FOOD COMPOSITION AND ADDITIVES

Preparation and Characterization of Standard Reference Material 1849 Infant/Adult Nutritional Formula

KATHERINE E. SHARPLESS, RICHARD M. LINDSTROM, BRYANT C. NELSON, KAREN W. PHINNEY, CATHERINE A. RIMMER, LANE C. SANDER, MICHELE M. SCHANTZ, RABIA O. SPATZ, JEANICE BROWN THOMAS, GREGORY C. TURK, STEPHEN A. WISE, and LAURA J. WOOD
National Institute of Standards and Technology, Analytical Chemistry Division, Gaithersburg, MD 20899-8390
JAMES H. YEN
National Institute of Standards and Technology, Statistical Engineering Division, Gaithersburg, MD 20899-8980

Standard Reference Material (SRM) 1849 Infant/Adult Nutritional Formula has been issued by the National Institute of Standards and Technology (NIST) as a replacement for SRM 1846 Infant Formula, issued in 1996. Extraction characteristics of SRM 1846 have changed over time, as have NIST's analytical capabilities. While certified mass fraction values were provided for five constituents in SRM 1846 (four vitamins plus iodine), certified mass fraction values for 43 constituents are provided in SRM 1849 (fatty acids, elements, and vitamins) and reference mass fraction values are provided for an additional 43 constituents including amino acids and nucleotides, making it the most extensively characterized food-matrix SRM available from NIST.

Infant formula is arguably the most regulated food in the United States. The Infant Formula Act of 1980 (Public Law 96-359) requires that manufacturers test their products to make sure that nutrients fall within specified limits (1). Since 1996, the National Institute of Standards and Technology (NIST) has provided Standard Reference Material (SRM) 1846 Infant Formula for use as a tool for assuring the quality of nutrient measurements (2). This paper describes the preparation and value assignment of SRM 1849, a more fully characterized replacement for SRM 1846. [SRM 1849 contains nutrients at levels that would not be in compliance with the U.S. Infant Formula Act of 1980 (1), and this material is not an infant formula. SRM 1846 was replaced with a product that is not an infant formula to provide broader material applicability.] As is true for all of NIST's food-matrix SRMs, SRM 1849 is intended for use as a primary control material when assigning values to in-house (secondary) control materials and for validation of analytical methods for the measurement of nutrients in similar matrixes.

Received January 27, 2010. Accepted by SG March 16, 2010.
Corresponding author's e-mail: katherine.sharpless@nist.gov
Mention of commercial entities is for information only; it does not imply recommendation or endorsement by NIST.

SRM 1846 was prepared in 1991 as a spray-dried base into which elements and encapsulated vitamins were dry-blended. The material was dry-blended to increase long-term stability of the encapsulated vitamins. Over time, the encapsulation hardened, making complete extraction of the originally certified fat-soluble vitamin content (retinol and -tocopherol) difficult if not impossible. At the end of its lifetime, only five analytes (ascorbic acid, riboflavin, niacin, pyridoxine hydrochloride, and iodine) remained as certified mass fractions in SRM 1846 (3).

When SRM 1846 was prepared, NIST offered a number of food-matrix materials with values assigned for elements and, therefore, did not measure elements in SRM 1846. Because of this, only reference values for element mass fractions, using collaborating laboratories' data, were assigned (4). In the early 1990s, NIST did not have methods in place to assign certified values for fatty acids, vitamins D and K, or many of the water-soluble vitamins; again, reference values were assigned for SRM 1846 using collaborating laboratories' data. Five certified, 38 reference, and nine information mass fraction values—52 values in total—were provided in SRM 1846 at the end of its lifetime. By contrast, SRM 1849 is provided with 43 certified and 43 reference mass fraction values—a total of 86 values. NIST made measurements of fatty acids, fat- and water-soluble vitamins, and elements. New methods were developed for the determination of vitamins in this material, in particular methods based on isotope-dilution MS (5).

