calculated intake for infants aged 0-<6 months

Study or Report (location)	Mean [Se] concentration breast milk			Se Intake
	µg/L	*µg/100 kcal*[1]	*µg/100 kJ*[1]	*µg/day*[2]
1992 Cumming (Aus)	11.9	1.8	0.44	9.3
1992 Dolamore (NZ)	13.4	2.1	0.49	10.5
1998 LSRO	5-22	2.1	0.50	10.5
2000 Daniels (Aus)	13	2.0	0.48	10
2000 IOM (US & Canada)	18	2.8	0.66	15
2002 Dorea (review of international data)	16	2.5	0.59	12.5
2003 EC SCF (citing Dorea 2002)	15	2.3	0.55	11.7
2005 Yamawaki (Japan)	17	2.6	0.63	13.3
2008 Daniels (Aus)	10.7	1.6	0.39	8.3
Average – all values	14	2.2	0.53	11

[1] Derived from reported concentrations using the mean energy content of breast milk (650 kcal/L or 2720 kJ/L).
[2] Calculated from the mean selenium concentration (µg/L) multiplied by the mean volume of breast milk intake of 0.8 L/day.

For infants aged 0–<6 months, the estimated selenium intake based on the Codex STAN 72-1981 minimum is 5.2 µg/day which does not meet the ANZ AI of 12 µg/day (NHMRC and MoH 2006). For infants 6–<12 months, the Codex STAN 72-1981 minimum would provide 3.9 µg/day which is also considerably less than half of the selenium AI (7.5 µg/day) for the 6–<12 months age group (assuming 50% of the AI would be met by formula and 50% from complementary foods).

For Australian infants, selenium status was assessed using plasma and erythrocyte concentrations and plasma erythrocyte glutathione peroxidase (GPx) activity (Daniels et al. 2000; Daniels et al. 2008). The latter assay is indicative of long-term selenium intakes. The studies demonstrated the lower selenium status of Australian infants relative to international populations but, importantly, this was not linked to clinical or adverse health outcomes.

The Daniels et al. study (2008) compared the effects of two selenium-supplemented formula (total selenium 13 and 21 µg/L selenium, or 0.46 and 0.75 µg/100 kJ) with formula containing a baseline selenium concentration (6 µg/L or 0.21 µg/100 kJ based on the reported energy content of 2800 kJ/L) and with a breastfed control group (mean pooled selenium concentration 10.7 µg/L). Study outcomes (plasma, erythrocyte and urinary selenium concentrations, plasma GPx activity, and growth) were measured at 16 weeks of age. Results of the study showed that selenium-supplemented formula gave plasma and erythrocyte selenium amounts that were greater than the breastfed group and infants fed the unsupplemented formula. Infants fed the formula containing the highest selenium amount (21 µg /L) had higher urinary selenium concentrations compared to other groups. This may indicate that infant selenium requirements were met and that excess selenium was excreted. Growth measures were not significantly different across the groups. The authors concluded that the selenium content of infant formula should be at least equivalent to that of breast milk in the same geographical area. Therefore, the minimum selenium amount in ANZ should be in the range of 10.7–13.4 µg /L to be consistent with the breast milk concentrations reported by Dolamore (1992) and Daniels (2008). That concentration range is equivalent to 0.39–0.48 µg/100 kJ using the midpoint energy content of 2725 kJ/L.

The United States Food and Drug Administration (FDA) recently published a Final Rule on the minimum and maximum amounts of selenium (United States Government 2015). This is a new composition requirement for infant formula sold within the United States. The rule requires the minimum amount of selenium to be 2.0 µg/100 kcal (or 0.48 µg/100 kJ), which was derived from the Daniels (2008) study and the amount that would be closest to the AI set by the IOM (15 µg/day and 20 µg/day for 0–6 and 7–12 months respectively) (IOM 2001).

Therefore, FSANZ concludes that the Codex STAN 72-1981 minimum selenium amount, which is aligned with Standard 2.9.1, does not meet the assessment criteria and use of this minimum could pose a risk to infant health. Recent studies indicate that the minimum selenium amount should be increased, at least to an amount that is comparable to the breast milk concentration of a selenium sufficient population. The minimum amount recently established by the FDA is two-fold greater than the minimum currently defined in Standard 2.9.1 and Codex STAN 72-1981, is comparable to breast milk concentration, and is closer to the amount that would meet the ANZ AI.

