National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1846

Infant Formula

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, calories, vitamins, minerals, and trace elements in infant formula and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 1846 consists of 10 single-use packets of spray-dried, milk-based infant formula, each containing approximately 30 g of material.

Certified Concentration Values: The certified concentration values of selected vitamins and iodine in SRM 1846 are provided in Table 1. Values were derived from the combination of results provided by NIST and collaborating laboratories. All assigned values are the equally weighted means of the measurements made by laboratories reporting results for a given analyte; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [1]. Values are reported on an as-received (not dry-mass) basis in both mass fraction units [2] and in the units specified by the Infant Formula Act of 1980 [3].

Reference Concentration Values: Reference concentration values for proximates, calories, additional vitamins, minerals, trace elements, and fatty acids are provided in Tables 2 through 5. Most of the reference values were derived from results reported by collaborating laboratories. Those for δ-tocopherol and γ- (plus β-) tocopherol were derived from measurements made by NIST using two analytical techniques over a six-year period. The reference values are noncertified values that do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple methods. Explanations in support of each reference value are given as a note in each table.

Information Values: Information values for additional trace elements and fatty acids are provided in Tables 6 and 7. These concentrations are also derived from analyses performed by the collaborating laboratories. These are noncertified values with no uncertainties reported as there is insufficient information to make an assessment of the uncertainties.

Expiration of Value Assignment: The value assignment of this SRM is valid until **30 September 2009**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this report. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Value Assignment: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division, E.R. Elkins of the National Food Processors Association (NFPA; Washington, DC), D.C. Woollard (AgriQuality New Zealand Ltd.; Auckland, New Zealand), H.E. Indyk (Anchor Products; Waitoa, New Zealand), M.K. Mountford of the Infant Formula Council (Atlanta, GA), J.T. Tanner of the U.S. Food and Drug Administration (FDA; Washington, DC), and W.R. Wolf of the U.S. Department of Agriculture (USDA; Beltsville, MD).

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Certificate Issue Date: 20 September 2007

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Analytical measurements at NIST were performed by J.B Thomas, S.A. Margolis, B.R. Norman, and K.E. Sharpless of the NIST Analytical Chemistry Division. Analyses for value assignment were also performed by Abbott Laboratories/Ross Products Division, Analytical Systems Research Corporation, Bristol Meyers Squibb/Mead Johnson Nutritionals, FDA, Covance Laboratories, Lancaster Laboratories, Medallion Laboratories, Nestlé USA, Southern Testing and Research Laboratories, University of Ljubljana Laboratory for Radiochemistry, University of Massachusetts Department of Chemistry, USDA Metabolism and Nutrient Interactions Laboratory, Wyeth-Ayerst Laboratories, members of the NFPA Food Industry Analytical Chemists Subcommittee (FIACS), and participants in an AOAC International collaborative study for vitamin K analysis.

Statistical analysis was provided by S.B. Schiller, L.M. Gill, and J.H. Yen of the NIST Statistical Engineering Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Storage: Until required for use, the infant formula should be stored at temperatures between approximately 20 °C and 25 °C in the original, sealed packets. **DO NOT** use the contents of previously opened packets as their stability has not been investigated. If reconstituted, the sample should be used immediately.

Warning: For laboratory use only. **NOT** for human consumption.

INSTRUCTIONS FOR USE

Before use, the contents of a packet should be mixed by gentle shaking. Test portions for analysis of fat-soluble vitamins should be reconstituted in hot water (greater than 90 °C but less than 100 °C) and homogenized (using a tissue homogenizer or blender) for 30 seconds to aid in breaking down the encapsulation around the vitamins. A minimum test portion of 5 g should be used for any analytical determination of vitamins in this material; a test portion of at least 250 mg should be used for proximate, minerals, and trace element measurements. (Users may prefer to reconstitute the contents of an entire packet in hot water, and subsequently remove a measured test portion for preparation and analysis.)

PREPARATION AND ANALYSIS

Preparation: SRM 1846 is a milk-based infant formula powder prepared by Analytical Systems Research Corporation, Indianapolis, IN. The SRM was manufactured by preparing a spray-dried formula base containing fat, protein, carbohydrate, and minerals, and then combining that formula base with a dry-blend vitamin premix that supplied all of the vitamins. Fat-soluble vitamins were incorporated in the premix in 200-μm particle diameter, cold-water-soluble powders; individual powders contained individual fat-soluble vitamins, i.e., one powder contained only vitamin A (as retinyl palmitate), one contained vitamin E (as RRR-α-tocopheryl acetate), etc. (The final product is composed of 95 % formula base and 5 % vitamin premix.) The finished blend was then passed through an agglomerator, which produces larger, faster-dissolving particles. The powdered infant formula was sealed under nitrogen in single-use foil packets, each containing approximately 30 g of material.