SRM 1849 was characterized by NIST and collaborating laboratories (Table 1), including the manufacturer of the material, to provide mass fraction values designated as certified, reference, or information. NIST used one method (for most of the elements measured) or two independent analytical methods (for vitamins and fatty acids). The means obtained using the individual NIST methods were combined with the manufacturer's mean result, the median of the individual collaborating laboratory means, and the mean of results provided by the U.S. Department of Agriculture (USDA), where available. The logic behind NIST's use of two independent methods is that biases inherent to one method are unlikely to occur in the other method; therefore, if results between the two methods agree, they are likely to represent

Table 1. The laboratories listed below performed measurements that contributed to the value assignment of constituents in SRM 1849 Infant/Adult Nutritional Formula

Abbott Nutrition, Columbus, OH
Campbell Soup Co., Camden, NJ[a]
Covance, Inc., Madison, WI[a]
Eurofins Danmark A/S, Kolding, Denmark
Eurofins Laboratories Ltd, Wolverhampton, UK
Fonterra, Palmerston North, NZ
Fonterra, Waitoa, NZ
General Mills, Inc., Minneapolis, MN[a]
Hormel Foods Corp., Austin, MN[a]
Kraft, East Hanover, NJ[a]
Kraft Foods, Glenview, IL[a]
Krueger Food Laboratories, Cambridge, MA[a]
Mead Johnson Nutritionals, Evansville, IN
Nestlé USA, Dublin, OH[a]
Novartis Nutrition Corp., St. Louis Park, MN[a]
PBM Nutritionals, Georgia, VT
U.S. Food and Drug Administration, Atlanta, GA

[a] These laboratories analyzed SRM 1849 as part of a GMA FIACC interlaboratory comparison exercise.

the true value. An analyst does not have to use one of the same analytical methods to obtain the certified value. A NIST-certified value is one in which NIST has the highest confidence in its accuracy, in that all known or suspected sources of bias have been fully investigated or taken into account (4). Certified values were provided for analytes that were measured by both NIST and the collaborating laboratories. NIST reference values represent a best estimate of the true value where all known or suspected sources of bias have not been fully investigated; reference values have associated uncertainties that may not include all sources of uncertainty and may represent only a measure of the of the measurement method(s)' precision (4). Reference values may be assigned if no NIST data are available, or if sources of bias in NIST measurements have not been fully resolved (4). Reference values in this material were provided for analytes measured only by collaborating laboratories, including the manufacturer.

Experimental

Material Preparation

SRM 1849 is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A base liquid containing all constituents was conventionally heat-processed, homogenized, then spray-dried. The ingredients used in preparation of SRM 1849 are provided in Table 2. The product was packaged by the manufacturer in single-use pouches, each containing 10 g powder and flushed with nitrogen. The materials were stored below 0 C following packaging, and stored at NIST at –80 C to enhance long-term stability. This material contains some nutrients at levels not permitted in infant formula and is not an infant formula.

Characterization and Value Assignment

(**a**) *Fatty acids*.—Value assignment of the mass fractions of fatty acids in SRM 1849 was based on the combination of measurements made at NIST using two different analytical methods, and by collaborating laboratories and the manufacturer. NIST provided results using two different