Maximum

Standard 2.9.1 specifies a mandatory maximum amount for selenium of 1.19 µg/100 kJ whereas Codex STAN 72-1981 specifies a GUL of 2.2 µg/100 kJ. The Standard 2.9.1 maximum was based on the LSRO recommendation (ANZFA 1999b). The Codex STAN 72-1981 GUL was based on the amount a 5 kg formula-fed infant would require to approach the UL of 47 µg/day established by the IOM (2000).

The ANZ UL is 45 µg/day for infants aged 0–6 months and 60 µg/day for ages 7–12 months (NHMRC and MoH 2006). The UL was derived from a study reported by Shearer & Hadjimarkos (1975) in which a breast milk concentration of 60 µg/L was not associated with adverse effects. The reported NOAEL of 47 µg/day was calculated from this breast milk concentration and mean intake volume (0.78 L/day). Based on a reference body weight of 7 kg and rounding, the NOAEL for selenium was reported to be 7 µg/kg body weight.

For infants aged 0–<6 months, the Codex STAN 72-1981 maximum selenium amount at the midpoint energy content (2725 kJ/L) and a mean intake volume (0.8 L/day) gives rise to an estimated intake of 48 µg/day. For infants 6–<12 months the estimated intake would be 36 µg/day. Thus, for both age groups, selenium at the Codex STAN 72-1981 maximum amount could give an estimated intake that exceeds the ANZ UL (assuming the UL for 6–<12 months would be 30 µg /day from formula consumption). Because the concentration of selenium in ANZ drinking water is generally low (NHMRC 2011), the potential exceedance of the UL for both age groups is likely to be insignificant.

The EFSA NDA Panel (2014) acknowledged that the maximum amount of 2.2 µg/100 kJ could exceed the UL but considered that there was also uncertainty in the UL. As no adverse effects have been reported with the Codex STAN 72-1981 amount, EFSA did not recommend a lower maximum amount.

The recent Final Rule published for the United States set maximum selenium amount of 1.7 µg/100 kJ which is approximately halfway between the Codex STAN 72-1981 and Standard 2.9.1 amounts (United States Government 2015). The Final Rule value was based on the evidence cited by the IOM in establishing the UL for selenium (IOM 2000). The Final Rule noted that the Codex STAN 72-1981 maximum was not adopted because it was based on history of safe use and not scientific data.

The higher Codex STAN 72-1981 amount as a GUL represents a less restrictive maximum and potentially allows exceedance of the UL. However, there is no international consensus on the appropriate maximum. In the absence of data indicating that the Codex STAN 72-1981 maximum selenium amount is unsafe, it is concluded that use of the Codex STAN 72-1981 maximum is unlikely to pose a risk to infant health.

3.7.10 Chromium and Molybdenum

Minimums

Neither Codex STAN 72-1981 nor Standard 2.9.1 set minimum amounts for chromium or molybdenum as there was no reliable biological or nutritional data to specify infant requirements or recommended intakes when these standards were reviewed.

AI amounts for chromium and molybdenum have been derived (NHMRC and MoH 2006). However, because no minimum amounts have been defined, calculating an estimated minimum intake to compare to AI amounts was not possible.

The EFSA (EFSA 2014) also considered that addition of chromium in infant formula was not necessary and did not recommend a minimum amount. For molybdenum, a minimum amount was proposed (0.1 µg/100 kJ) based on dietary intakes reported for European children. This minimum corresponds to estimated intakes of 2.2 µg/day and 1.6 µg/day for infants aged 0–<6 months and 6-<12 months, respectively, which would meet the ANZ AI amounts set for both age groups (2 µg/day and 3 µg/day for 0–6 and 7–12 months, respectively, based on the assumption that the older age group would receive 50% of nutrient intake from formula and 50% from complementary foods).