NIST Analyses for Fat-Soluble Vitamins: In the original value-assignment analyses, δ-tocopherol, γ- (plus β-) tocopherol, α-tocopherol, and α-tocopheryl acetate were measured in two packets from each of 12 randomly selected boxes over a four-day period. (The infant formula may contain β-tocopherol, but the chromatographic systems described below are incapable of resolving β-and γ-tocopherol; the instrument was calibrated using only γ-tocopherol.) Test portions of approximately 6 g were saponified in a 9 % (mass/volume) potassium hydroxide solution. The samples were saponified for 45 minutes at 37 °C to 40 °C. Analytes were extracted into diethyl ether/hexane, which was subsequently evaporated, and the analytes were redissolved in ethanol. Samples were injected onto a monomeric C_{18} column [4] and analytes were eluted using a gradient of methanol and ethyl acetate. (Additional methodological detail is provided in reference 5.) Both a programmable UV/visible absorbance detector set to 292 nm for measurement of the tocopherols and 422 nm for measurement of *trans*-β-apo-10'-carotenal oxime (the internal standard) and a fluorescence detector (excitation wavelength of 295 nm, emission wavelength of 335 nm) were used for quantitation of the tocopherols.

In the years following the original value-assignment analyses, the tocopherols were also measured using a different C_{18} column, and a gradient of acetonitrile, methanol, and ethyl acetate, and this revised Certificate of Analysis reflects the inclusion of tocopherol data obtained using this chromatographic method. In these analyses, 6-g test portions were reconstituted in 6 mL sub-boiling (approximately 90 °C water), and were homogenized for 30 s. Test portions were saponified in a 1.7 % (mass/volume) methanolic potassium hydroxide solution at 55 °C for 60 min. Analytes were extracted into diethyl ether/petroleum ether, which was subsequently evaporated, and analytes were dissolved in ethanol/ethyl acetate.

In the original certification analyses, single packets from each of the same 12 randomly selected boxes in which the tocopherols were analyzed were also analyzed for vitamin A (*trans*-retinol and *trans*-retinyl palmitate) over a six-day period. Extraction and chromatography were performed as described above, with the exception that test portions were saponified overnight. Measurement of retinol and retinyl palmitate was at 325 nm using a programmable UV/visible absorbance detector; measurement of *trans*-β-apo-10'-carotenal oxime (the internal standard) was at 422 nm.

NIST Analyses for Water-Soluble Vitamins: Vitamin C was measured in duplicate packets from each of 12 randomly selected boxes over a three-day period. Test portions of approximately 5 g were combined with dithiothreitol to reduce dehydroascorbic acid to ascorbic acid. Proteins were coagulated, and the clear solutions were analyzed by anion exchange chromatography using a cross-linked polyamine column [6]. A chromatographic solvent consisting of a phosphate buffer in acetonitrile and water eluted the ascorbic acid to an electrochemical detector set at 700 mV [6].

Riboflavin (vitamin B_2) and pyridoxine (vitamin B_6) were measured in duplicate test portions from one packet from each of 10 randomly selected boxes over a four-day period. Nicotinamide (niacinamide) was measured in each of these packets over a two-day period. Test portions of 3.5 g to 5 g were dissolved in water, and the proteins were coagulated overnight; the clear solution was removed for analysis. A monomeric C_{18} column [4] separated the analytes using a gradient of aqueous potassium acetate and acetonitrile. A fluorescence detector was used to measure pyridoxine (excitation wavelength of 295 nm, emission wavelength of 405 nm) and riboflavin (excitation wavelength of 269 nm, emission wavelength of 520 nm); an absorbance detector was used for measurement of nicotinamide at 260 nm.

NIST Analysis for Iodine: Iodine was measured by neutron activation analysis in single packets from each of five randomly selected boxes. In a class-100 clean bench, test portions of approximately 3.5 g were combusted in a quartz combustion tube under flowing oxygen, and iodine was removed from the gas stream onto activated charcoal traps. The sealed charcoal traps and iodine standards were then irradiated for 60 s in the NBSR pneumatic facility RT-4 at 20 MW reactor power. Both the standards and test portions were counted for 20 min at a distance of 20 cm after a 10-min decay period.

