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(分担)研究報告書

健康食品における安全性確保を目的とした基準等作成のための行政的研究

分担研究：コーデックス委員会における乳児用調製粉乳の成分分析方法の選定状況

分担研究者　山田和彦　(独)国立健康・栄養研究所 食品保健機能プログラム
研究協力者　遠藤　香　(独)国立健康・栄養研究所 食品保健機能プログラム

研究要旨
コーデックス委員会における乳児用調製粉乳の食品成分分析方法の選定状況について調査した。乳児用調製粉乳の規格に関する議論は、コーデックス委員会の栄養・特殊用途食品部会(Codex Committee on Nutrition and Foods for Special Dietary Uses、以下 CCNFSDU)において 1995 年に開始された。分析方法については現在も議論が進行中である。コーデックス委員会 CCNFSDU における乳児用調製粉乳の分析方法は、明確な原則のもとに議論されていること、専門部会の協力体制があること、議論の概要は文書でたどることが可能であることが分かった。これらはわが国において、乳児用調製粉乳の分析方法を検討する際にも参考になるものと思われる。

A. 目的

現在の特別用途食品の制度は、昭和 27 年に旧栄養改善法によって定められた枠組みが基本的に維持されたままとなっている。近年、高齢化の進展や生活習慣病患者の増加に伴う医療費の増大とともに、医学や栄養学の著しい進歩や栄養機能表示に関する制度の定着など、特別用途食品制度を取り巻く状況は大きく変化している。こうした状況を踏まえ、平成 19 年 11 月より 7 回に渡り「特別用途食品制度のあり方に関する検討会」が開催され、対象食品の範囲等が見直され、乳児用調製粉乳の許可基準も大きく見直された。平成 21 年 4 月より「特別用途食品の表示許可等について」(食安発 0212001 号)が施行される。新しい基準では特に定めがない場合、乳児用調製粉乳に含まれる成分は、栄養表示基準における栄養成分等の分析方法によって試験される。ただし、α-リノレン酸とリノール酸については、栄養表示基準における栄養成分等の分析方法には分析方法が記載されていないことから、試験方法の整備が早急に必要である。

コーデックス委員会は、消費者の健康の保護、食品の公正な貿易の確保等を目的として、1962 年に国際連合食糧農業機関及び世界保健機関により設置された国際的な政

府間機関であり、国際食品規格（コーデックス規格）の作成等を行っている。乳児用調製粉乳の許可基準見直しの際には、コーデックス規格が参考にされたことから、コーデックス委員会における乳児用調製粉乳の分析方法の選定状況について調査した。

B. 方法

コーデックス委員会における乳児用調製粉乳の規格とその分析方法は、栄養・特殊用途食品部会(Codex Committee on Nutrition and Foods for Special Dietary Uses、以下CCNFSDU)において議論されていることから、主にCCNFSDUとコーデックス委員会(Codex Alimentarius Commission)のホームページから入手した。

参照資料

・Codex Alimentarius Comission Procedural Manual, 17th Edition

・Standard For Infant Formula And Formulas For Special Medical Purpose Intended For Infants (CODEX STAN 72-1981)

・Alinorm 07/30/23、07/30/26-Rev、08/31/23、08/31/26、09/32/26

・CX/NFSDU 08/30/2-Add.1、

・CX/MAS 07/28/6-Add.1、08/29/6

・CCNFSDU 2007 CRD10、CRD17、2008 CRD2、CRD15

C. 結果

CCNFSDUにおける乳児用調製粉乳の規格見直しの経緯

1995年の第19回CCNFSDU会議において、乳児用調製粉乳のコーデックス規格(CODEX STAN 72-1981)は、最新の科学情報や各国における状況を考慮し、改訂されることになった。10年以上に及ぶ議論を経て、2007年にコーデックス規格(“Standard For Infant Formula And Formulas For Special Medical Purposes Intended For Infants”、CODEX STAN 72-1981)として採択された。

この規格は、1.Scope、2.Description、3.Essential Composition and Qulity Factors、4.Food Additives、5.Contaminants、6.Hygiene、7.Packaging, 8.Fill Concainters、9.Labelling、10.Methods of Analysis and Sampling の10のsectionから構成されている。ただし、section 10 の”Methods of Analysis and Sampling”については、乳児用調製粉乳の議論が開始されてから10年あまり経過した2006年の第28回会議の際に、それまでの古い分析方法の見直しが提案され、現在も議論が進められている。現在のところ、CODEX STAN 72-1981 の section.10 に関しては”to be finalized”となっている。