No evidence has emerged indicating that formula fed infants are at risk of chromium or molybdenum deficiency or low intakes. Therefore, the absence of a minimum amount as set in both Standard 2.9.1 and Codex STAN 72-1981 was determined to be unlikely to pose a risk to infant health.

Maximums

Voluntary maximum amounts for chromium (2 µg/100 kJ) and molybdenum (3 µg/100 kJ) have been specified in Standard 2.9.1 but Codex STAN 72-1981 specifies no limit for these minerals. The voluntary maximum was set as a precaution (ANZFA 1999a). The EC SCF did not recommend setting maximum amounts as there are no known adverse effects associated with high intakes of chromium or molybdenum from food. Therefore, removing the voluntary maximum amounts for chromium and molybdenum would be unlikely to pose a risk to infant health.

3.7.11 Conclusion – minerals and electrolytes

Table 19 summarises results of the comparative analysis for minerals and electrolytes in infant formula. For some chloride, sodium, and magnesium, no specific issues were found in relation to any of the assessment criteria (represented by ✓ in Table 18) and it was concluded that use of Codex STAN 72-1981 for these nutrients would be unlikely to pose a risk to infant health.

Some issues were identified for the remaining minerals or electrolytes (potassium, calcium, phosphorus, iodine, copper, zinc, manganese, chromium, molybdenum; issues identified represented by ✗ in Table 19). In these cases, further analysis indicated that use of Codex STAN 72-1981 for these nutrients also would be unlikely to pose risk to infant health.

Only iron and selenium did not meet the assessment criteria and further analysis indicated that use of Codex STAN 72-1981 for these nutrients could pose a risk to infant health. For both of these nutrients, multiple issues were identified:

- Iron: potential risk of iron deficiency using the Codex STAN 72-1981 minimum which is lower than Standard 2.9.1; debate about the bioavailability of iron in infant formula; Codex STAN 72-1981 does not set maximum amount [11]

- Selenium: Codex STAN 72-1981 minimum is aligned with Standard 2.9.1 but does not meet AI, current science indicates that the minimum amount should be increased; Codex STAN 72-1981 maximum potentially exceeds UL but there is no international consensus on the appropriate value for the UL.

Table 19: Summary of comparative analysis: minerals and electrolytes[1]

Mineral or electrolyte	Codex STAN 72-1981					Further analysis	Conclusion
	Minimum			Maximum			
	Is it aligned with Std 2.9.1*?*	*Does it compare with breast milk?*	*Does intake from min meet AI[2]?*	*Is it aligned with* Std 2.9.1*?*	*Is intake from max less than UL[2]?*	*Issue examined:*	*Use of Codex STAN 72-1981:*
Sodium	✓	✓	✓	✓	#	No issues.	Unlikely to pose a risk to infant health.
Chloride	✓	✓	#	✓	#	No issues.	Unlikely to pose a risk to infant health.
Potassium	×	✓	×	✓	#	Codex min is substantially less than Std 2.9.1 and does not meet AI	Unlikely to pose a risk to infant health.
Calcium	×	✓	✓	✓	#	Calcium: phosphorus ratio is different in Codex	Unlikely to pose a risk to infant health.
Phosphorus	×	✓	✓	✓	#	Calcium: phosphorus ratio is different in Codex	Unlikely to pose a risk to infant health.
Magnesium	✓	✓	✓	✓	#	No issues.	Unlikely to pose a risk to infant health.
Iron	×	×	✓	#	✓	Codex min substantially lower than Std 2.9.1, does not meet AI; bioavailability; no max in Codex	Could pose risk to infant health
Zinc	✓	✓	✓	×	#	Codex max lower than Std 2.9.1 and GUL; ratio to other nutrients	Unlikely to pose a risk to infant health.
Iodine	×	×	×	×	#	Codex min and max different from Std. 2.9.1; min does not compare to BM and does not meet AI	Unlikely to pose a risk to infant health.
Copper	×	✓	✓	×	#	Codex min and max substantially different from Std 2.9.1	Unlikely to pose a risk to infant health.
Manganese	✓	✓	✓	×	#	Codex max is a GUL	Unlikely to pose a risk to infant health.
Selenium	✓	×	×	×	×	Codex min does not meet AI; Codex max exceeds UL	Could pose risk to infant health
Chromium	#	✓	✓	×	#	Codex max is GUL	Unlikely to pose a risk to infant health.
Molybdenum	#	✓	#	×	#	Codex max is GUL	Unlikely to pose a risk to infant health.