Analyses by Collaborating Laboratories: Data from eight additional sources were used for certification of this material: nine laboratories selected to assist in the certification (Appendix A); an interlaboratory comparison exercise organized by the NFPA FIACS (20 participating laboratories); the manufacturer of the material; the University of Ljubljana Laboratory for Radiochemistry (Ljubljana, Slovenia); the University of Massachusetts Department of Chemistry (Amherst, MA); the USDA Metabolism and Nutrient Interactions Laboratory (Beltsville, MD); four additional collaborating laboratories (Appendix B); and 34 laboratories participating in an AOAC collaborative study for the analysis of vitamin K. Not every laboratory measured every analyte. The nine laboratories listed in Appendix A were asked to use AOAC methods or their equivalent, to make duplicate measurements from each of six packets, and to report detailed methodological information with their results. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in Appendix C. The methods used by the laboratories listed in Appendices A and B, NIST, USDA, the University of Massachusetts, the University of Ljubljana, the manufacturer, and the AOAC collaborative study are included in this listing; NFPA FIACS methods are not included.

Homogeneity Assessment: The homogeneity of ascorbic acid (vitamin C), riboflavin (vitamin B_2), pyridoxine (vitamin B_6), and vitamin E was assessed at NIST using the methods described above. While no statistically significant heterogeneity was found for vitamins B_2 (3.5 g to 5 g test portions) and E (6 g test portions), a 2 % relative standard deviation of the material was observed for vitamins C (5 g test portions) and B_6 (3.5 g to 5 g test portions). Similarly, in an assessment of the homogeneity of zinc and selenium by the USDA, 1 % and 2 % relative standard deviations, respectively, were observed for 0.25 g test portions. Therefore, a 2 % heterogeneity contribution has been included in all vitamin, mineral, and trace element uncertainties. Proximate and fatty acid data have been treated as homogeneous.

Value Assignment: The nine laboratories listed in Appendix A reported six mean values for their six duplicate analyses. The mean for each laboratory was determined from these six values. For the calculation of assigned values, for analytes other than the individual fatty acids and vitamin K, each of these laboratory means was weighted equally with the mean of the NFPA interlaboratory comparison exercise, the mean from a NIST analysis (when available), and the means from analyses by the manufacturer, the University of Ljubljana, and the University of Massachusetts (also when available).

The four laboratories listed in Appendix B reported results for the analysis of fatty acids in four packets of infant formula, and assigned values are calculated from the equally weighted means of these results.

Two of the nine laboratories listed in Appendix A reported six mean values for their six duplicate analyses of vitamin K. The FDA laboratories reported values for 10, 10, and 31 analyses, respectively; two sets of data were received from the FDA laboratory in Atlanta. The 34 AOAC collaborative study laboratories reported results for duplicate analyses. The reference value is calculated from the equally weighted means of these results.

Table 1. Certified Concentration Values for Selected Vitamins and Iodine[a,b]

	Mass Fraction (mg/kg)	Units Specified by Infant Formula Act [3]
Vitamin C	1146 ± 66	(22.2 ± 1.3) mg/100 kcal
Vitamin B_2	17.4 ± 1.0	(337 ± 21) µg/100 kcal
Vitamin B_6 (pyridoxine hydrochloride)	8.4 ± 1.0	(162 ± 20) µg/100 kcal
Niacin	63.3 ± 7.6	(1230 ± 150) µg/100 kcal
Iodine	1.11 ± 0.17	(21.4 ± 3.3) µg/100 kcal

[a] Each certified concentration value, expressed as a mass fraction of the material (as received) for selected vitamins and iodine, is the equally weighted mean of results from analyses by NIST, the NFPA FIACS interlaboratory comparison exercise, the Universities of Ljubljana and Massachusetts (for iodine), and laboratories listed in Appendix A. The expanded uncertainty, computed according to the CIPM method [1], is at the 95 % level of confidence, and includes within- and between-laboratory uncertainties as well as material variability. Each certified value and expanded uncertainty defines a range of values within which the true concentration is expected to lie with 95 % confidence. The certified concentration values have been converted to the units specified by the Infant Formula Act; the uncertainties on values expressed relative to 100 kcal have been expanded to include the uncertainty in the kcal measurements. Analytical methodology information is provided in Appendix C.

