codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 2 **CX/NFSDU 08/30/2-Add.1**

September 2008

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES

30th Session,

Cape Town, South Africa

REPORT OF THE ELECTRONIC WORKING GROUP ON METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS (CODEX STAN 72-1981)

SUMMARY OF RECOMMENDATIONS

- The eWG method recommendations are in TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72. Method recommendations have been made for Type I, Type II, Type III and Type IV methods.
- For a number of the Type III methods the eWG has not been able to select one Type II Reference Method. The eWG proposes that guidance should be requested from CCMAS on selection criteria, which may enable CCNFSDU to recommend which of these Type III methods can be selected as Type II.
- The eWG recommends the methods of analysis for infant formula are made clear in Codex STAN 234 in the section titled “Foods for Special Dietary Uses” by using the description “Infant Formula” in the column titled “Commodity Standard”.
- The eWG recommends that when method of analysis recommendations are for specific forms of vitamins, this information should be included in Codex STAN 234 when the method is endorsed, and that CCNFSDU should review this information to ensure that results obtained by different methods covering different forms of the same vitamin adequately express the intention of the Committee in STAN 72.
- The eWG recommends CCNFSDU should provide clarification on the units of expression for a number of other vitamins and choline in STAN 72 by including a note with the methods stating the units of expression for these substances.
- The eWG recommends that methods in the proposed “Infant Formula” list of Codex Stan 234 are periodically reviewed to keep them up to date.

BACKGROUND

The EWG was established by the 29th session of CCNFSDU (ALINORM 08/31/26)

Paragraph 153) The electronic working group (EWG) should prepare a list of methods of analysis for infant formulae to be considered at the 30th Session of the CCNFSDU in 2008. In preparing this list, the EWG should:

- *Review methods of analysis for provisions listed in Section 3.1 of the Codex Revised Standard for Infant Formula and Formulas for Special Medical Purposes Intended for infants;*
- *Follow the Principles for the establishment of Codex Methods of Analysis in the Codex Procedural Manual, including the General Criteria for the Selection of Methods of Analysis:*
- *The electronic working group, chaired by New Zealand, would be open to all members and observers, and would work in English*

Expressions of interest to participate in the eWG were received from twenty-one countries and organisations in January 2008. The members of the eWG are from Brazil, Canada, Croatia, Czech Republic, Denmark, European Community, France, Germany, Hungary, Israel, Japan, Malaysia, Norway, New Zealand, Republic of Korea, Thailand, The Netherlands, USA, IDF, ISDI and ISO. A background paper providing information, asking questions and requesting method details and supporting criteria was sent to the working group members in March 2008. Responses were received from eWG participants, summarised and distributed to eWG members in July for review. Working group members provided further information for certain methods of analysis and the process for selection of methods of analysis was reviewed. The method recommendations were revised and circulated to the eWG in early September and the report was finalised at the end of September.

The eWG chair would like to thank all working group members for their participation in the working group, the co-operative manner in which information was provided, and the extensive provision of method recommendation material.

CRITERIA FOR SELECTION OF METHODS OF ANALYSIS BY THE EWG ON METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL DIETARY USES (CODEX STAN 72 -1981 REVISION 2007)

The terms of reference in para 153 of ALINORM 08/31/26, state the eWG is to "follow the Principles for the Establishment of Codex Methods of Analysis in the Codex Procedural Manual, including the General Criteria for the Selection of Methods of Analysis." The Codex Procedural Manual (17th edition) lists the criteria shown in italics on pages 74 and 75.

General Criteria for the Selection of Methods of Analysis

(a) *Official methods of analysis elaborated by international organizations occupying themselves with a food group or group of foods should be preferred*.

(b) Preference should be given to methods of analysis, the reliability of which have been established in respect of the following criteria, selected as appropriate:

(i) specificity

(ii) accuracy

(iii) precision; repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories)

(iv) limit of detection

(v) sensitivity

(vi) practicability and applicability under normal laboratory conditions

(vii) other criteria which may be selected as required.

(c) The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.

(d) All proposed methods of analysis must have direct pertinence to the Codex Standard for which they are directed.

(e) *Methods of analysis which are applicable uniformly to various groups of commodities should be given preference over methods which apply only to individual commodities*.

The eWG received nearly 200 method proposals for the provisions in Section 3.1 of the Codex Revised Standard for Infant Formula and Formulas for Special Medical Purposes Intended for infants.

The methods most directly pertinent for analysis of infant formula are those that have been validated for infant formula. When a method for a provision elaborated by an international organization, such as AOAC, CEN, ISO, and IDF was proposed, the eWG noted that these organizations follow rigorous method selection processes for their purposes of methods evaluation; however, the international organizations have not evaluated their methods for the purpose of recommending a Type II method for analysis of infant formula. The eWG was able to recommend that a method be considered as a Type II or Type III method for infant formula if the validation included collaborative laboratory studies on infant formulas or similar and applicable food matrices.

The eWG did not recommend Type I, Type II, or Type III methods of analysis for infant formula and formulas for special medical purposes intended for infants that are not elaborated by international organisations.

AOAC and EN methods for chromium and molybdenum were recommend as Type IV (tentative methods) since, although EN and AOAC methods were proposed, these had not included infant formula in the validation. Type IV (tentative method) recommendations are also made for trans fatty acids, total phospholipids, folic acid and selenium.

A Type I (defining method) recommendation is made for total fat.

Type II (reference methods) recommendations are made for total carbohydrates, iron, calcium, phosphorus, magnesium, chloride, manganese, iodine, copper, zinc and choline.

Type III (alternative approved methods) method recommendations are made for calories, vitamin A, vitamin D, vitamin K, thiamine (Type III or IV), niacin, vitamin B_6, pantothenic acid, folic acid, iron, calcium, phosphorus, magnesium, manganese, copper and zinc.

When several methods are recommended as Type III the eWG has not made a recommendation to select one as Type II unless information provided to the working group clearly distinguishes one method for selection as Type II. The ability to recommend Type III methods but not select the Type II method was the case for the provisions in section 3.1, for fatty acids, trans fatty acids, vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, niacin, vitamin B_6, vitamin B_{12}, vitamin C and biotin. The eWG referred to the Codex Procedural Manual (17^{th} edition) for guidance on the criteria for selection of a Type II reference method and found that the procedure was not sufficiently clear for the eWG to agree on criteria. Consequently the eWG has recommended these methods to CCMAS as Type III methods and proposes that further guidance should be sought from CCMAS on selection criteria for the Type II reference method.

The Codex Procedural Manual, page 74, defines Type II Reference Methods, which are methods recommended for use in case of disputes and for calibration purposes, and Type III Alternative Approved Methods, which are methods that may be used for control, inspection or regulatory purposes. A Type II method is the one designated Reference Method, which should be selected from Type III methods, but the Procedural Manual does not explain the selection process. The procedure also describes the Criteria Approach where method criteria may be identified and values quantified for incorporation into the Codex commodity standard, as an alternative to recommending specific methods of analysis.

The Procedural Manual explains on page 107 that the normal practice when Codex Committees have included provisions on methods of analysis is the Codex Committee should provide information for each method to the Codex Committee on Methods of Analysis and Sampling.

The terms of reference for the eWG in paragraph 153 of ALINORM 08/31/26 are to review methods of analysis for provisions listed in section 3.1 and to follow the Principles for the Establishment of Codex Methods of Analysis in the Codex Procedural Manual, including the General Criteria for the Selection of Methods of Analysis.

One member of the eWG proposed an approach to applying the General Criteria to facilitate making recommendations on Type II methods which involved use of a template to compile the relevant selection criteria parameters and provide a basis to compare the available scientific information.

The eWG proposes that CCNFSDU should request comment from CCMAS on the CCNFSDU approach to applying the general criteria for selection of methods as well as any additional guidance from CCMAS on the selection criteria for a Type II method that could be applied by CCNFSDU to recommend a Type II method. In the case that CCNFSDU is still unable to select a Type II method for any of these provisions, consideration may be given to the Criteria Approach as an alternative

The eWG provides detailed information in the TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72 as this may be useful for CCMAS endorsement of the method recommendations.

CODEX STAN 234-1999 RECOMMENDED METHODS OF ANALYSIS AND SAMPLING

Codex Stan 234-1999 Methods of Analysis by Alphabetical Order of Commodity Categories and Names, Part A includes Foods for Special Dietary Uses. Although methods of analysis for infant formula were previously listed in this section, there are no methods currently listed for infant formula.

The eWG recommends the methods of analysis for infant formula are made clear in Codex Stan 234 in the section titled “Foods for Special Dietary Uses” by using the description “Infant Formula” in the column titled “Commodity Standard”. If the CCNFSDU believes it is necessary to provide further clarification, a footnote should be added to indicate that the analytical methods listed specifically for infant formula should be used for infant formula and formulas for special medical purposes intended for infants.

Some methods recommended are for specific forms of the provision in section 3.1 and this information should be included in Codex Stan 234 when the method is endorsed. In addition, the method listed in Codex STAN 234 should include a note clarifying the units of expression when this is part of the provisions in Codex STAN 72 e.g. Vitamin C (expressed as ascorbic acid).

The eWG recommends that methods in the proposed “Infant Formula” list should be periodically reviewed to keep them up to date.

RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72

The eWG method recommendations are in TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72.

Method recommendations have been made for Type I, Type II, Type III and Type IV methods.