[1] ✓= Codex amount meets this criteria; × = Codex amount does not meet this criteria (see text in Section 3.7); # = not defined. Abbreviations: St.291 = Standard 2.9.1; min = minimum amount; max = maximum amount; AI = adequate intake; UL = upper level of intake; GUL = guidance upper level; BM = breast milk.
[2] *See Section 2.2.3 for assumptions that were applied in this comparison*

3.8 Other optional substances

Other optional substances[12] in infant formula are choline, L-carnitine, and inositol which are set as optional nutrients in Standard 2.9.1 but are listed as mandatory nutrients in Codex STAN 72-1981.

Choline was classified as an essential nutrient by the IOM in 1998 and by the NHMRC and MoH in 2006. Carnitine and inositol are classified as conditionally essential. Conditionally essential refers to nutrients that are normally synthesised endogenously but must be supplied exogenously for specific populations that cannot synthesise them in adequate amounts. Carnitine and inositol are considered conditionally essential for infants mainly because they may lack the developmental maturity for endogenous synthesis.

When infant formula regulations in Australia and New Zealand were last reviewed (1995–2002), permission to add choline, carnitine, and inositol as optional substances was consistent with international standards and regulations at the time. Since then, Codex and European regulations, as well as the recommendations of key reviews, have changed so that addition of these substances is now mandatory (Table 20).

Table 20: Recommendations and regulations for the addition of choline, L-carnitine, and inositol

Review or Regulation	Regulatory provision or recommendation		
	Choline	***L-Carnitine***	***Inositol***
Standard 2.9.1	optional	optional	optional
Codex STAN 72-1981	mandatory	mandatory	mandatory
CFR Title 21 part 107	mandatory for non-milk based	not specified	mandatory for non-milk based
1991 EC Directive *	optional	optional; mandatory for soy protein isolate	optional
Reviews			
1998 LSRO	mandatory	mandatory	mandatory
1998 ANZFA P93	optional	optional	optional
2003 EC SCF	mandatory	mandatory for soy protein isolate and hydrolysed protein	mandatory
2006 EC Directive	mandatory	mandatory for soy protein isolate and hydrolysed protein	mandatory
2014 EFSA	mandatory	mandatory	mandatory

* Precedes the current EC Directive 2006

Although optional, Standard 2.9.1 defines minimum and maximum amounts such that if choline, carnitine or inositol is added, then it must be added within a prescribed range. Standard 2.9.1 amounts are based on the recommendations of the LSRO (1998) and are generally comparable to amounts in Codex STAN 72-1981 (detailed below). The minimum and maximum amounts permitted in Standard 2.9.1 and Codex STAN 72-1981 refers to the

[12] Nucleotides and taurine are substances that are optional additions to infant formula in both Standard 2.9.1 and Codex. As such, and because they are not essential nutrients, these have not been included in this assessment.

total amount, i.e. it includes any naturally occurring substance present in source ingredients (i.e. cow's milk).

No adverse effects in infants consuming choline, carnitine and inositol-supplemented formulas have been reported. Thus, the prescribed current amounts have an extended history of safe use both in ANZ and overseas. Therefore, the safety aspect of infant formula supplemented with these nutrients has not been examined further in this assessment.

The sections below describe the evidence that supports the mandatory inclusion of choline, carnitine, and inositol to infant formula. Data tables summarising the comparative analysis for these substances is provided in Appendix 2.

3.8.1 Choline

Choline is an amine that is a precursor for the neurotransmitter acetylcholine and phospholipids (such as phosphatidyl choline). Choline also functions as a methyl group donor in methyl transfer reactions (Zeisel and da Costa 2009). It is required for cell membrane biosynthesis and therefore is essential for tissue growth and neurodevelopment. Choline can be synthesised *de novo* but dietary sources are needed in response to greater demand or impaired synthesis. Breastfed infants are thought to obtain most of their choline from breast milk.