[b] **NOTE:** NIST has removed the certified value for vitamin E as α-tocopherol due to the increasing difficulty in extracting this analyte from the material.

Table 2. Reference Concentration Values for Proximates and Calories[a]

	Mass Fraction (%)	g/100 kcal
Solids	98.02 ± 0.27	19.01 ± 0.16
Ash	2.913 ± 0.048	0.565 ± 0.010
Fat	27.1 ± 0.6	5.25 ± 0.12
Nitrogen	1.739 ± 0.058	0.337 ± 0.012
Protein	11.10 ± 0.37	2.153 ± 0.073
Carbohydrate (by difference)	57.2 ± 1.0	11.10 ± 0.21
Calories[b]	(515.5 ± 4.2) kcal/100 g	

[a] Each reference concentration value, expressed as a mass fraction of the material (as received) for proximates and calories, is an equally weighted mean of method-specific results from the NFPA FIACS interlaboratory comparison exercise and the laboratories listed in Appendix A. The expanded uncertainty, computed according to the CIPM method [1], is at the 95 % level of confidence, and includes within- and between-laboratory uncertainties. Each reference value and expanded uncertainty defines a range of values within which the concentration is expected to lie with 95 % confidence when analyzed using AOAC Official Methods of Analysis. The reference values have been converted to express concentrations relative to 100 kcal, and the uncertainties on these values have been expanded to include the uncertainty in the kcal measurements. Analytical methodology information is provided in Appendix C. **NOTE:** NIST has replaced the previously used term "noncertified" with "reference value."

[b] The value for calories is the mean of individual caloric calculations from the laboratories listed in Appendix A and the NFPA FIACS interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 517 kcal/100 g.

Table 3. Reference Concentration Values for Selected Vitamins[a,b]

	Mass Fraction (mg/kg)	Units Specified by Infant Formula Act [3]
Vitamin D[c]	0.117 ± 0.011	(90.5 ± 8.7) IU[d]/100 kcal
δ-Tocopherol[e]	20.19 ± 1.69	not required by Infant Formula Act
γ-Tocopherol[e,f]	75.01 ± 5.07	not required by Infant Formula Act
Vitamin K[g]	0.944 ± 0.041	(18.32 ± 0.81) µg/100 kcal
Vitamin B_1 hydrochloride	10.9 ± 1.5	(212 ± 28) µg/100 kcal
Vitamin B_{12}	0.039 ± 0.003	(0.746 ± 0.051) µg/100 kcal
Folic Acid[h]	1.29 ± 0.28	(25.1 ± 5.5) µg/100 kcal
Pantothenic acid[h]	48.7 ± 7.3	(940 ± 140) µg/100 kcal
Biotin[h]	0.411 ± 0.066	(8.0 ± 1.3) µg/100 kcal
Choline (ion)	1250 ± 120	(24.2 ± 2.6) mg/100 kcal
Inositol[h]	940 ± 190	(18.2 ± 3.7) mg/100 kcal

[a] Each reference concentration value, expressed as a mass fraction for selected vitamins, is an equally weighted mean of results from the NFPA FIACS interlaboratory comparison exercise and laboratories listed in Appendix A. The value for vitamin K is the equally weighted mean of results reported by laboratories listed in Appendix A and the AOAC International collaborative study laboratories. The expanded uncertainty, computed according to the CIPM method [1], is at the 95 % level of confidence and includes within- and between-laboratory uncertainties as well as material variability. Each reference value and expanded uncertainty defines a range of values within which the concentration is expected to lie with 95 % confidence. The reference values have been converted to the units specified by the Infant Formula Act; the uncertainties on values expressed relative to 100 kcal have been expanded to include the uncertainty in the kcal measurements. Analytical methodology information is provided in Appendix C. **NOTE:** NIST has replaced the previously used term "noncertified value" with "reference value."

[b] **NOTE:** NIST has removed the reference value for vitamin A as *trans*-retinol due to the increasing difficulty in extracting this analyte from the material.

[c] Includes pre-vitamin D

[d] IU = International Units

[e] Reference values, expressed as mass fractions for δ-tocopherol and γ-tocopherol, are the means of results obtained by NIST using two analytical techniques. The uncertainty in the reference values is expressed as an expanded uncertainty, *U*, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effect of within-laboratory components of uncertainty as well as material variability. The coverage factor, *k*, is determined from the Student's
t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. **NOTE:** Concentration values have been revised due to the inclusion of additional data.