For a number of the Type III methods (fatty acids, trans fatty acids, vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, niacin, vitamin B_6, vitamin B_{12}, vitamin C and biotin) the eWG has not been able to select one Type II Reference Method. The eWG proposes that guidance should be requested from CCMAS on selection criteria, which may enable CCNFSDU to recommend which of these Type III methods can be selected as Type II.

The eWG includes answers to the questions raised by the 28th and 29th sessions of CCMAS in the Table 1 with further clarification provided as follows;

In March 2007 the 28th session of CCMAS identified that the CCNFSDU needed to further consider the proposed methods since many required updating and agreed to refer all methods back to CCNFSDU. This is recorded in the CCMAS ALINORM (07/30/23) paras 81 to 86. In particular the Committee made the following comments:

Paragraph 83) In general, methods using microbioassay as a principle should be reviewed, as well as the methods for determination of PER, carbohydrates and fat in order to replace then with more modern methods.

The eWG has reviewed methods using microbioassay and made recommendations in the table. The eWG continues to support the recommendation of certain methods based on microbioassay. In March 2008, the 29th session of CCMAS, ALINORM 08/31/23 agreed to delete methods for dietary fibre and PER. The eWG recommends methods for carbohydrates and fat in the table.

Paragraph 84) Clarification was required as to how Vitamin C was expressed and on the differences between the methods proposed for Vitamin K, B12 and B6.

The eWG provides clarification in the table as to how vitamin C is expressed for the recommended methods and clarification on the differences between methods proposed for Vitamin K, B12 and B6.

Paragraph 85) It was recommended that the method for sodium and potassium be replaced with the ISO 8070/IDF 119.2007 method (atomic absorption).

Paragraph 86) As regards crude protein, the committee agreed that the conversion factors included in the method proposed corresponded to the earlier standard and recommended that the CCNFSDU correct the conversion factor for soy protein to 5.71 in the description in the method in order to be consistent with the provision in the revised standard.

In March 2008, the 29th session of CCMAS, ALINORM 08/31/23 deliberated on dietary fibre and endorsed the ISO and IDF method for sodium and potassium.

Paragraph 55) The Committee noted the replies by the Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) and agreed to delete methods for dietary fibre and PER. While agreeing to the request from CCNFSDU to delete the methods for dietary fibre, the Committee noted out that a method for dietary fibre was necessary to calculate total energy and agreed to request the CCNFSDU to reconsider inclusion of methods for dietary fibre.

The eWG recommends dietary fibre is not necessary to calculate the total energy as there is insignificant indigestible carbohydrate in infant formula. A method recommendation for dietary fibre has not been made. The recommended method for total carbohydrates includes any dietary fibre present, and this in turn will be included in the calculation of energy.

Paragraph 56) The Committee endorsed the ISO and IDF method for sodium and potassium as Type II and the AOAC 984.27 method as Type III and agreed to replace the current method for crude protein with AOAC 991.20 or ISO 8968-1/2:2001|IDF 20-1/2:2001 with a footnote on the use of the appropriate conversion factors as proposed by the CCNFSDU.

The eWG recommends no change to these methods as adopted.

NOTE ON THE FORM OF THE VITAMIN IN THE PROVISIONS LISTED IN SECTION 3.1 OF CODEX STAN 72 AND THE VITAMIN FORM DETERMINED BY THE METHOD OF ANALYSIS

Vitamins and related substances exist in various chemical forms such as esters, amides and isomers in foods and in vitamin supplements. The various forms may differ in their bioavailability and their physiological activity. In some but not all cases STAN 72 specifies the form of the vitamin or the form in which the vitamin content is to be expressed.

Many methods of analysis determine the specific forms of vitamins. In order to clarify exactly what form each method determines the recommendations for some of the vitamins in TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72 are accompanied by a note stating the form of the vitamin determined by the method. The eWG recommends that these notes should be adopted with the methods.

It is understood that the intent of Codex STAN 72 is to have forms of the vitamin that are biologically active for the infant. As such, appropriate method specificity must be applied during the selection of methods. The eWG recommends the CCNFSDU should review the notes stating the form of the vitamin to ensure that results obtained by different methods covering different forms of the same vitamin adequately express the intention of the Committee

in STAN 72. The review of the notes should focus on vitamin A, vitamin D, vitamin K, riboflavin, niacin, folic acid, vitamin C, biotin and choline.

The compilation of the recommended methods of analysis has indicated the need for further clarification of the units of expression or conversion factors for some vitamins (e.g. vitamin E, thiamine, vitamin B6, and pantothenic acid) and choline. The eWG recommends the CCNFSDU should provide clarification on the units of expression in STAN 72 by including a note with the methods stating the units of expression for these substances.

The comments in TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72 on the form of vitamins (the first row of the entries for each vitamin) is provided in the eWG report for information to CCNFSDU and is not intended to be passed on to CCMAS.

TABLE 1; RECOMMENDED METHODS OF ANALYSIS FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS, CODEX STAN 72

Points that need particular attention by CCNFSDU are highlighted.

Type III method recommendations which are suitable for consideration as the Type II Reference Method using the guidance on selection criteria requested from CCMAS..*

Provision	Requirement	Method	Principle	Status and eWG Recommendation
Calories (by calculation)	Minimum 60kcal (250kJ), maximum 70kcal (295kJ), per 100ml prepared ready for consumption. Compositional provisions are generally specified per 100 kcal or 100 kJ.	Method described in CAC/Vol IX-Ed.1, Part III	Calculation method	**The eWG recommends this method as Type III** *Notes* 1. Currently adopted as a Type III method for Special foods in CODEX STAN 234-1999, amended 2007. 2. The references in this method (methods of analysis and conversion factors for specific food ingredients) need to be updated.
Protein (Crude protein)	Minimum 1.8g/100kcal (0.45g/100kJ) cows' milk protein or minimum 2.25g/100kcal (0.5g/104kJ) soy protein; maximum limit 3.0g/100kcal (0.7g/100kJ); no	AOAC 991.20 ISO 8968-1/2 | IDF20-1/2: 2001	Titrimetry (Kjeldahl)[2]	**This method should be retained as adopted** *Notes* 1. This method has been adopted (2008) as a defining method (Type I). It was proposed by CCNFSDU 29 and endorsed by CCMAS (08/31/23 para 56), with a footnote on the use of the appropriate conversion factors as proposed by CCNFSDU. 2. Results of interlaboratory study parameters obtained in collaborative study of this method, r value = 0.038 and R value = 0.049. Reference: JAOAC 73: 849 -859 (1990). 3. The provision in the Standard for Infant Formula is "protein"; the method of analysis has been adopted as "crude protein". The method measures total nitrogen. 4. Test portions should remain below 1.5 g for powder product and 5 g for ready-to-feed and concentrated products. These recommendations are based on Appendix III.- Titrant normality, test portion size and measurement of residual sulfuric acid, as part of a publication by Lynch and Barbano in 1999 (JAOAC 1999, vol. 82:6, pp. 1389-1398) and a

[1] Guidance upper levels

	GUL[1].			survey of the composition values from the USDA nutrient database for infant formula (http://www.nal.usda.gov/fnic/foodcomp/search/).
Amino acid profile	For an equal energy value, IF[3] must contain an available quantity of each essential and semi-essential amino acid equal to that of breast milk (defined in Annex 1)	**No suitable published methods are available**		
Total fat	Minimum 4.4g/100kcal (1.05g/100kJ); maximum 6.0g/100kcal (1.4g/100kJ).	AOAC 989.05 ISO 8381:2000/ IDF 123A:1988	Gravimetry (Röse-Gottlieb)	**The eWG recommends this method should apply to milk-based infant formula containing ≤ 5% starch or dextrin, Type I** *Notes* 1. Validated for milk-based infant formulae, except formulae containing starch or dextrin. Reference: Bulletin of the IDF (1988), N°235, J Eisses, *Methods for the determination of the fat content, part 3, Infant foods, edibles ices, milk and milk products (special cases), Determination of the fat content according to Röse-Gottlieb or Weibull-Berntrop* 2. Normally regarded as a defining method (Type I). 3. Note from IDF/ISO: this standard will be published as ISO 8381|IDF 123:2008 by the end of this year.
Total fat	Minimum 4.4g/100kcal (1.05g/100kJ); maximum 6.0g/100kcal (1.4g/100kJ).	ISO 8262-1 |IDF 124-1: 2005	Gravimetry (Weibull-Berntrop)	**The eWG recommends this method should apply to milk-based infant formula, Type I** *Notes* 1. Validated. References: Schuller, P.L. *Report of the collaborative study of CX/MAS on fat determination in infant foods.* Codex Committee on Methods of Analysis and Sampling, CX/MAS 75/10,1975 Bulletin of the IDF (1988), N°235, J Eisses, *Methods for the determination of the fat content, part 3, Infant foods, edibles ices, milk and milk products (special cases), Determination of the fat content according to Röse-Gottlieb or Weibull-Berntrop* 2. Normally regarded as a defining method (Type I).

[2] The calculation of the protein content of infant formulas prepared ready for consumption may be based on N x 6.25, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The value of 6.38 is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of 5.71 as a specific factor for conversion of nitrogen to protein in other soy products.