Table 2. Ingredients in SRM 1849 as indicated by the manufacturer

Lactose	Magnesium phosphate	L-Carnitine
Nonfat dry milk	Ferrous sulfate	-Carotene
High oleic safflower oil	Choline chloride	Retinyl palmitate
Soy oil	Zinc sulfate	Thiamine (vitamin B_1) chloride hydrochloride
Coconut oil	Taurine	Pyridoxine (vitamin B_6) hydrochloride
Whey protein concentrate	Myo-inositol	Riboflavin (vitamin B_2)
Sodium caseinate	Cytidine monophosphate	Chromium chloride
Magnesium sulfate	*RRR*- -tocopheryl acetate	Sodium fluoride
Potassium citrate	Guanosine monophosphate	Sodium molybdate
Sodium chloride	Manganese sulfate	Folic acid
Calcium phosphate	Uridine monophosphate	Biotin
Docosahexaenoic acid (DHA)	Adenosine monophosphate	Sodium selenate
L-Methionine	Niacinamide	Phylloquinone (vitamin K_1)
Arachidonic acid (AA)	Copper sulfate	Cholecalciferol (vitamin D_3)
Ascorbic acid (vitamin C)	d-Calcium pantothenate	Cyanocobalamin (vitamin B_{12})

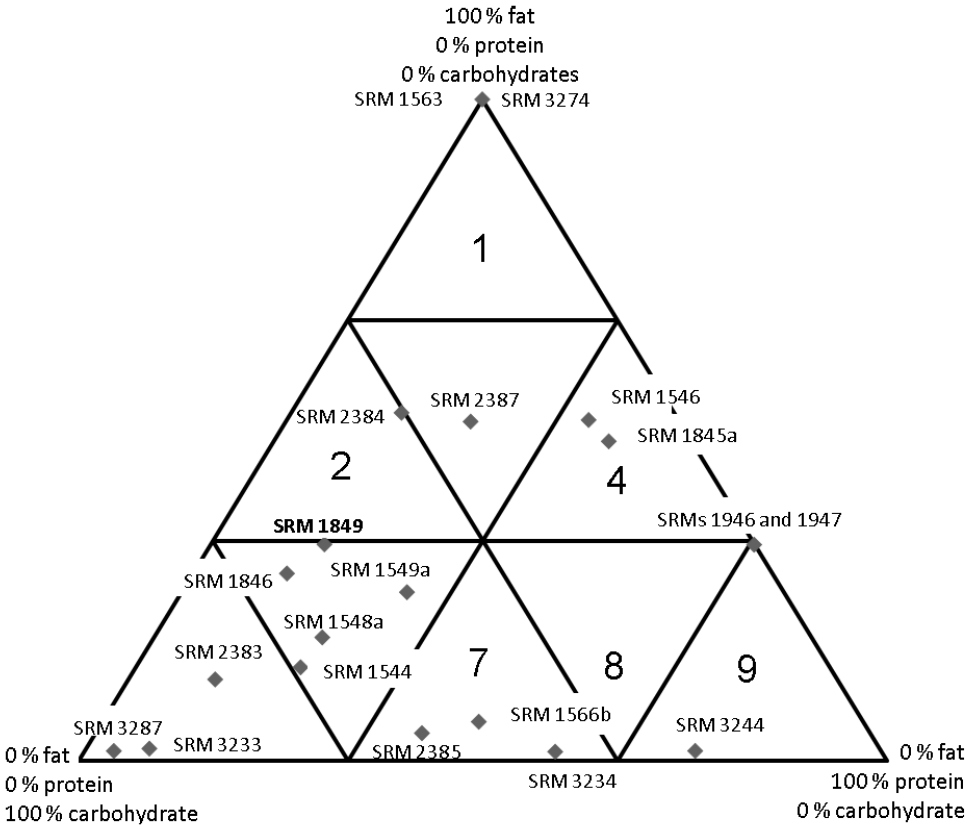

Figure 1. Location of SRM 1846 Infant Formula and SRM 1849 Infant/Adult Nutritional Formula in the fat-protein-carbohydrate triangle developed by AOAC INTERNATIONAL for categorization of food matrixes. Other food-matrix SRMs available from NIST or in preparation, with values assigned for proximates, are shown. SRM 1544 and SRM 1548 diet composites, SRM 1546 meat homogenate, SRM 1549 whole milk powder (in preparation), SRM 1563 coconut oil, SRM 1566b oyster tissue, SRM 1845a whole egg powder (in preparation), SRM 1946 and 1947 fish tissue, SRM 2383 baby food composite that is being replaced, SRM 2384 baking chocolate, SRM 2385 spinach, SRM 2387 peanut butter, SRM 3274 botanical oils with values assigned for fatty acids, SRM 3233 fortified breakfast cereal (in preparation), SRM 3234 soy flour (in preparation), SRM 3244 protein powder drink mix, and SRM 3287 blueberries (in preparation).

analytical methods: GC with a flame ionization detector (FID) or MS detector.