[f] Includes β-tocopherol

[g] Contains both *cis* and *trans* vitamin K. One and two laboratories, respectively, reported values for *cis* and *trans* vitamin K; mean values were 0.098 mg/kg *cis* and 0.93 mg/kg *trans* vitamin K.

[h] Collaborating laboratories reported measuring either total or free analyte; results were indistinguishable and have been combined for value assignment.

Table 4. Reference Concentration Values for Minerals and Trace Elements[a]

	Mass Fraction (mg/kg)	Units Specified by Infant Formula Act [3]
Calcium	3670 ± 200	(71.1 ± 4.0) mg/100 kcal
Phosphorus	2610 ± 150	(50.7 ± 2.9) mg/100 kcal
Magnesium	538 ± 29	(10.43 ± 0.56) mg/100 kcal
Iron	63.1 ± 4.0	(1.225 ± 0.079) mg/100 kcal
Zinc	60.0 ± 3.2	(1.164 ± 0.063) mg/100 kcal
Copper	5.04 ± 0.27	(97.7 ± 5.2) µg/100 kcal
Sodium	2310 ± 130	(44.8 ± 2.6) mg/100 kcal
Potassium	7160 ± 380	(138.9 ± 7.5) mg/100 kcal
Chloride	4920 ± 300	(95.5 ± 5.9) mg/100 kcal

[a] Each reference concentration value, expressed as a mass fraction for minerals and trace elements, is an equally weighted mean of results from the NFPA FIACS interlaboratory comparison exercise, the USDA, the manufacturer, and laboratories listed in Appendix A. The expanded uncertainty, computed according to the CIPM method [1], is at the 95 % level of confidence and includes within- and between-laboratory uncertainties as well as material variability. Each reference value and expanded uncertainty defines a range of values within which the concentration is expected to lie with 95 % confidence. The reference values have been converted to the units specified by the Infant Formula Act; the uncertainties on values expressed relative to 100 kcal have been expanded to include the uncertainty in the kcal measurements. Analytical methodology information is provided in Appendix C. **NOTE:** NIST has replaced the previously used term "noncertified value" with "reference value."

Table 5. Reference Concentration Values for Fatty Acids (as Triglycerides)[a]

	Mass Fraction (%)
Dodecanoic acid (C12:0) (Lauric acid)	3.65 ± 0.56
Tetradecanoic acid (C14:0) (Myristic acid)	1.54 ± 0.13
Hexadecanoic acid (C16:0) (Palmitic acid)	2.90 ± 0.15
(Z)-9-Hexadecenoic acid (C16:1) (Palmitoleic acid)	0.0208 ± 0.0025
Octadecanoic acid (C18:0) (Stearic acid)	2.84 ± 0.14
(Z)-9-Octadecenoic acid (C18:1) (Oleic acid)	6.01 ± 0.15
9-Octadecenoic acid (C18:1) (Elaidic acid)	4.00 ± 0.54
(Z,Z)-9,12-Octadecadienoic acid (C18:2) (Linoleic acid)	3.48 ± 0.40
(Z,Z,Z)-9,12,15-Octadecatrienoic acid (C18:3) (Linolenic acid)	0.0982 ± 0.0048
Eicosanoic acid (C20:0) (Arachidic acid)	0.088 ± 0.011
Docosanoic acid (C22:0) (Behenic acid)	0.0566 ± 0.0075

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is an equally weighted mean of results provided by the laboratories listed in Appendix B. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO and NIST Guides [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix C.

Table 6. Information Concentration Values for Manganese and Selenium[a]

	Mass Fraction (mg/kg)	µg/100 kcal
Manganese	0.4	7
Selenium	0.08	1

[a] Information values for manganese and selenium are the equally weighted means of results from the analysis by the USDA, the NFPA FIACS interlaboratory comparison exercise, and laboratories listed in Appendix A. The information values have also been converted to express concentrations relative to 100 kcal. Analytical methodology information is provided in Appendix C.

Table 7. Information Concentration Values for Fatty Acids (as Triglycerides)[a]

	Mass Fraction (%)
Hexanoic acid (C6:0) (Caproic acid)	0.063
Octanoic acid (C8:0) (Caprylic acid)	0.60
Decanoic acid (C10:0) (Capric acid)	0.47
Pentadecanoic acid (C15:0)	0.011
Heptadecanoic acid (C17:0) (Margaric acid)	0.023
9-Eicosenoic acid (C20:1) (Gadoleic acid)	0.031
Tetracosanoic acid (C24:0) (Lignoceric acid)	0.039

[a] Each information concentration value, expressed as a mass fraction on an as-received basis, is an equally weighted mean of results provided by the laboratories listed in Appendix B. Analytical methodology information is provided in Appendix C.