[3] Infant formula

Fatty acids	Lauric and myristic fatty acids combined <20% total fatty acids. Erucic acid <1% total fatty acids. LA[4] minimum 300mg/100kcal (70mg/100kJ); no maximum; GUL 1400mg/100kcal (330mg/100kJ). ALA[5] minimum 50mg/100kcal (12mg/100kJ); no maximum limit nor GUL specified. PUFA[6] is needed for calculation of α-TE content (see vitamin E).	AOAC 996.06	Gas chromatography	**The eWG recommends this method should be considered for adoption as Type III*** *Notes* 1. Proposed by CCNFSDU 28 and recommended in CRD 10 2. Validated (but not for infant formulas). References: J.AOAC Int. 80: 555 - 563 (1997). J.AOAC Int. 82: 1146 - 1155 (1999). 3. Adopted as Type II for determination of saturated fat for nutrition labelling purposes. 4. Information should be adequate for listing as a reference method (Type II), or if not, a tentative method (Type IV).
Trans fatty acids	≤ 3% of total fatty acids	AOCS Ce 1h-05	Gas liquid chromatography	**The eWG recommends this method as Type III*, for infant formulae not containing milkfat** *Notes* 1. Method for Determination of *cis*, *trans*, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-Ruminant Animal Oils and Fats. 2. Validated (but not for infant formula). Performance statistics were extracted from the collaborative study report and are included with the method. 3. Adopted as Type II for the purposes of the Guidelines for Nutrition Labelling 4. The method states "The method is not suitable for the analysis of dairy, ruminant, marine, long chain polyunsaturated (PUFA) fats and oils, or products supplemented with conjugated

[4] Linoleic acid

[5] Alpha-linoleic acid

[6] Polyunsaturated fatty acids

				linoleic acid (CLA)." The method should therefore be recommended for infant formulae not containing milkfat.
Trans fatty acids	≤ 3% of total fatty acids	AOAC 996.06	Gas chromatography	**The eWG recommends this method as Type IV, with optimisation for the determination of TFAs** *Notes* 1. Method for quantitation of individual fatty acids, including trans 2. A publication describing an improved procedure for the determination of trans fatty acids is available under "Proposed Modifications to AOAC 996.06, Optimizing the Determination of Trans Fatty Acids: Presentation of Data; Rozemat at.: *J. AOAC Int'l*, VOL. 91, NO. 1, 2008" 3. Validated (but not for infant formulas). References: J.AOAC Int. 80: 555 - 563 (1997). J.AOAC Int. 82: 1146 - 1155 (1999).
Total phospholipids	≤ 300mg/100kcal (72mg/100kJ)	AOCS Ja7b-91	Gas liquid chromatography	**The eWG recommends this method as Type IV with suitable extraction and preparation procedures** *Notes* 1. The method is applicable to oil-containing lecithins, deoiled lecithins, lecithin fractions; not applicable to lyso-PC and lyso-PE. 2. Validated. Reference Pure Appl. Chem. 64: 447 - 454 (1992). A summary of statistics from the IUPAC phospholipid collaborative study is included with the method. 1. Suitable extraction and preparation procedures applicable to infant formulae are needed in conjunction with this method. The Walstra & de Graaf procedure for the extraction of the fat is suitable. Reference: Walstra, P. & de Graaf, J. J. (1962) *Note on the determination of the phospholipid content of milk products*. Netherlands Milk & Dairy J., 16, 283-287. 2. Recommended as a tentative method (Type IV) since the method is not validated for infant formula.
Total carbohydrates	Minimum 9.0g/100kcal (2.2g/100kJ); maximum 14.0g/100kcal (3.3g/100kJ).	AOAC 986.25	Determination by difference, i.e. the remainder after deducting fat, ash and crude protein from total solids.	**The eWG recommends this method as Type II.** *Notes* 1. Validated. Reference: JAOAC 69: 777 - 785 (1986). 2. Recommended in CRD 10
Dietary fibre		**The eWG recommends Dietary fibre is not necessary to calculate the total energy as there is insignificant indigestible carbohydrate in infant formula.** *Notes* 1. CCNFSDU29 considered methods for dietary fibre (paragraph 156) and did not recommend including a method for any substance or provision that was not included in section 3.1. 2. CCMAS 29 (paragraph 55) noted the replies of the CCNFSDU and agreed to delete methods for dietary fibre. While agreeing to the request from CCNFSDU to delete the methods for dietary fibre, CCMAS noted that a method for dietary fibre was necessary		

<table>
<tr><td></td><td></td><td colspan="3">to calculate total energy and agreed to request the CCNFSDU to reconsider inclusion of methods for dietary fibre.
3. The recommended method for total carbohydrates includes any dietary fibre present, and this in turn will be included in the calculation of energy.
4. ISDI notes the lack of a definition for dietary fibre, and comments that there is no need to have methods for dietary fibre for infant formula.
5. A method for dietary fibre, total in Special foods (AOAC 985.29) is listed in the current version of Codex Stan 234, and a method for dietary fibre, total in infant formula (AOAC 991.43) was listed in Codex Stan 234 (2006 revision).</td></tr>
<tr><td>Moisture/Total Solids</td><td></td><td colspan="3">The eWG requests CCNFSDU to consider whether a method is needed for moisture/total solids.
Notes
1. CCNFSDU29 (paragraph 156) did not recommend including a method for any substance or provision that was not included in section 3.1.
2. However estimation of moisture content (total solids) is needed for calculation of carbohydrates and calories.
3. If a method is included, it would be a defining method (Type I or Type IV depending on the adequacy of validation). Alternative methods would need to be calibrated against this method.
4. Types of methods: The Karl Fischer method measures water, including water of crystallisation, whereas drying methods (roughly speaking) do not include water of crystallisation. If a Karl Fischer method is adopted this may necessitate reconsideration of the compositional provisions.
5. Terminology: In powders the results are generally reported as moisture, and in liquids as total solids or dry matter.
6. Methods are listed in Codex Stan 234 for “loss on drying” in special foods (AOAC 934.01, AOAC 925.23; milk based special foods 925.23, IDF Standard 21B:1987 and ISO 6731:1989).</td></tr>
<tr><td>Ash</td><td></td><td colspan="3">The eWG requests CCNFSDU to consider whether a method is needed for ash.
Notes
1. CCNFSDU29 (paragraph 156) did not recommend including a method for any substance or provision that was not included in section 3.1.
2. However estimation of ash content is needed for calculation of carbohydrates and calories.
3. A method is listed in Codex Stan 234 for ash in special foods (AOAC 942.05).</td></tr>
<tr><td>Vitamin A</td><td colspan="4">Note on the form of Vitamin A in Codex Standard 72
Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin A
Vitamin A: expressed as retinol equivalents (RE).
1 µg RE = 3.33 IU Vitamin A = 1 µg all-trans retinol. Retinol contents shall be provided by preformed retinol, while any contents of carotenoids should not be included in the calculation and declaration of vitamin A activity.
<u>Comment:</u> Carotenoids are unequivocally excluded from declaration of vitamin A content.
The requirement that vitamin A content shall be provided by “preformed retinol” implies only naturally present retinol, and excludes the common vitamin A acetate and palmitate supplements. These forms are physiologically active and may be quantified either specifically as intact esters and aggregated with natural retinol, or converted to retinol during analysis. It would seem that the standard should provide for all forms of retinol present in infant formula, whether preformed or derived from supplemental acetate and/or palmitate forms. It does not make sense to exclude vitamin A added for nutrient purposes from this provision and it seems at the least, that “preformed’ should be removed from the standard</td></tr>
<tr><td></td><td>Minimum 60µg/100kcal</td><td>AOAC 992.04 (retinol isomers)</td><td>High performance liquid chromatography</td><td>The eWG recommends this method as Type III*</td></tr>
</table>

Vitamin A	(14µg/100kJ); maximum 180µg/100kcal (43µg/100kJ).	Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)		*Notes* 1. Currently adopted as Type II method for follow-up formula in CODEX STAN 234-1999 rev 2007 and previously listed for infant formula in rev 2006. 2. Proposed by CCNFSDU 28 and recommended in CRD 10 3. Validated: Study matrices included powdered infant formula, powdered milk, and liquid infant formula **4.** Reference: J.AOAC Int. 76: 399 - 413 (1993).
Vitamin A	Minimum 60µg/100kcal (14µg/100kJ); maximum 180µg/100kcal (43µg/100kJ).	AOAC 992.06 (retinol) Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)	High performance liquid chromatography	**The eWG recommends this method as Type III*** *Notes* 1. Currently adopted as Type II method for follow-up formula in CODEX STAN 234-1999, amended 2007. 2. Proposed by CCNFSDU 28 and recommended in CRD 10 3. Reference: J. AOAC Int. 76: 399- 413 (1993).
Vitamin A	Minimum 60µg/100kcal (14µg/100kJ); maximum 180µg/100kcal (43µg/100kJ).	EN 12823-1:2000 (all-trans-retinol and 13-cis-retinol) Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)	High performance liquid chromatography	**The eWG recommends this method as Type III** *Notes* 1. Recommended in CRD 15 2. Validated. Precision data for various foods is in CRD 15. 3. Collaboratively tested according to ISO 5725, among others an enriched milk powder was included in the validation. In accordance with the EU MAT Certification Study Guidelines, the parameters for margarine (CRM 122) and milk powder (CRM 421) have been defined in an interlaboratory test. The study was organised by the Institute of Food Research, Norwich, United Kingdom. 4. Reference: Finglas, P.M., van den Berg, H. & de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg.
Vitamin D	Note on the form of Vitamin D in Codex Standard 72 Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin D *Calciferol. 1 µg calciferol = 40 IU vitamin D* Comment: Calciferol is not specific and conceivably includes all forms of vitamin D. This currently generic descriptor could therefore include the parent forms of vitamin D2 and D3 and the physiologically antirachitic hydroxylated metabolites. For food nutritional labelling requirements it is however implicit that the parent cholecalciferol (vitamin D3) is the target nutrient, given that this is the form commonly added to infant formulas. The current definition does not discriminate ergocalciferol (vitamin D2) which is rarely added to foods.			