Minimum and comparison to breast milk

The total choline concentration in breast milk is about 1250 µmol/L or in the range of 160-210 mg/L (EFSA 2014) with similar concentrations in cow's milk (Holmes-McNary et al. 1996; Ilcol et al. 2005). Choline in milk is present in several forms: free choline, phosphocholine, glycerophosphocholine, phosphatidyl choline, and sphingomyelin. The amounts of these forms vary considerably in breast milk and in different types of bovine-derived infant formulas (Holmes-McNary et al. 1996). Variability in breast milk is attributed to gestational age, stage of lactation, maternal choline intake, and genetic factors that alter milk choline concentrations. Bovine-derived formulas contain less phosphocholine than breast milk (Holmes-McNary et al. 1996). In formula, the forms of choline vary as a result of processing where lipid soluble (phosphatidylcholine and sphingomyelin) and water soluble (free choline and phosphocholine) forms may be fractionated differently during processing of cow's milk. Specific functional roles of the different forms of choline have not been shown so nutritional requirements are specified in terms of total choline.

Standard 2.9.1 currently permits optional addition of choline in the range of 1.7–7.1 mg/100 kJ based on the range recommended by the LSRO (1998). The LSRO minimum was derived from the lower end of the range for choline content in breast milk. The range in Codex STAN 72-1981 is 1.7–12 mg/100 kJ with the minimum (also based on LSRO) set as a mandatory minimum and the maximum as a GUL. The derivation of the higher maximum in Codex STAN 72-1981 was explained but is probably based on extrapolation of the adult safe level of intake and correcting for potential age differences in metabolism (EC Scientific Committee on Food 2003).

The ANZ AI is 125 mg/day for infants aged 0–6 months and 150 mg/day for infants aged 7–12 months (NHMRC and MoH 2006). For infants aged 0–<6 months, the estimated intake based on the Standard 2.9.1 and Codex STAN 72-1981 minimum (1.7 mg/100 kJ) and assuming the midpoint energy content (2725 kJ/L) and the mean intake volume (0.8 L/day) would be 37 mg/day. Similarly, the estimated intake for infants aged 6–<12 months would be 28 mg/day assuming a mean intake volume of 0.6 L/day. Thus, neither age group would meet the ANZ AI. However, the typical amount would likely to be in the mid-range of 1.7–12 mg/100 kJ (Codex STAN 72-1981 amounts for choline) and using the midpoint of this range

(6.9 mg/100 kJ), estimated intakes would be 150 mg/day and 113 mg/day for infants 0–<6 and 6–<12 months respectively. Thus, the Codex STAN 72-1981 midpoint choline concentration could meet the ANZ AI for both age groups (assuming the older infants obtain 50% of their choline intake from formula and 50% from complementary foods).

The EFSA (2014) recommendation for choline was to increase the minimum content to 6 mg/100 kJ on the basis that it would meet the AI of 130 mg/day estimated from intakes for breastfed infants.

Changing choline from an optional addition to a mandatory nutrient as in Codex STAN 72-1981 will increase choline intakes for formula-fed infants. In consideration of the recent EFSA recommendation, there is some uncertainty in the evidence base that underpins the appropriate minimum amount of choline. However, in the absence of evidence showing choline insufficiency in the ANZ population, it is concluded that the mandatory inclusion of choline in the range in Codex STAN 72-1981 is unlikely to pose a risk to infant health.

Maximum

The Codex STAN 72-1981 maximum amount (12 mg/100 kJ) is a GUL and higher than the Standard 2.9.1 maximum (7.1 g/100 kJ). The Codex STAN 72-1981 maximum was based on based on extrapolation from adult data on the safe level of intake and allowing for potential age differences in metabolism (EC SCF 2003). However this basis predates more recent evidence that excess choline consumption may be linked to development of cardiovascular disease (Tang and Hazen 2014). The new research has shown the choline, a quaternary amine, may not be completely absorbed and amounts that pass through to the large bowel are metabolised by gut microbes to trimethylamine. Trimethylamine is absorbed and metabolised to trimethylamine oxide which is the metabolite associated with increased cardiovascular disease risk. The new evidence has not been demonstrated in infants or children but does suggest uncertainty in the safety of excess dietary choline.