REFERENCES

[1] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Result*;, NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[2] Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811, U.S. Government Printing Office: Washington, DC 1995 Ed. (1994); available at http://physics.nist.gov/Pubs/.

[3] Infant Formula Act of 1980, Public Law 96–359 [H.R. 6940] (1980).

[4] Sander, L.C.; Wise, S.A.; *Evaluation of Shape Selectivity in Liquid Chromatography*; LC·GC 5, pp. 378–390 (1990).

[5] Sharpless, K.E.; Schiller, S.B.; Margolis, S.A.; Brown Thomas, J.; Iyengar, G.V.; Colbert, J.C.; Gills, T.E.; Wise, S.A.; Tanner, J.T.; Wolf, W.R.; *Certification of Nutrients in Standard Reference Material 1846: Infant Formula*; J. AOAC Vol. 80, pp. 661–621 (1997).

[6] Margolis, S.A.; Schapira, R.M.; *The Measurement of L-Ascorbic Acid and D-Ascorbic Acid in Biological Samples*; J. Chromatogr. B; Vol. 690, pp. 25–33 (1997).

Certificate Revision History: 20 September 2007 (Update of expiration date and editorial changes); 22 March 2006 (Editiorial changes to clarify the form of vitamins B_1 and B_6 and extension of the certification period); 24 January 2006 (This revision reports a change in vitamin B_1 in Table 3 and Appendix C to vitamin B_1 hydrochloride, and editorial changes); 25 August 2004 (This technical revision reports the removal of the certified value for vitamin E (α-tocopherol) and the reference value for vitamin A (retinol) because of increasing difficulty in extracting these analytes from the material. The expiration date for the material has been extended); 14 January 2004 (The expiration date for the material has been extended); 17 October 2003 (This revision reports an extension in the value assignment date); 25 September 2001 (This technical revision reports a change from certified to reference value for vitamin A (because of increasing difficulty in extracting this analyte from the material) and a change in the concentration values for δ- and γ-tocopherol (because of the inclusion of more data); 04 October 1999 (This technical revision reports the addition of reference and information values for fatty acids and a change from information to reference value for vitamin K); 02 June 1998 (This technical revision reports a change from information to certified value for iodine; reports a reference value for choline (ion) instead of choline; and reports a revision in the "Instructions for Use" to aid in breaking down the encapsulation around the fat-soluble vitamins); 08 May 1996 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

The laboratories listed below performed measurements that contributed to the certification of SRM 1846 Infant Formula.

Abbott Laboratories, Ross Products Division; Columbus, OH, USA.
Bristol Meyers Squibb, Mead Johnson Nutritionals; Zeeland, MI, USA.
Covance Laboratories; Madison, WI, USA.
Food and Drug Administration, Atlanta Center for Nutritional Analysis; Atlanta, GA, USA.
Food and Drug Administration, Center for Food Safety and Applied Nutrition; Washington, DC, USA.
Lancaster Laboratories; Lancaster, PA, USA.
Nestlé USA; Dublin, OH, USA.
Southern Testing and Research; Wilson, NC, USA.
Wyeth-Ayerst Laboratories; Radnor, PA, USA.

APPENDIX B

The laboratories listed below performed measurements of individual fatty acids in SRM 1846.

Covance Laboratories; Madison, WI, USA.
Lancaster Laboratories; Lancaster, PA, USA.
Medallion Laboratories; Minneapolis, MN, USA.
Southern Testing and Research Laboratories; Wilson, NC, USA.

APPENDIX C

The methodological information reported by collaborating laboratories whose results were used for value assignment is summarized below. The number of laboratories using a particular method is provided in parentheses. The methods used by the laboratories listed in Appendices A and B, USDA, the manufacturer, the University of Ljubljana, the University of Massachusetts, the AOAC collaborative study laboratories, and NIST are included in this appendix; NFPA FIACS methods are **NOT** included.