Vitamin D	Minimum 1µg/100kcal (0.25µg/100kJ); maximum 2.5µg/100kcal (0.6µg/100kJ).	AOAC 992.26 (D2 and/or D3 measured as single components. Hydroxylated forms not measured.)	High performance liquid chromatography	**The eWG recommends this method as Type III, and notes limitations on applicability to infant formula containing 488-533 IU/L The minimum requirement for vitamin D in Codex STAN 72 is 280 IU/L** *Notes* 1. Recommended in CRD 10 2. Validated. The method is applicable to ready-to-feed milk-based infant formulas containing 488 to 533 IU/L vitamin D3. **3.** References: J. AOAC 68: 177- 182 (1985) J. AOAC Int. 76: 1042 - 1056 (1993). **4.** The method was listed for use with milk based infant formula in CODEX STAN 234-1999, rev. 2006. **5.** The minimum requirement for vitamin D3 (1 µg /100 kcal = 40 IU/100 kcal) means 280 IU/L vitamin D3, calculating the maximal energy density (70 kcal/100 ml prepared ready for consumption infant formula) laid down by Codex Stan 72. This concentration is outside of the applicable concentration range of method AOAC 992.26 (488-533 IU/L). **6.** D_2 and/or D_3 measured as single component. Method cannot discriminate if both present. Hydroxylated forms not measured.
Vitamin D	Minimum 1µg/100kcal (0.25µg/100kJ); maximum 2.5µg/100kcal (0.6µg/100kJ).	EN 12821:2000 (D2 and/or D3 measured as single components. Hydroxylated forms not measured.)	High performance liquid chromatography	**The eWG recommends this method as III*.** *Notes* 1. Precision data for various foods is in CRD 15 2. Validated. Collaboratively tested according to ISO 5725, among others an enriched milk powder was included in the validation. 3. Reference: EN 12821:2000. Foodstuffs - Determination of vitamin D by high performance liquid chromatography - Measurement of cholecalciferol (D3) and ergocalciferol (D2) 4. The parameters on margarine (CRM 122) and milk powder (CRM 421) have been defined in an interlaboratory test, in accordance with the EU MAT Certification Study Guidelines. The study was organized by the Laboratory of the Government Chemist, UK. Reference: Finglas, P.M., van den Berg, H. and de Froidmont-Görtz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg. 5. The parameters on milk, liquid infant, formula, cooking oil, margarine, infant formula and fish oil have been defined in an interlaboratory test according to AOAC Guidelines for

				collaborative study procedures to validate characteristics of a method of analysis. The study was organized by NMKL (Nordic Committee on Food Analysis). Reference: Staffas A, Nyman A. JAOAC Int., 2003, 86:400-406 6. D2 and D3 measured as single component. Method cannot measure the content of vitamin D if both forms are present. Hydroxylated forms not measured. The method is capable to quantitate D2 and D3 in the same sample, it is just not described.
Vitamin D	Minimum 1µg/100kcal (0.25µg/100kJ); maximum 2.5µg/100kcal (0.6µg/100kJ).	AOAC 995.05 (D_2 or D_3. Method can discriminate if both present. Hydroxylated forms not measured).	High performance liquid chromatography	**The eWG recommends this method as Type III*** *Notes* 1. Official Methods of AOAC Int. (18th ed., 2005): 50.1.23. 2. References: J. AOAC Int. 75: 566 - 571 (1992). J. AOAC Int. 79 : 73 - 80 (1996). 3. Validated. The method is applicable to the determination of 8 to 2600 IU (International Unit; 1 microgram vitamin D = 40 IU) vitamin D/quart (1 quart = 0.946 L) in infant formulas and enteral products. The results of the interlaboratory study supporting acceptance of the method are included in the method. 4. Method can discriminate between D_2 or D_3, if both present. Hydroxylated forms not measured.
Vitamin E	Note on the form of Vitamin E in Codex Standard 72 Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin E *1 mg α-TE (alpha-tocopherol equivalent) = 1 mg d-α-tocopherol* *Vitamin E content shall be at least 0.5 mg α-TE per g PUFA, using the following factors of equivalence to adapt the minimal vitamin E content to the number of fatty acid double bonds in the formula: 0.5 mg -TE/g linoleic acid (18:2 n-6); 0.75 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).* Comment: The standard does not provide conversion factors to determine tocopherol equivalents derived from the multiple vitamin E congeners potentially present in an infant formula. Neither the congeners (□,□,□,□), their tocotrienol equivalents or the supplemental □-tocopheryl acetate form are specified.			
Vitamin E	Minimum 0.5mg/100kcal (0.12mg/100kJ); no maximum limit. GUL 5mg/100kcal (1.2mg/100kJ). Minimum 0.5mg α-TE per g	AOAC 992.03 (Measures all-rac-vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual □-congeners)	High performance liquid chromatography	**The eWG recommends this method as Type III*** *Notes* 1. Recommended in CRD 10 **2.** Reference: J. AOAC Int. 76: 399 - 413 (1993). 3. Validated. The results of the interlaboratory study supporting acceptance of the method (milk-based liquid, ready-to-feed) are stated in the method. 4. The method was listed for use with infant formula in CODEX STAN 234-1999, rev. 2006.

	PUFA[6] using specified factors of equivalence.			5. Measures all-rac-vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual □-congeners.
Vitamin E	Minimum 0.5mg/100kcal (0.12mg/100kJ); no maximum limit. GUL 5mg/100kcal (1.2mg/100kJ). Minimum 0.5mg α-TE per g PUFA[6] using specified factors of equivalence.	EN 12822: 2000 (Measures Vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual tocopherol congeners (□□□□).	High performance liquid chromatography	**The eWG recommends this method as Type III*** *Notes* 1. Validated. Precision data for various foods incl. milk powder is in CRD 15. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation. 2. The parameters on margarine (CRM 122) and milk powder (CRM 421) of different methods for the determination of Vitamin E (α-tocopherol) have been defined in an international comparison study organised by the European Commissions Standard, Measurement and Testing program. Reference: Finglas, P.M., van den Berg, H. and de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg. 3. In accordance with ISO 5725 : 1986 [19], the validation data on milk powder and oat powder have been defined in an inter-laboratory test. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany. Reference: Untersuchung von Lebensmitteln - Bestimmung von Tocopherolen und Tocotrienolen in dietätischen Lebensmitteln L 49.00-5 September 1998 (Food Analysis - Determination of tocopherols and tocotrienols in dietetic foodstuffs L 49.00-5 September 1998) in: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG: Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedargsgegenständen/Bundesgesundheitsamt (In: Collection of official methods under article 35 of the German Federal Foods Act, Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office), Loseblattausgabe September 1998, Bd. 1 (Loose leaf edition as of 1998-09, Vol.1) Berlin, Köln: Beuth Verlag GmbH 4. Measures Vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual tocopherol congeners (□□□□).

Vitamin K	Note on the form of Vitamin K in Codex Standard 72. The standard provides no qualification on the definition of forms of vitamin K. Comment: Vitamin K present in infant formulas can include cis and/or trans K1, dihydro-K1, and the menaquinone series, and a more rigorous definition may be required.			
Vitamin K	Minimum 4µg/100kcal (1µg/100kJ); no maximum limit; GUL 27µg/100kcal (6.5µg/100kJ)	AOAC 992.27 (trans-K_1).	High performance liquid chromatography.	**The eWG recommends this method as Type III, and notes limitations on applicability to ready-to-feed milk-based infant formulas containing 75 to 130 micrograms/L *trans*-vitamin K1,the minimum requirement for vitamin K in CODEX STAN 72 is 28 µg/L** *Notes* 1. Recommended in CRD 10. 2. The method was listed for use with infant formula in CODEX STAN 234-1999, rev. 2006. 3. Validated. The method is applicable to ready-to-feed milk-based infant formulas containing 75 to 130 micrograms/L *trans*-vitamin K1. 4. Possible quantification problem; The minimum requirement for vitamin K (4 µg /100 kcal) means 28 µg /L vitamin K, calculating the maximal energy density (70 kcal/100 ml prepared ready for consumption infant formula) laid down by the Codex Standard. This concentration is outside of the applicable concentration range of method AOAC 992.27 (75-130 µg /L). 5. References: J. AOAC 68: 684 - 689 (1985) J. AOAC Int. 76: 1042 - 1056 (1993) AOAC 992.27 6. Measures trans-K_1.
Vitamin K	Minimum 4µg/100kcal (1µg/100kJ); no maximum limit; GUL 27µg/100kcal (6.5µg/100kJ)	AOAC 999.15 (Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column. Can also discriminate and measure dihydro-K_1 and menaquinones).	High performance liquid chromatography with C30 column to separate the cis- and the trans- K vitamins	**The eWG recommends this method as Type III*** *Notes* 1. Validated. The method is applicable to the determination of total vitamin K1 (phylloquinone) in infant formula and milk (fluid, ready-to-feed, and powdered) containing > 1 microgram vitamin K1/100 g solids). 2. Reference: J. AOAC Int. 83: 121- 130 (2000). 3. Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column. Can also discriminate and measure dihydro-K_1 and menaquinones. Proposed by CCNFSDU 28. CCMAS 28 asked for clarification of the differences from AOAC 992.27. Consideration needs to be given to i) ability to discriminate the cis and trans- forms of K1 which can be accomplished with a