CCNFSDUにおける乳児用調製粉乳の分析方法の見直し

2006年11月の第28回CCNFSDU会議において、分析方法を更新するべきとの提案がなされたことから、分析方法の一覧を更新し、分析・サンプリング法部会(Codex Committee on Methods of Analysis and Sampling、以下CCMAS)に送った。2007年3月の第28回CCMAS部会では、CCNFSDUから送られた分析方法について、(1)総食物繊維の

分析方法が2種類掲載されているが、使い分けを明確にする、(2)微生物学的測定法、炭水化物、脂質およびタンパク質効率の分析方法は新しい方法に変えるべきである、(3)ビタミンCの表現方法、ビタミンK、ビタミンB_6およびビタミンB_{12}の分析方法の違いを明確にすること、(4)ナトリウムとカリウムの分析方法をISO8070|IDF 119.2007に変更するべきである、(5)窒素換算係数は従来どおりとすることに同意するが、大豆たんぱく質については5.71に修正すること等のコメントがあった。また、CCNFSDUからCCMASに送られたリストの分析方法の多くは古いので、更新が必要であるとの意見が出されたことから、この分析方法は承認されることなくCCNFSDUに戻された。

この意見を受け、2007 年 11 月に開催された第 29 回 CCNFSDU 会議では、CCMAS からの質問に対して、(1)、(2)CODEX STAN 72-1981 の Section3.1 Essential Composition に食物繊維とタンパク質効率が記載されていないので分析方法は記載しないことが推奨される、(4)ナトリウムとカリウムの分析方法は ISO8070|IDF 119.2007 に変更する、(5)窒素換算係数の使用方法は分析方法のリストか脚注に記載することになった。ニュージーランドを議長国とする電子ワーキンググループが設置され、第 30 回 CCNFSDU 会議で議論するための分析方法のリストを更新し、CCMAS からの質問に対しても検討することとなった。電子ワーキンググループは、分析方法のリストをコーデックスの手続きマニュアル(Codex Procedure Manual, 17th edition)における”Pricnciples for the Establishment of Codex Methods of Analysis”に従って作成した。

2008 年 3 月の第 29 回 CCMAS 会議において、CCNFSDU からの返答に対し、(1)、(2)食物繊維とタンパク質効率を削除することに合意するが、食物繊維は総エネルギー量を計算するのに必要なので、分析方法に記載することを再度検討すること、(4)ナトリウムとカリウムの分析方法は ISO と IDF 法を TypeⅡとして、AOAC984.27 法を TypeⅢとして承認した。(5)粗タンパク質の分析方法は窒素換算係数を脚注に入れ、AOAC991.20 または、
ISO 8968-1/2:2001|IDF 20-1/2:2001 に変更し、窒素換算係数の使用方法を脚注に入れることを CCNFSDU に提案した。

2008 年 11 月の第 30 回 CCNFSDU 会議ではニュージーランドを議長国とする電子ワーキンググループから更新した分析方法のリストが提出された。また、電子ワーキンググループは、CCMAS からの質問への返答に関して、(1)乳児用調製粉乳には食物繊維がほとんど含まれないため、総エネルギー量を算出するのに必要ない、(2)微生物学的測定法を用いた方法を見直し、また、総炭水化物については AOAC 986.25、脂肪については AOAC 989.05 と ISO 8381|IDF 123:2008 もしくは ISO 8262-1|IDF 124-1:2005 が推奨される、(3)ビタミン C はアスコルビン酸と表現し、ビタミン K、ビタミン B_6、ビタミン B_{12} の分析方法の違いについては表に記載した、と回答するように提案した。委員会は電子ワーキンググループからの提案を受け入れ、更新した分析方法のリスト(表 1)と CCMAS からの質問に対する回答を転送し、現在は、分析・サンプリング部会からの返答を待っている状況

である。

D. 考察

コーデックス委員会における分析方法の選定の特徴としては、コーデックスの手続きマニュアル(Codex Procedure Manual, 17th edition)における”Principles for the Establishment of Codex Methods of Analysis”に従って作成されることである。具体的な内容としては、(a)食品分析に関する国際組織によって作られた公式な方法を優先すること、(b)特異性、正確性、再現性、検出限界、感度、通常の設備のある研究室で実施適用可能であること (c)ルーチン分析に適していること、(d)コーデックスの基準にとって適切であること、(e)1つの商品にしか適応できない方法よりも多種類の商品に適用できる方法を優先させること、が基本的な原則である。この原則に従って、分析方法がリストアップされて議論が行われる。ただし、CCNFSDU のような部会において判断できない場合には、分析方法を専門に扱う部会である CCMAS に対して助言を求めることができる。製品についての専門の部会と、分析方法に関する専門の部会が協力することにより、適切な分析方法を確立することが可能になっていると思われる。