Proximates, Fatty Acids, and Calories

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (2) Vacuum oven (6) Thermogravimetric analysis (1)
Ash	Mass loss after ignition in muffle furnace (8) Thermogravimetric analysis (1)
Fat	Alkali pretreatment to break fat emulsion, extract with ether: Roese-Gottlieb - petroleum ether (5) Mojonnier - diethyl ether/petroleum ether (3) Acid digestion, ether extraction (1)
Fatty Acids	Hydrolysis followed by gas chromatography (4)
Nitrogen	Kjeldahl (6) Thermal conductivity (2) Pyrolysis, gas chromatography (1)
Protein	Calculated; a factor of 6.38 was used to calculate protein from nitrogen results
Carbohydrate	Calculated; solids - (protein + fat + ash)
Calories	Calculated; 9(fat) + 4(protein) + 4(carbohydrate)

Fat-Soluble Vitamins

Vitamin D	Saponification - NPLC - absorbance detection (3) Saponification - RPLC - absorbance detection (3)
δ-Tocopherol, γ-Tocopherol	Saponification - RPLC - fluorescence detection (NIST) Saponification - RPLC - absorbance and fluorescence detection (NIST)
Vitamin K	Enzymatic digestion - RPLC - absorbance detection (2) Enzymatic digestion - solid-phase extraction - RPLC - post-column reduction - fluorescence detection (1) Matrix solid-phase dispersion - RPLC - post-column reduction - fluorescence detection (1) Enzymatic digestion - RPLC - post-column reduction - fluorescence detection (35)

Water-Soluble Vitamins

Vitamin C	Fluorescence detection (6) Electrochemical titration (1) Colorimetric titration (1) Ion exchange chromatography - electrochemical detection (NIST)
Vitamin B_1 hydrochloride	Microbiological (1) Digestion - fluorescence detection (3) Extraction - RPLC - fluorescence detection (2) Extraction - ion pairing chromatography - fluorescence detection (1)
Vitamin B_2	Microbiological (1) Digestion - fluorescence detection (4) Extraction - RPLC - fluorescence detection (2 + NIST)
Vitamin B_6 (pyridoxine hydrochloride)	Microbiological (6) Extraction - RPLC - fluorescence detection (NIST)
Vitamin B_{12}	Microbiological (6)
Niacin	Microbiological (6) Acid digestion - absorption spectrophotometry (1) Extraction - RPLC - fluorescence detection (NIST)
Folic acid	Microbiological (6)
Pantothenic acid	Microbiological (6)
Biotin	Microbiological (6)
Choline	Acid digestion - absorption spectrophotometry (4) Acid digestion - electrochemical detection (2)
Inositol	Microbiological (3) Ion exchange chromatography - electrochemical detection (1)

Minerals and Trace Elements

Calcium	Flame atomic absorption spectrometry (2) Inductively coupled plasma atomic emission spectrometry (7)
Chloride	Electrochemical titration (5) Inductively coupled plasma atomic emission spectrometry (1) Absorption spectrophotometry (1)
Copper	Flame atomic absorption spectrometry (2)

	Inductively coupled plasma atomic emission spectrometry (7)
Iodine	Colorimetric titration (1) Absorption spectrophotometry (1) Gas chromatography - electron capture detection (1) Ion-selective electrode (1) Inductively coupled plasma atomic emission spectrometry (2) Radiochemical neutron activation analysis (1 + NIST) Inductively coupled plasma - mass spectrometry (1)
Iron	Flame atomic absorption spectrometry (2) Inductively coupled plasma atomic emission spectrometry (7)
Magnesium	Flame atomic absorption spectrometry (2) Inductively coupled plasma atomic emission spectrometry (7)
Manganese	Flame atomic absorption spectrometry (1) Inductively coupled plasma atomic emission spectrometry (6)
Phosphorus	Absorption spectrophotometry (3) Inductively coupled plasma atomic emission spectrometry (6)
Potassium	Flame atomic absorption spectrometry (1) Flame atomic emission spectrometry (1) Inductively coupled plasma atomic emission spectrometry (7)
Selenium	Graphite furnace atomic absorption spectrometry (2) Hydride generation atomic absorption spectrometry (1) Gas chromatography - isotope dilution mass spectrometry (1)
Sodium	Flame atomic absorption spectrometry (1) Flame atomic emission spectrometry (1) Inductively coupled plasma atomic emission spectrometry (7)
Zinc	Flame atomic absorption spectrometry (2) Inductively coupled plasma atomic emission spectrometry (7) Inductively coupled plasma - isotope dilution mass spectrometry (1)