There is no ANZ UL derived for infants because choline supply for infants should be solely from breast milk, formula, and food. A value of 1000 mg/day has been set for children 1–3 years old (based on the adult value of 3500 mg/day and corrected for body weight difference) (NHMRC and MoH, 2006). Applying a similar body weight correction (using the WHO reference weights in Table 17) the upper limit would range from approximately 340–716 mg/day. The estimated intakes based on the Codex STAN 72-1981 maximum would be 155 mg/day and 196 mg/day for infants aged 0–<6 months and 6–<12 months, respectively. Because these amounts are well under an approximated UL, it is concluded that the maximum amount in Codex STAN 72-1981 is unlikely to pose a risk to infant health. However, based on new research cited above, this amount should be mandated.

3.8.2 L-Carnitine

L-carnitine is a quaternary amine that is a co-factor for acyl-transferases. These enzymes function in the transport of FAs across the mitochondrial membrane which is required before FAs can be oxidised and metabolised to produce energy. Carnitine is endogenously synthesised from lysine and methionine but in newborns, an enzyme required for carnitine synthesis (γ-butyrobetaine hydroxylase) has been reported to have 12% of the activity measured in adults (Crill and Helms 2007). The milk of all mammals contains L-carnitine mainly as an unesterified form (referred to as “free” carnitine). As a result, the concentration may be decreased during fractionation and dilution of milk protein in manufacturing.

Carnitine deficiency is assessed by measuring free and total carnitine concentrations in serum. As reviewed by Crill and Helms (2007), infants fed unsupplemented soy-based formula (which contains little or no carnitine) had lower serum carnitine concentration

compared to infants fed carnitine-supplemented soy-based formula suggesting infants lack capacity to synthesise sufficient carnitine. In addition, higher serum FA concentrations (indicating less utilisation of dietary fats for energy) were reported in some studies for infants on unsupplemented formula, although consequences for growth or development is unclear. Because of the critical role of carnitine in lipid metabolism and the decreased rate of carnitine biosynthesis in infants, L-carnitine has now been considered to be a necessary addition to infant formula with a minimum amount corresponding to breast milk (Crill and Helms 2007; EFSA 2014).

Standard 2.9.1 permits L-carnitine to be added as an optional substance in the range of 0.21–0.8 mg/100 kJ which is comparable to the amount measured in breast milk (0.27–0.42 mg/100 kJ). The LSRO (1998) recommended a range of 0.28–0.48 g/100 kJ based on breast milk concentration. The previous Australian standard (ANZFA 1999a) specified a minimum (0.27 mg/100 kJ) with no maximum specified, consistent with European recommendations in the early 1990's (LSRO 1998, p.62). The maximum amount prescribed in Standard 2.9.1 was increased from the LSRO recommendation (0.48 mg/100 kJ) to accommodate the natural amount of carnitine that is typically found in cow's milk infant formulas (ANZFA 1999a).

The Codex STAN 72-1981 minimum amount (0.3 mg/100 kJ) was derived to allow for a presumed decrease in bioavailability in formula (EC SCF 2003) and is a mandatory inclusion in infant formula. The recent EFSA review (2014) reached the same conclusion on the basis that no new data had emerged to suggest that this amount was insufficient to support growth and development. Therefore, it is concluded that the mandatory inclusion of carnitine at the amount in Codex STAN 72-1981 is unlikely to pose a risk to infant health.

A maximum amount has not been specified in Codex STAN 72-1981 and no discussion about this was provided in the EC SCF review (2003). There is no evidence in infants or in children indicating that they consume excess carnitine or that carnitine consumption is linked with adverse health outcomes. However, recent studies suggests that, similar to choline, non-absorbed carnitine is metabolised by microbes in the large bowel to trimethylamine, a compound that may be associated with the development of cardiovascular disease (Koeth et al. 2013)The new research indicates uncertainty in the safety of excess carnitine consumption. Therefore, it is concluded that the lack of a specification for the maximum amount in Codex STAN 72-1981 may pose a risk to infant health.