				C30 column, ii) whether the menaquinones (K2) be included. AOAC 999.15 is a more specific fluorescence method than AOAC 999.27 and has a better sample preparation with enzyme instead of a labor-intensive multistep procedure. AOAC 995.15 & EN 14148 are based on a joint AOAC/EN collaborative study. The main weakness with this procedure is that both cis- and trans- K1 (total K1) are determined. The cis-form is inactive. To overcome this problem, the C18 HPLC column must be replaced by a C30 HPLC column which separates the two vitamers.
Vitamin K	Minimum 4µg/100kcal (1µg/100kJ); no maximum limit; GUL 27µg/100kcal (6.5µg/100kJ)	EN 14148:2003 (vitamin K_1) (Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column.)	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Recommended in CRD 15. Precision data for various foods including a range of infant formulae is in CRD 15. 2. Validated. The precision data have been defined in an international collaborative study: 3. Reference: Indyk, H. E. and Woollard, D. C.: Vitamin K in Milk and Infant Formulas by Liquid Chromatography: Collaborative study. J. AOAC intern. 83, 2000, 121-130. 4. Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column.
Thiamin	Note on the form of Thiamin in Codex Standard 72. The standard provides no qualification on the definition of forms of thiamine. Comment: Several endogenous phosphorylated forms exist in infant formulas, although vitamin B1 is usually dominated by the supplement thiamine hydrochloride. In this case, units of expression (free base vs hydrochloride salt) need to be defined.			
Thiamin	Minimum 60µg/100kcal (14µg/100kJ); no maximum limit; GUL 300µg/100kcal (72µg/100kJ)	AOAC 942.23 (Measures all vitamin B_1 forms and aggregates as thiamine)	Fluorimetry	**The eWG recommends this method as Type III or IV** 1. Recommended in CRD 10. 2. Currently adopted as Type II method for Special foods in CODEX STAN 234-1999, rev 2007. 3. Validated on many food matrixes, but not infant formula or similar food matrixes. 4. The method has been used traditionally 5. The method is not applicable in presence of materials that adsorb thiamin or which contain extraneous materials which affect thiochrome. 6. References: JAOAC 25: 456- 458 (1942); JAOAC 27: 534 - 537 (1944) ; JAOAC 28: 554 - 559 (1945); JAOAC 31: 455 - 459 (1948); JAOAC 43: 45 - 46 (1960); JAOAC 43: 55 - 57 (1960); AND

				JAOAC 64: 1336 - 1338 (1981). 7. Measures all vitamin B_1 forms and aggregates as thiamine. Subject to significant spectral interference.
Thiamin	Minimum 60µg/100kcal (14µg/100kJ); no maximum limit; GUL 300µg/100kcal (72µg/100kJ)	AOAC 986.27 (Measures all vitamin B_1 forms as thiamine)	Fluorimetry	**The eWG recommends this method as Type III*** **1.** Recommended in CRD 10. 2. Validated 3. Reference: JAOAC 69: 777 - 785 (1986). 4. Measures all vitamin B_1 forms as thiamine. Subject to significant spectral interference.
Thiamin	Minimum 60µg/100kcal (14µg/100kJ); no maximum limit; GUL 300µg/100kcal (72µg/100kJ)	EN 14122:2003 (Measures all vitamin B_1 forms (natural and added free, bound and phosphorylated) following extraction and conversion to thiamine)	High performance liquid chromatography with pre-or post column derivatization to thiochrom	**The eWG recommends this method as Type III*** **1.** Recommended in CRD 15 2. Validated. Precision data for various foods is in CRD 15 3. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation. In accordance with the EU SMT Certification Study guidelines, the data given for CRM 121 (wholemeal flour), CRM 421 (milk powder), CRM 485 (mixed vegetables) and CRM 487 (pig's liver) have been defined in an interlaboratory test. The Institute of Food Research, Norwich, UK on behalf of the EU Community Bureau of Reference, conducted the study. Reference: Finglas, P. M., Scott, K. J., Witthoft, C. M., van den Berg, H. and de Froidmont-Gortz, I.: The certification of the mass fractions of vitamins in four reference materials: Wholemeal flour (CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pig's liver (CRM 487). EUR-report 18320, Office for Official Publications of the European Communities, Luxembourg, 1999. 4. The data given for tube feeding solution, baby food, powdered milk, meal with fruits, yeast and cereal, chocolate powder and food supplement have been defined in a French interlaboratory test.. Reference: Arella, F., Lahély, S., Bourguignon, J. B. and Hasselmann, C.: Liquid chromatographic determination of vitamin B1 and B2 in foods. A collaborative study. Food Chem. 56, 1996, 81-86. 5. Measures all vitamin B_1 forms (natural and added free, bound and phosphorylated) following extraction and conversion to thiamine.
Riboflavin	Note on the form of Riboflavin in Codex Standard 72. The standard provides no qualification on the form of riboflavin. Comment: Several endogenous phosphorylated forms exist in infant formulas, eg free and/or bound riboflavin, FMN, FAD etc. Vitamin B2 is			

	generally enhanced through supplementation with either free riboflavin or FMN.			
Riboflavin	Minimum 80µg/100kcal (19µg/100kJ); no maximum limit; GUL 500µg/100kcal (119µg/100kJ)	AOAC 985.31 (Measures free and bound forms. Uncertain whether phosphorylated forms captured)	Fluorimetry	**The eWG recommends this method as Type III*** 1. Recommended in CRD 10 2. Validated 3. AOAC 985.31 Riboflavin in Ready-to-Feed Milk-Based Infant Formula, Fluorometric Method. First Action 1985; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.07. 4. Reference: JAOAC 68: 514 - 522 (1985). Official Methods of AOAC Int. (18th ed., 2005) cross-references AOAC 985.31 to AOAC 970.65 [45.1.08; Riboflavin (Vitamin B2) in Foods and Vitamin Preparations, Fluorometric method, First Action 1970; Final Action 1971]. AOAC 970.65 dates from the 1970s. 5. Literature references for AOAC 970.65 date to 1940 and are not included here. 6. Measures free and bound forms. Uncertain whether phosphorylated forms captured. Subject to significant spectral interference.
Riboflavin	Minimum 80µg/100kcal (19µg/100kJ); no maximum limit; GUL 500µg/100kcal (119µg/100kJ)	EN 14152:2003 (Measures natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as riboflavin.)	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Recommended in CRD 15 2. Validated. Precision data for various foods is in CRD 15. 3. Collaboratively tested according to ISO 5725, an enriched milk powder was included in the validation. The parameters on CRM 421 (milk powder) and CRM 487 (pig liver)of different methods for the determination of riboflavin (Vitamin B2) have been defined in an international comparison study organised by the European Commissions Standard, Measurement and Testing programme. Reference: Finglas, P. M., Scott, K. J., Witthoft, C. M., van den Berg, H. & de Froidmont-Gortz, I.: The certification of the mass fractions of vitamins in four reference materials: Wholemeal flour (CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pig's liver (CRM 487). EU Report 18320, Office for Official Publications of the European Communities, Luxembourg, 1999. 4. Both natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as riboflavin.
Niacin	Note on the form of Niacin in Codex Standard 72. *Niacin refers to preformed niacin.* Comment: Niacin is the generic descriptor for two vitamers, nicotinic acid and nicotinamide. However terminology differs between the USA			

	and Europe for this vitamin and this standard needs to be unambiguous. Other forms also exist, eg NAD, NADH etc. It is therefore unclear what is meant by "preformed niacin".			
Niacin	Minimum 300µg/100kcal (70µg/100kJ); no maximum limit; GUL 1500µg/100kcal (360µg/100kJ)	AOAC 985.34 (niacin (preformed) and nicotinamide)	Microbioassay and turbidimetry	**The eWG recommends this method as Type III** 1. CCMAS recommended review and replacement with a more modern method. 2. Recommended in CRD 10. 3. Validated 4. AOAC 985.34 Niacin and Niacinamide (Nicotinic Acid and Nicotinamide) in Ready-to-Feed Milk-Based Infant Formula; Microbiological-turbidimetric method. First Action 1985; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.19. 5. Reference: JAOAC 68: 514 - 522 (1985). 6. The method is applicable to baby foods (meat based), beverages, juices, cereal products, cheese, dairy products, fruits and potato products. 7. Free and bound forms aggregated and measured as nicotinic acid.
Niacin	Minimum 300µg/100kcal (70µg/100kJ); no maximum limit; GUL 1500µg/100kcal (360µg/100kJ)	prEN 15652:2007 (Free and bound and phosphorylated forms measured either as aggregate of nicotinic acid + nicotinamide, or as individual forms)	High performance liquid chromatography	**The eWG recommends for consideration as Type III* when published as EN method** **1.** Recommended in CRD 15. 2. Validated. Precision data for various foods is in CRD 15 3. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation.The precision data for the determination of niacin were established according to ISO 5725-2 in 2002 by an international collaborative study organised by AéRIAL (CRT: Centre de Ressources technologiques) and the CGd'UMA (Commission Générale d'Unification des Méthodes d'Analyses) according to ISO 5725-2 in 1999 by a French collaborative study organized by CGd'UMA, 4. Reference: • To be published: Bergantzlé M., Validation study on the determination of niacin by HPLC in several matrices; • Lahély S., Bergantzlé M., Hasselmann, C.: Fluorimetric determination of niacin in foods by highperformance liquid chromatography with post-column derivatization Food chem., 65, 129-133 (1999) 5. Free and bound and phosphorylated forms measured either as aggregate of nicotinic acid + nicotinamide, or as individual forms
Vitamin B_6	Note on the form of Vitamin B_6 in Codex Standard 72.			