本研究で調査の対象とした文書には、コーデックス委員会での議論の概要が記されている。これらの文書のほとんどは公開されており、分析方法が選定された経緯をたどることができる。また、分析方法は選定されてから一定期間が経過したら更新する必要がある。過去の分析方法が決められた経緯が明らかにされていることは、分析方法を更新する際にも有益な情報になると思われる。

以上より、コーデックス委員会 CCNFSDU における乳児用調製粉乳の分析方法は、明確な原則のもとに議論されていること、専門部会の協力体制があること、議論の概要は文書でたどることが可能であり、日本において乳児用調製粉乳の分析方法を選定する際にも参考になるものと思われる。

E. 結論

コーデックス委員会 CCNFSDU における乳児用調製粉乳の分析方法の選定状況について調査した。分析方法に関する議論は現在も進行中である。コーデックス委員会 CCNFSDU における乳児用調製粉乳の分析方法は、明確な原則のもとに議論されていること、専門部会の協力体制があること、議論の概要は文書でたどることができる。これらはわが国において、乳児用調製粉乳の分析方法を検討する際にも参考になるものと思われる。

F. 研究発表

1. 論文発表

なし

2. 学会発表

なし

3. その他

なし

G. 知的所有権の取得状況

1. 特許取得

なし

2. 実用新案登録

なし

表1　更新された分析方法案のリスト (CX/MAS 09/30/6)

A. COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES

Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants, CODEX STAN 72-1981

Provision	Method	Principle	Notes and Type Proposed
Calories (by calculation)	Method described in CAC/Vol IX-Ed.1, Part III[2]	Calculation method	**Type III** 1. Currently adopted as a Type III method for Special foods. 2. The references in this method (methods of analysis and conversion factors for specific food ingredients) need to be updated.
Total fat	AOAC 989.05 ISO 8381|IDF 123:2008	Gravimetry (Röse-Gottlieb)	**Type I.** This method should apply to milk-based infant formula containing ≤ 5% starch or dextrin
Total fat	ISO 8262-1 |IDF 124-1: 2005	Gravimetry (Weibull-Berntrop)	**Type I**, this method should apply to milk-based infant formula
Fatty acids	AOAC 996.06	Gas chromatography	**Type III*** 1. Validated (but not for infant formulas). . 2. Adopted as Type II for determination of saturated fat for nutrition labeling purposes. 3. Information should be adequate for listing as a reference method (Type II), or if not, a tentative method (Type IV).
Trans fatty acids	AOCS Ce 1h-05	Gas liquid chromatography	**Type III*,** for infant formulae not containing milkfat 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

[1] ALINORM 09/32/26, Appendix VI

[2] Section 9. Calories by calculation – Section 9.2 Conversion Factors

(a) protein 4 kcal per g
(b) carbohydrate 4 kcal per g
(c) fat 9 kcal per g
(d) monosaccharides 3.75 kcal per g
(e) specific food ingredients See "Energy and Protein Requirements"(FAO Nutrition Meeting 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

			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 linoleic acid (CLA).≤The method should therefore be endorsed for infant formulae not containing milkfat.
Trans fatty acids	AOAC 996.06	Gas chromatography	**Type IV, with optimisation for the determination of TFAs**
Total phospholipids	AOCS Ja7b-91	Gas liquid chromatography	**Type IV with suitable extraction and preparation procedures** 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. 3. 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. 4. Recommended as a tentative method (Type IV) since the method is not validated for infant formula.
Total carbohydrates	AOAC 986.25	Determination by difference	**Type II**. Determination by difference, i.e. the remainder after deducting fat, ash and crude protein from total solids.
Moisture/Total Solids	AOAC 934.01 AOAC 925.23, or IDF 12B:1987 ISO 6731:1989	Gravimetry	**Type I** No provision for moisture/total solids, however estimation of moisture content (total solids) is needed for calculation of carbohydrates and calories.
Ash	AOAC 942.05	Gravimetry	**Type I** -No provision for ash, however estimation of ash content is needed for calculation of carbohydrates and calories

Vitamin A[3]	AOAC 992.04 (retinol isomers)	High performance liquid chromatography	**Type III*** - Currently adopted as Type II method for follow-up formula Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)
Vitamin A	AOAC 992.06 (retinol)	High performance liquid chromatography	**Type III*-** Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)
Vitamin A	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	**Type III** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15. 2. 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. 3. 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[4]	AOAC 992.26	HPLC	**Type III,** with 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 D_2 and/or D_3 measured as single component. Method cannot discriminate if both present. Hydroxylated forms not measured.