3.8.3 Inositol

Inositol is a sugar alcohol that is phosphorylated to various phosphoinositides functioning in transmembrane receptor signalling and lipid synthesis. Inositol is present in human tissues predominantly as *myo*-inositol [13] as free or phosphorylated forms which are endogenously synthesised from glucose. Although human deficiency has not been demonstrated, infants may have a dietary requirement for inositol based on (1) high *myo*-inositol content in breast milk; (2) high inositol concentrations measured in the serum and tissues of neonates; and (3) evidence suggesting a role for inositol and/or phosphoinositides in infant lung maturation and surfactant synthesis (Cavalli et al. 2006; Brown et al. 2008; Howlett et al. 2012).

Decreased capacity of endogenous inositol synthesis has not been shown in infants. However, studies measuring the plasma appearance rate of inositol in response to feeding supplemented and unsupplemented formula suggest that both *de novo* and dietary inositol are required to meet metabolic requirements for inositol in healthy, term infants (Brown 2009).

[13] Myo-inositol refers to one of nine possible stereoisomers of inositol.

Cavalli et al (2006) reported the free inositol concentration in breast milk collected over 3–29 days after delivery from term pregnancies to be 991 µmol/L which is equivalent to 178 mg/L or 6.57 mg/100 kJ (based on formula with the midpoint energy content of 2725 kJ/L). The concentration measured in mature breast milk (> 4 weeks post-partum) was reported by EFSA (EFSA 2014) to be 130–325 mg/L. However, breast milk inositol concentrations have been noted to be highly variable and to decline with time of lactation (Pereira et al. 1990).

Standard 2.9.1 specifies the optional addition of inositol of 1.0–9.5 mg/100 kJ based on the recommendation of the LSRO (1998). Codex STAN 72-1981 prescribes the same minimum amount but as a mandatory amounts and the same maximum amount but as GUL. The recent EFSA review on infant formula composition also recommended the mandatory inclusion of inositol at the existing Codex STAN 72-1981 amounts in infant formula because it is uncertain whether infants have capacity to synthesise adequate endogenous inositol (2014). Neither standard has set a mandatory maximum but it has been suggested that the upper level should be around that reported for breast milk (9.6 mg/100 kJ) (LSRO 1998). No safety data or negative health effects related to inositol in infants or children have been reported.

On the basis of the above evidence, inclusion of inositol according to Codex STAN 72-1981 is unlikely to pose a risk to infant health.

3.8.4 Conclusion – other permitted substances

Codex STAN 72-1981 prescribes mandatory inclusion of carnitine, choline and inositol, whereas these three components are optional permitted substances in Standard 2.9.1. These substances, which are required for growth and development, may be synthesised *de novo* but the efficacy of these pathways in infants is not completely known.

To date, only choline has achieved the status of being classed as an essential nutrient in some countries, including ANZ (NHMRC and MoH, 2006). The minimum choline amount is insufficient to meet the AI set for ANZ. However changing choline to be a mandatory addition is likely to improve intakes for formulated infant even based on a low minimum amount.

Carnitine and inositol are conditionally essential. Evidence supporting their mandatory addition includes presence in breast milk, low serum concentrations and physiological or biochemical outcomes suggesting inadequacy in infants fed unsupplemented formulas.

3.9 Summary

Infant formula nutrient composition was assessed to determine whether the compositional requirements in Codex STAN 72-1981 would pose a nutritional health risk to ANZ infants. Using a systematic comparative approach using several defined criteria, all essential nutrients and several nutritive substances were assessed for nutritional safety and adequacy. Overall, most nutrient amounts and other prescribed factors such as permitted forms and nutrient ratios were found to be consistent with scientific evidence and were concluded to unlikely pose a risk to infant health.

The evidence base for certain aspects for three nutrients – linoleic acid, selenium and iron – was found to be uncertain. For these nutrients, it was concluded that Codex STAN 72-1981 could pose a risk to infant health and that the current Standard 2.9.1 would be less likely to impose risk.

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