	The standard provides no qualification on the form of vitamin B6. Comment: This means all forms are potentially included, i.e. pyridoxine, pyridoxal, pyridoxamine and the related phosphorylated forms. Vitamin B6 is generally enhanced through supplementation with pyridoxine, and could be expressed as either the free base or hydrochloride salt. Methods for vitamin B6 can therefore measure and report single or aggregate forms.			
Vitamin B_6	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 175µg/100kcal (45µg/100kJ).	AOAC 985.32 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine and measures as pyridoxine.)	Microbioassay	**The eWG recommends this method as Type III** 1. Recommended in CRD 10. 2. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods, and asked for clarification of the differences from AOAC 961.15. 3. Validated AOAC Method 985.32. (Pyridoxine, Pyridoxal, Pyridoxamine) in Ready-to Feed Milk-Based Infant Formula Microbiological Method. First Action 1985; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.18. Reference: JAOAC 68: 514 - 522 (1985). 4. Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine and measures as pyridoxine.
Vitamin B_6	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 175µg/100kcal (45µg/100kJ).	AOAC 2004.07 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Recommended in CRD 10 2. Validated. The method is applicable to the determination of vitamin B6 in milk- and soy based liquid infant formula at 0 - 1mg/100g. Reference: JAOAC Int. 88: 30 - 37 (2005) Results of the interlaboratory study for vitamin B6 in reconstituted infant formula (milk- and soy-based) are included with the method. Measures free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.
Vitamin B_6	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 175µg/100kcal (45µg/100kJ).	EN 14166:2008 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and measures as pyridoxine.)	Microbioassay	**The eWG recommends this method as Type III** 1. Recommended in CRD 15 2. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 3. Validated. Precision data for various foods is in CRD 15 Foodstuffs - Determination of vitamin B6 by microbiological assay

				The following data were obtained in an interlaboratory trial held in 1996 between participating European laboratories. Reference: The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121),milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pigs liver (CRM 487). Finglas, P.M., Scott, K.J., Witthoft, C., van den Berg, H. & Froidmont-Görtz, I. (1999); EUR-report 18320, Office for Official Publications of the European Communities, Luxembourg. 4. Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and measures as pyridoxine.
Vitamin B_6	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 175µg/100kcal (45µg/100kJ).	EN 14663:2005 (includes glycosylated forms) (Free and bound phosphorylated and glycosylated forms measured as the individual forms pyridoxal, pyridoxine and pyridoxamine.)	High performance liquid chromatography	**The eWG recommends this method as Type III** 1. Recommended in CRD 15 2. Validated. Precision data for various foods (semolina with milk, powder; potato puree, powder; vegetables with ham (baby food); multi vitamin drink) is in CRD 15 The precision data for the determination of vitamin B6 were established in an interlaboratory test according to ISO 5725 carried out by the former BgVV (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, German Federal Institute for Consumer protection and veterinary medicine). Reference: Bognár, A.: Bestimmung von Vitamin B6 in Lebensmitteln mit Hilfe der Hochdruckflüssig- Chromatographie (HPLC). Z Lebensm Unters Forsch A, 1985, 181: 200 – 205 3. Free and bound phosphorylated and glycosylated forms measured as the individual forms pyridoxal, pyridoxine and pyridoxamine.
Vitamin B_6	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 175µg/100kcal (45µg/100kJ).	EN 14164:2008 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Precision data for the determination of vitamin B6 in baby food, biscuit, cereal, yeast, tube-feeding solution, chocolate powder and powdered milk were established in an interlaboratory test according to ISO 5725 carried out by DGCCRF (Direction Génerale de la Concurrence, de la Consommation et de le Repression des Fraudes). Reference: Bergaentzlé M., Arella F., Bourguignon J.B., Hasselmann C., Determination of vitamin B6 in foods by HPLC: a collaborative study. Food Chem (1995), 52, 81-86

				2. The precision data for the determination of vitamin B6 in reconstituted infant formula were established in an interlaboratory test according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis. Reference: Mann D.L., Ware G.W., Bonnin E. Liquid Chromatographic analysis of vitamin B6 in reconstituted infant formula: Collaborative Study. JAOAC (2005), 88,1:30-37 3. Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.
Vitamin B_{12}	Note on the form of Vitamin B_{12} in Codex Standard 72. The standard provides no qualification on the form of vitamin B_{12}. Comment: This means all forms are potentially included. However cyanocobalamin is the form used in food supplementation and most extraction conditions employed will convert multiple endogenous forms to a single cyano form.			
Vitamin B_{12}	Minimum 0.1µg/100kcal (0.025µg/100kJ); no maximum limit; GUL 1.5µg/100kcal (0.36µg/100kJ)	AOAC 986.23 (Measures total vitamin B_{12} as cyanocobalamin	Turbidimetric Method	**The eWG recommends this method as Type III*** 1. Recommended in CRD 10. 2. CCMAS asked for clarification of the differences from AOAC 952.20. A great difference between AOAC 952.20 and AOAC 986.23 methods is the sample matrix; the first is applicable in vitamin preparations, but not in infant formulae. 3. Validated AOAC Method 986.23 Cobalamin (Vitamin B12 Activity) in Milk-Based Infant Formula. Turbidimetric method (microbiological). First Action 1986; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.20. Reference: JAOAC 69: 777 - 785 (1986). 4. Measures total vitamin B_{12} as cyanocobalamin.
Pantothenic acid	Note on the form of Pantothenic acid in Codex Standard 72. The standard provides no qualification on the form of pantothenic acid. Comment: This means all forms are potentially included eg the free calcium pantothenate supplement and that derived from Coenzyme A. It is important to define units of expression either as pantothenic acid or the calcium salt.			
Pantothenic acid	Minimum 400µg/100kcal (96µg/100kJ); no maximum limit; GUL 2000µg/100kcal	AOAC 992.07 (Measures total pantothenate (free pantothenic acid + CoA- + ACP-bound) and measured as D-pantothenic acid (or calcium D-pantothenate).)	Microbioassay	**The eWG recommends this method as Type III.** In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which has been used traditionally should currently be recommended as Type III and recommended as **Type IV** when another method can be recommended as Type II or III

	(478µg/100kJ)			1. The method was listed for use with infant formula in CODEX STAN 234-1999, rev. 2006. 2. Recommended in CRD 10. 3. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 4. Validated. Results of the interlaboratory study supporting acceptance of the method (milk-based liquid, ready-to-feed) are presented in the method. Reference: J. AOAC Int. 76: 399 - 413 (1993). 5. Measures total pantothenate (free pantothenic acid + CoA- + ACP-bound) and measured as D-pantothenic acid (or calcium D-pantothenate).
Folic acid	Note on the form of Folic acid in Codex Standard 72. The standard is specific for folic acid. Comment: Currently the provision is specific for folic acid which implies that only the free supplemental form should be quantified during analysis, and expressed as ug (despite DFE gaining common usage). However, such a test method would exclude all natural forms present in milk, and therefore invalidate currently recommended microbiological assay methods.			
Folic acid	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 50µg/100kcal (12µg/100kJ)	AOAC 992.05 (Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.)	Microbioassay	**The eWG recommends this method**. In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which has been used traditionally should currently be recommended as Type III and recommended as **Type IV** when another method can be recommended as Type II or III. 1. Recommended in CRD 10 2. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 3. Validated. Results of the interlaboratory study supporting acceptance of the method (milk-based, ready-to-feed) are listed in the method. Reference: J. AOAC Int. 76: 399 - 413 (1993). 4. Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.
Folic acid	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 50µg/100kcal (12µg/100kJ)	EN 14131:2003 (Total folate (free + bound), aggregated and measured as folic acid.)	Microbioassay	**The eWG recommends this method**. In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which has been used traditionally should currently be recommended as Type III and recommended as **Type IV** when another method can be recommended as type II or III 1. Recommended in CRD 15

				2. Validated. Precision data for various foods is in CRD 15 The precision of the method was established by interlaboratory tests conducted within the European Union's Standards, Measurement and Testing (EU SMT) programme, and carried out in accordance with ISO 5725. Reference: Finglas, P.M., et al., The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121), milk powder (CRM 421), lyophilized mixed vegetables (CRM 485) & lyophilized pig's liver (CRM 487). B1, B6 & folate in CRM 121; B1, B2, B6, B12 & folate in CRMs 421 & 487, and B1, B6, folate & carotenoids in CRM 485. 1999, Luxembourg: Office for Official Publications of the European Communities. 3. Equivalent to AOAC 992.05. Note that these methods quantify total folate, including folates of natural source and not folic acid alone, which is used as source for fortification. 4. Measures total folate (free + bound), aggregated and measured as folic acid.
Folic acid	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 50µg/100kcal (12µg/100kJ)	J AOAC Int. 2000:83; 1141-1148 (Measures free folic acid + proportion of free, natural folate)	Optical Biosensor Immunoassay	**The eWG does not recommend this method as Type III as it is not established as official methodology.** In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which is recently introduced and currently under AOAC collaborative study should be considered as **Type IV** 1. Reference: Indyk HE, Evans EA, et al. J AOAC Intl. 2000:83:1141-1148, Determination of Biotin and Folate in Infant Formula and Milk by Optical Biosensor-Based Immunoassay. http://www.atyponlink.com/AOAC/doi/abs/10.5555/jaoi.2000.83.5.1141 2. Measures free folic acid + proportion of free, natural folate.
Folic acid	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 50µg/100kcal (12µg/100kJ)	J Chromatogr. A., 928, 77-90, 2001 (Measures total folates after conversion to, and measurement as 5-Me-H4PteGlu)	High performance liquid chromatography, incorporating immunoaffinity clean-up and conversion to 5-methyltetrahydrofolate	**eWG does not recommend this method as Type III as it is not established as official methodology.** In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which is recently introduced and currently under AOAC collaborative study should be considered as **Type IV** 1. Under evaluation by CEN TC275/WG9 2. Measures total folates after conversion to, and measurement as 5-Me-H_4PteGlu.
Vitamin C	Note on the form of Vitamin C in Codex Standard 72.			