[3] 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.*

Comment: 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

Vitamin D	EN 12821:2000 (D2 and/or D3 measured as single components. Hydroxylated forms not measured.)	HPLC	**Type III*** 1. Precision data for various foods is in CCNFSDU 29th session 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	AOAC 995.05	HPLC	**Type III*** (D_2 or D_3. Method can discriminate if both present. Hydroxylated forms not measured).

[4]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 Es	AOAC 992.03	HPLC	**Type III*** Measures all-rac-vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual α -congeners.
Vitamin E	EN 12822: 2000 (Measures Vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual tocopherol congeners (α, β, γ, δ).	HPLC	**Type III*** 1. Validated. Precision data for various foods incl. milk powder is in CCNFSDU 29th session 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

[5] 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 (α, β, γ, δ), their tocotrienol equivalents or the supplemental α -tocopheryl acetate form

			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 (α,.β,.γ, δ).
Vitamin K[6]	AOAC 992.27 (trans-K_1).	HPLC.	**Type III***
Vitamin K	AOAC 999.15	HPLC with C30 column to separate the cis- and the trans- K vitamins	**Type III*** - Proposed by CCNFSDU 28. CCMAS 28 asked for clarification of the differences from AOAC 992.27. 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). 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	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	**Type III*** 1. Precision data for various foods including a range of infant formulae is in CCNFSDU 29th session 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

[6] 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.

			and trans forms depending on LC column.
Thiamin[7]	AOAC 942.23 (Measures all vitamin B_1 forms and aggregates as thiamine)	Fluorimetry	**Type III or IV** - Currently adopted as Type II method for Special foods. 1. Validated on many food matrixes, but not infant formula or similar food matrixes. 2. The method has been used traditionally 3. The method is not applicable in presence of materials that adsorb thiamin or which contain extraneous materials which affect thiochrome. 4. Measures all vitamin B_1 forms and aggregates as thiamine. Subject to significant spectral interference.
Thiamin	AOAC 986.27	Fluorimetry	**Type III** *- (Measures all vitamin B_1 forms as thiamine)
Thiamin	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	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 2. 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. 3. 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.,

[7] 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.

			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. 4. Measures all vitamin B_1 forms (natural and added free, bound and phosphorylated) following extraction and conversion to thiamine.
Riboflavin*	AOAC 985.31	Fluorimetry	**Type III* - .** Measures free and bound forms. Uncertain whether phosphorylated forms captured. Subject to significant spectral interference.
Riboflavin	EN 14152:2003 (Measures natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as riboflavin.)	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15. 2. 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. 3. Both natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as 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.

Niacin[9]	AOAC 985.34 (niacin (preformed) and nicotinamide)	Microbioassay and turbidimetry	**Type III** 1. CCMAS recommended review and replacement with a more modern method. 2. Validated 3. 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. 4. Reference: JAOAC 68: 514 -522 (1985). 5. The method is applicable to baby foods (meat based), beverages, juices, cereal products, cheese, dairy products, fruits and potato products. 6. Free and bound forms aggregated and measured as nicotinic acid.
Niacin	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	**Type III* when published as EN method** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 2. 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, 3. 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) 4. Free and bound and phosphorylated forms measured either as aggregate of nicotinic acid + nicotinamide, or as individual forms

[9] 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".

Vitamin B_6 [10]	AOAC 985.32 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine and measures as pyridoxine.)	Microbioassay	**Type III** 1. 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	AOAC 2004.07 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**Type III*** 1. 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	EN 14166:2008 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and	Microbioassay	**Type III** 1. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 2. Validated. Precision data for various foods is in CCNFSDU 29th session 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

[10] 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.