	"expressed as ascorbic acid" Comment: Further clarification of form(s) of vitamin C is required, eg ascorbic acid (AA), oxidised dehydroascorbic acid (DHA), or total ascorbate (AA + DHA), since both forms are physiologically active. However, the enantiomeric D-forms are not antiscorbutic and need to be discriminated.			
Vitamin C	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 70µg/100kcal (17µg/100kJ)	AOAC 985.33 (measures ascorbic acid (AA))	2,6-dichloroindophenol titrimetry	**The eWG recommends this method as Type III*** 1. Recommended in CRD 10. 2. CCMAS asked for clarification on how vitamin C was expressed. Determines only L(+) ascorbic acid and not the total amount for which the amount of dehydroascorbic acid has to be included. This method is specific for reduced ascorbic acid only 3. Validated References: J. AOAC 68: 514 - 522 (1985).
Vitamin C	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 70µg/100kcal (17µg/100kJ)	EN 14130:2003 (Measures ascorbic acid + dehydroascorbic acid).	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Recommended in CRD 15. 2. Validated. Precision data for various foods is in CRD 15. Validated Collaboratively tested according to ISO 5725, an enriched milk powder was included in the validation. The precision parameters for orange juice, liquid soup, powder milk, freeze-dried soup, breakfast cereals and fruits baby food have been defined in a collaborative study 3. Reference: Arella F., Deborde J.L., Bourguignon J.B., Hasselmann C., (1997), Ann. Fals. Exp. Chim., 90,N°940:217-233. 4. Measures total L-ascorbate (Ascorbic acid + dehydroascorbic acid).
Biotin	Note on the form of Biotin in Codex Standard 72 The standard provides no qualification on the form of biotin. Comment: Free d-biotin is generally used as a supplement. However, endogenous biotin is mostly present as a protein-bound form, which may be liberated as bioactive d-biocytin. Attention needs to be given to which forms are to be quantified.			
Biotin	Minimum 1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)	EN 15607:2008 (d-biotin) (Measures total D-biotin (free + D-biocytin)	High performance liquid chromatography	**The eWG recommends this method as Type III*** 1. Recommended in CRD 15 2. Validated. Precision data for various foods including infant milk powder is in CRD 15. Collaboratively tested according to ISO 5725, among others, an enriched infant milk powder was included in the validation. The data were obtained in an interlaboratory study organized by CGd'UMA (Commission Générale d'Unification des Méthodes d'Analyses) in 2000. It was organized in accordance with ISO 5725-2.

				Reference: Arella, F., Deborde, J.L., Bourguignon, J.B., Bergaentlze, M., Ndaw, S., Hasselmann, C.: Liquid chromatographic determination of biotin in foods. A collaborative study. Ann. Fals. Exp. Chim., 93, 951,193-200 (2000) 3. Measures total D-biotin (free + D-biocytin)
Iron	Minimum 0.45mg/100kcal (0.1mg/100kJ); no maximum limit. GUL footnote: "levels may need to be determined by national authorities".	AOAC 985.35	Atomic absorption spectrophotometry	**The eWG recommends this method as Type II** The method is applicable to the determination of Ca, Mg, Fe, Zn, Cu, Mn, Na, and K. Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method. References: JAOAC 68: 514 - 522 (1985), J. AOAC Int. 80: 834 - 844 (1997).
Iron	Minimum 0.45mg/100kcal (0.1mg/100kJ); no maximum limit. GUL footnote: "levels may need to be determined by national authorities".	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Validated Reference: JAOAC 67: 985 - 992 (1984).
Calcium	Minimum 50mg/100kcal (12mg/100kJ); no maximum limit; GUL 140mg/100kcal (35mg/100kJ). Calcium to phosphorus ratio: minimum 1:1 and maximum 2:1	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**The eWG recommends this method as Type II** Current Codex method for special foods, and adopted by CAC 31 for infant formula, Type II, for determination of Na and K. Reference of the collaborative study: International Dairy Journal, Volume 18, Issue 9, September 2008, Pages 899-904, Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): A joint ISO/IDF collaborative study, Laurent Noël, Michael Carl, Christelle Vastel and Thierry Guérin

Calcium	Minimum 50mg/100kcal (12mg/100kJ); no maximum limit; GUL 140mg/100kcal (35mg/100kJ). Calcium to phosphorus ratio: minimum 1:1 and maximum 2:1	AOAC 985.35	Atomic absorption spectroscopy	**The eWG recommends this method as Type III** Recommended in CRD 10 Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method. References: JAOAC 68: 514 - 522 (1985), J. AOAC Int. 80: 834 - 844 (1997).
Calcium	Minimum 50mg/100kcal (12mg/100kJ); no maximum limit; GUL 140mg/100kcal (35mg/100kJ). Calcium to phosphorus ratio: minimum 1:1 and maximum 2:1	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Current Codex method (Type III) for Special foods. Proposed by CCNFSDU 28 and recommended in CRD 10. Validated Reference: JAOAC 67: 985 - 992 (1984).
Phosphorus	Minimum 25mg/100kcal (6mg/100kJ); no maximum limit; GUL 100mg/100kcal (24mg/100kJ)	AOAC 986.24	Spectrophotometry (molybdovanadate)	**The eWG recommends this method as Type II** Current Codex method for special foods. Proposed by CCNFSDU 28 and recommended in CRD 10. Validated. The collaborative study was performed with soy powder infant formula, whey powder infant formula, soy ready-to-feed formula and enteral formula. The results of the interlaboratory study supporting acceptance of the method are included in the method. References: JAOAC 69: 777-785 (1986) J. AOAC Int. 80: 834-844 (1997)
Phosphorus	Minimum 25mg/100kcal (6mg/100kJ); no maximum limit;	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc in Infant Formula. In this method, a test portion is digested in HNO_3 / $HClO_4$ and

	GUL 100mg/100kcal (24mg/100kJ)			elements are determined by ICP emission spectroscopy. Official Methods of AOAC Int. (18th ed., 2005): 50.1.15. Reference: JAOAC 67: 985 - 992 (1984).
Magnesium	Minimum 5mg/100kcal (1.2mg/100kJ); no maximum limit. GUL 15mg/100kcal (3.6mg/100kJ)	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**The eWG recommends this method as Type II** Current Codex method for special foods and infant formula, Type II, for determination of Na and K. Reference of the collaborative study: International Dairy Journal, Volume 18, Issue 9, September 2008, Pages 899-904, Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): A joint ISO/IDF collaborative study, Laurent Noël, Michael Carl, Christelle Vastel and Thierry Guérin
Magnesium	Minimum 5mg/100kcal (1.2mg/100kJ); no maximum limit. GUL 15mg/100kcal (3.6mg/100kJ)	AOAC 985.35	Atomic absorption spectroscopy	**The eWG recommends this method as Type III** Recommended in CRD 10 Validated for infant formula. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method. References: JAOAC 68: 514 - 522 (1985), J. AOAC Int. 80: 834 - 844 (1997).
Magnesium	Minimum 5mg/100kcal (1.2mg/100kJ); no maximum limit. GUL 15mg/100kcal (3.6mg/100kJ)	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Recommended in CRD 10 Validated Reference: JAOAC 67: 985 - 992 (1984).
Sodium and potassium	Sodium minimum 20mg/100kcal (60mg/100kJ); maximum 60mg/100kcal (14mg/100kJ); no GUL. Potassium	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**This method should be retained as adopted** Adopted by CAC31 for sodium and potassium as Type II. Reference of the collaborative study: International Dairy Journal, Volume 18, Issue 9, September 2008, Pages 899-904, Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): A joint ISO/IDF collaborative study, Laurent Noël, Michael Carl, Christelle Vastel and Thierry Guérin

	minimum 60mg/100kcal (14mg/100kJ); maximum 180mg/100kcal (43mg/100kJ).			
Sodium and potassium	Sodium minimum 20mg/100kcal (60mg/100kJ); maximum 60mg/100kcal (14mg/100kJ); no GUL. Potassium minimum 60mg/100kcal (14mg/100kJ); maximum 180mg/100kcal (43mg/100kJ).	AOAC 984.27	ICP emission spectroscopy	**This method should be retained as adopted** Adopted by CAC31 for sodium and potassium as Type III. Validated Reference: JAOAC 67: 985 - 992 (1984).
Chloride	Minimum 50mg/100kcal (12mg/100kJ); maximum 160mg/100kcal (38mg/100kJ); no GUL	AOAC 986.26	Potentiometry	**The eWG recommends this method as Type II** Validated Reference: JAOAC 69: 777 - 785 (1986).
Manganese	Minimum 1µg/100kcal (0.25µg/100kJ); no maximum limit. GUL 100µg/100kcal (24µg/100kJ)	AOAC 985.35	Atomic absorption spectrophotometry	**The eWG recommends this method as Type II** Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as 986.24 phosphorus). References: JAOAC 68: 514 - 522 (1985) J. AOAC Int. 80: 834 - 844 (1997).
Manganese	Minimum 1µg/100kcal (0.25µg/100kJ); no maximum	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Validated Reference: JAOAC 67: 985 - 992 (1984).