	measures as pyridoxine.)		(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. 3. Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and measures as pyridoxine.
Vitamin B_6	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	**Type III** 1. Validated. Precision data for various foods (semolina with milk, powder; potato puree, powder; vegetables with ham (baby food); multi vitamin drink) is in CCNFSDU 29th session 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 2. Free and bound phosphorylated and glycosylated forms measured as the individual forms pyridoxal, pyridoxine and pyridoxamine.
Vitamin B_6	EN 14164:2008 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**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} [11]	AOAC 986.23 (Measures total vitamin B_{12} as cyanocobalamin	Turbidimetric Method	**Type III*** 1. 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. 2. 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). 3. Measures total vitamin B_{12} as cyanocobalamin.
Pantothenic acid[12]	AOAC 992.07 (Measures total pantothenate (free pantothenic acid + CoA- +ACP-bound) and measured asD-pantothenic acid (or calcium D-pantothenate).)	Microbioassay	**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 endorsed as Type III and recommended as **Type IV** when another method can be recommended as Type II or III 1. The method was listed for use with infant formula in CODEX STAN 234-1999, rev. 2006. 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 liquid, ready-to-feed) are presented in the method. Reference: J. AOAC Int. 76: 399 - 413 (1993). 4. Measures total pantothenate (free pantothenic acid + CoA- + ACP-bound) and measured as D-pantothenic acid (or calcium D-pantothenate).
Folic acid[13]	AOAC 992.05 (Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.)	Microbioassay	**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 endorsed as Type III and recommended as **Type IV** when another method can be recommended as Type II or III. 1. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 2. Validated. Results of the interlaboratory study supporting acceptance of the method (milk-based, ready-to-feed) are listed in the method.

[11] 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. [12] 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.

			3. Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.
Folic acid	EN 14131:2003 (Total folate (free + bound), aggregated and measured as folic acid.)	Microbioassay	**Type III** In line with the CCMAS 28 request to review methods using microbioassay as a principle, this method which has been used traditionally should currently be endorsed as Type III and recommended as **Type IV** when another method can be recommended as type II or III 1. 2. Validated. Precision data for various foods is in CCNFSDU 29th session 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	J AOAC Int. 2000:83; 1141 1148 (Measures free folic acid + proportion of free, natural folate)	Optical Biosensor Immunoassay	**Not recommended 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,this method which is recently introduced and currently under AOAC collaborative study should be endorsed 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	J Chromatogr. A., 928, 77-90, 2001	High performance liquid	**Not recommended 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, this method

[11] 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.

	(Measures total folates after conversion to, and measurement as 5 Me-H4PteGlu)		
Vitamin C[14]	AOAC 985.33 (measures ascorbic acid (AA))	2,6 dichloroindophenol titrimetry	**Type III*** 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.
Vitamin C	EN 14130:2003 (Measures ascorbic acid + dehydroascorbic acid).	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session 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 2. Reference: Arella F., Deborde J.L., Bourguignon J.B., Hasselmann C., (1997), Ann. Fals. Exp. Chim., 90, N°940:217-233. 3. Measures total L-ascorbate (Ascorbic acid + dehydroascorbic acid).

[14] 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.

Biotin[15]	EN 15607:2008 (d biotin) (Measures total D-biotin (free + D-biocytin)	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods including infant milk powder is in CCNFSDU 29th session 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) 2. Measures total D-biotin (free + D-biocytin)
Iron	AOAC 985.35	Atomic absorption spectrophotometry	**Type II** - The method is applicable to the determination of Ca, Mg, Fe, Zn, Cu, Mn, Na, and K. Currently listed for copper determination in edible casein products and whey powders (Type II) 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.
Iron	AOAC 984.27	ICP emission spectroscopy	**Type III**
Calcium	ISO 8070 | IDF 119: 2007	Flame atomic Absorption spectrophotometry	**Type II** - Current Codex method for special foods, and adopted by CAC 31 for infant formula, Type II, for determination of Na and K.
Calcium	AOAC 985.35	Atomic absorption spectroscopy	**Type III** 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).

[15] 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.

Calcium	AOAC 984.27	ICP emission spectroscopy	**Type III** - Current Codex method (Type III) for Special foods.
Phosphorus	AOAC 986.24	Spectrophotometry (molybdovanadate)	**Type II** - Current Codex method for special foods. .
Phosphorus .	AOAC 984.27	ICP emission spectroscopy	**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 elements are determined by ICP emission spectroscopy.
Magnesium	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**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	AOAC 985.35	Atomic absorption spectroscopy	**Type III** 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.
Magnesium	AOAC 984.27	ICP emission spectroscopy	**Type III**
Chloride	AOAC 986.26	Potentiometry	**Type II**
Manganese	AOAC 985.35	Atomic absorption spectrophotometry	**Type II**
Manganese	AOAC 984.27	ICP emission spectroscopy	**Type III**
Iodine	AOAC 992.24	Ion-selective potentiometry	**Type II, for milk-based formula** - Current Codex method for milk-based follow-up formula 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