	limit. GUL 100µg/100kcal (24µg/100kJ)			
Iodine	Minimum 10µg/100kcal (2.5µg/100kJ); no maximum limit; GUL 60 µg/100kcal (14µg/100kJ)	AOAC 992.24	Ion-selective potentiometry	**The eWG recommends this method as Type II, for milk-based formula** Current Codex method for milk-based follow-up formula, and was listed in Codex Stan 234 (2006 revision) for milk-based infant formula (Type II method). Validated. The method is applicable to ready-to-feed milk-based infant formula containing 75-150 microgram/L iodide. The results of the interlaboratory study supporting acceptance of the method (ready-to-feed milk-based infant formula) are stated in the method. Reference: J AOAC Int. 76: 1042 - 1056 (1993).
Selenium	Minimum 1µg/100kcal (0.24µg/100kJ); no maximum limit; GUL 9µg/100kcal (2.2µg/100kJ)	AOAC 996.17	Continuous hydride generation atomic absorption spectrometry (HGAAS)	**The eWG recommends this method as Type IV** Validated (not with infant formula). Interlaboratory study included samples with selenium levels from 0.25 to 5,450 micrograms/g. Accuracy of method was substantiated by in-house analyses of NIST SRMs (1657 Wheat Flour; 1577a Bovine Liver; 1643c Trace Elements in Water). The results of the interlaboratory study supporting acceptance of the method are listed in the method. Reference: J. AOAC Int. 80: 469 - 480 (1997).
Selenium	Minimum 1µg/100kcal (0.24µg/100kJ); no maximum limit; GUL 9µg/100kcal (2.2µg/100kJ)	EN 14627	Hydride generation atomic absorption spectrometry (HGAAS)	**The eWG recommends this method as Type IV** Foodstuffs. Determination of trace elements. Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) after pressure digestion Not validated for infant formulas
Selenium	Minimum 1µg/100kcal (0.24µg/100kJ); no maximum limit; GUL 9µg/100kcal (2.2µg/100kJ)	AOAC 2006.03	ICP emission spectroscopy	**The eWG recommends this method as Type IV** Validated (not with infant formula). Interlaboratory study included samples with selenium levels from 0.25 to 257 micrograms/g. The results of the interlaboratory study supporting acceptance of the method are included in the method. Reference: J. AOAC Int. 89: 1447 - 1466 (2006).

Copper	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 120µg/100kcal (29µg/100kJ). Footnote: "adjustment may be needed in these levels for IF made in regions with a high content of copper in the water supply".	AOAC 985.35	Atomic absorption spectroscopy	**The eWG recommends this method as Type II** Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method. References: JAOAC 68: 514 - 522 (1985) J. AOAC Int. 80: 834 - 844 (1997).
Copper	Minimum 35µg/100kcal (8.5µg/100kJ); no maximum limit. GUL 120µg/100kcal (29µg/100kJ). Footnote: "adjustment may be needed in these levels for IF made in regions with a high content of copper in the water supply".	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Validated for infant formula Reference: JAOAC 67: 985 - 992 (1984).
Zinc	Minimum 0.5mg/100kcal (0.12mg/100kJ); no maximum limit. GUL 1.5mg/100kcal (0.36mg/100kJ)	AOAC 985.35	Atomic absorption spectroscopy	**The eWG recommends this method as Type II** Applicable to Ca, Mg, Fe, Zn, Cu, Mn, Na, and K. Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method

				References: JAOAC 68: 514 - 522 (1985) J. AOAC Int. 80: 834 - 844 (1997).
Zinc	Minimum 0.5mg/100kcal (0.12mg/100kJ); no maximum limit. GUL 1.5mg/100kcal (0.36mg/100kJ)	AOAC 984.27	ICP emission spectroscopy	**The eWG recommends this method as Type III** Validated for infant formula. Reference: JAOAC 67: 985 - 992 (1984).
Choline	Note on the form of Choline in Codex Standard 72. The standard provides no qualification on the form of choline. Comment: Free choline is one of a number of salts used as supplement. However a number of bound forms are also present in infant formulas including added lecithin and endogenous components of milk phospholipid. Units of expression also require definition (eg as choline hydroxide).			
Choline	Minimum 7mg/100kcal (1.7mg/100kJ); no maximum limit; GUL 50mg/100kcal (12mg/100kJ)	AOAC 999.14	Enzymatic Colorimetric Method	**The eWG recommends this method as Type II, with limitations on applicability due to choline and ascorbate concentration.** 1. Recommended in CRD 10 2. Validated. 3. The method is applicable to the determination of choline in milk and infant formula containing 45-175 mg solids/100 g. The method does not apply to powdered infant formula/milk containing more than 100 mg vitamin C/100 g solids because of ascorbate suppression of color development. The results of the interlaboratory study supporting acceptance of the method are included in the method. 4. References: J.AOAC Int. 83: 131 - 138 (2000). JAOAC 87: 1297-1304 (2004)
Myo-Inositol	Note on the form of Myo-Inositol in Codex Standard 72. The standard is reasonably specific for myo-inositol. Comment: The current standard implies that both free and/or phospholipid-bound myo-inositol may be included and requires clarification.			
Myo-Inositol	Minimum 4mg/100kcal (1mg/100kJ); no maximum limit. GUL 40mg/100kcal (9.5mg/100kJ)	**No suitable published methods are available**		

L-Carnitine	Note on the form of L-Carnitine in Codex Standard 72. The standard specifies the L-form of carnitine. Comment: Despite defining the L-enantiomer, it does not distinguish between the supplemental and endogenous free carnitine and the naturally occurring acylcarnitines present at significant levels in milk.			
L-Carnitine	Minimum 1.2mg/100kcal (0.3mg/100kJ); maximum limit not specified	**No suitable published methods are available**		
Chromium (Section B of STAN 72 only)	Minimum 1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)	EN 14082	AAS after dry ashing	**The eWG recommends this method as Type IV** Foodstuffs. Determination of lead, cadmium, zinc, copper, iron, and chromium by AAS after dry ashing. Infant formula was not included in the validation.
Chromium (Section B of STAN 72 only)	Minimum 1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)	EN 14083	Graphite furnace AAS after pressure digestion	**The eWG recommends this method as Type IV.** Foodstuffs. Determination of lead, cadmium, chromium and molybdenum by GF-AAS after pressure digestion. Infant formula was not included in the validation.
Chromium (Section B of STAN 72 only)	Minimum 1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)	AOAC 2006.03	ICP emission spectroscopy	**The eWG recommends this method as Type IV** Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers (Microwave Digestion and Inductively Coupled Plasma-Optimal Emission Spectrometry). Infant formula was not included in the validation. Reference: J. AOAC Int. 89: 1447 - 1466 (2006).
Molybdenum (Section B of STAN 72 only)	Minimum 1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)	EN 14083	Graphite furnace AAS after pressure digestion	**The eWG recommends this method as Type IV** Foodstuffs. Determination of lead, cadmium, chromium and molybdenum by GF-AAS after pressure digestion. Infant formula was not included in the validation.
Molybdenum	Minimum	AOAC 2006.03	ICP emission	**The eWG recommends this method as Type IV**

(Section B of STAN 72 only)	1.5µg/100kcal (0.4µg/100kJ); no maximum limit. GUL 10µg/100kcal (2.4µg/100kJ)		spectroscopy	Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers (Microwave Digestion and Inductively Coupled Plasma-Optimal Emission Spectrometry). Reference: J. AOAC Int. 89: 1447 - 1466 (2006).

Annex

CODEX ALIMENTARIUS, VOLUME IX (CAC/VOL IX-ED.1)

PART III, METHODS OF ANALYSIS FOR FOODS FOR INFANTS AND CHILDREN

9. CALORIES BY CALCULATION

9.1 This section describes the method of calculation of the amount of energy, expressed in Calories (kcal) and/or kilojoules (kJ) of the food for the purpose (a) of expressing analytical results in terms of "100 available Calories (or 100 available kilojoules)" and (b) of declaring the amount of energy (in kcal or kJ) per stated amount of food.

9.2 Conversion Factors

(a)	Protein	4 kcal per g	(see Method 6)
(b)	Carbohydrate	4 kcal per g	(see Method 1)
(c)	Fat	9 kcal per g	(see Method 2)
(d)	Monosaccharides	3.75 kcal per g	(determined as such where known to be present)
(e)	Specific food ingredients	See "Energy and Protein Requirements" (FAO Nutrition Meetings Report Series No. 52 or WHO Technical Report Series No. 522)	

(f) Other specific Calorie conversion factors may be used where the formulation of the food and the nutrient content are known and where such specific conversion factors are physiologically more meaningful than the factors listed above.

9.3 Expression of Results

Results are expressed as the total number of kcal per 100 g of the food as sold. To convert from number of kcal to number of kJ per 100 g of food, use the conversion factor: 1 kJ is equivalent to 0.239 kcal.