(**b**) *Vitamins*.—Value assignment of the mass fractions of the vitamins in SRM 1849 was based on the combination of results provided from several different analytical methods at NIST, collaborating laboratories, and the manufacturer. NIST provided measurements by using a combination of different LC methods with different detection (5). Vitamin A was measured by using two LC methods with absorbance (LC/abs) or MS detection. Retinyl palmitate-d_4 was used as the internal standard for isotope dilution (ID) LC/MS. Tocopherols, including -tocopheryl acetate, were measured using LC/abs or LC with fluorescence (LC/FL) detection. Vitamins D and K (cholecalciferol and phylloquinone, respectively) were measured at NIST using only ID-LC/MS with vitamin D_3-d_3 and vitamin K_1-d_4 used as internal standards (5). -Carotene was measured at NIST by using two LC/abs methods. Results for -carotene from all sources, including collaborating laboratories, ranged from 2 to 12 g/g; therefore, a value could not be assigned.

Water-soluble vitamins were measured by using two LC methods with absorbance, MS, or MS/MS detection (5). Vitamins B_1, B_2, B_6, niacinamide, and pantothenic acid were measured in one set of analyses by using LC/MS with isotopically labeled internal standards: $^{13}C_3$-thiamine chloride; $^{2}H_4$-niacinamide; calcium $^{13}C_3$, ^{15}N-pantothenate; and $^{13}C_4$-pyridoxine hydrochloride. Vitamins B_1, B_6, and niacinamide were also measured by LC/abs (vitamins B_1 and niacinamide) and FL (vitamin B_6). Folic acid measurements were made by using negative- and positive-ion mode LC/MS/MS with $^{13}C_5$-folic acid as the internal standard (6). Biotin was measured using negative-ion mode LC/MS and positive-ion mode LC/MS/MS with $^{2}H_2$-biotin as an internal standard (7).

The USDA's Food Composition and Methods Development Laboratory (Beltsville, MD) provided results for water-soluble vitamins (8, 9) and vitamin D (10) using LC/abs or LC/FL (as appropriate) and LC/MS methods.

(**c**) *Elements*.—Value assignment of the mass fractions of the elements in SRM 1849 was based on the combination of

Table 3. Certified (bold) and reference mass fractions (normal typeface) for proximates, fatty acids as triglycerides, lactose, cholesterol, and calories in SRM 1849[a]

Fraction	Units[b]	Collaborating laboratories' median	MADe[c]	Manufacturer's mean	SD	NIST GC/MS mean	SD	NIST GC-FID mean	SD	Assigned value	U^a
Solids	%	98.4	0.1							98.4	0.1
Ash	%	4.49	0.01	4.55	0.01					4.52	0.04
Protein	%	13.2	0.1	13.3	0.1					13.3	0.1
Total fat	%	30.6	0.2	31.3	0.2					31.0	0.5
Carbohydrates	%	50.2	0.1							50.2	0.3
Lactose monohydrate	%	49.8	1.3							49.8	1.8
Calories[d]	kcal/100 g	527.1	2.0							527.0	4.0
Cholesterol	mg/g	0.127	0.068							0.127	0.015
C6:0	%	0.061	0.005							0.061	0.011
C8:0	%	0.620	0.016	0.712	0.014	0.618	0.016	0.602	0.027	**0.638**	**0.067**
C10:0	%	0.480	0.012			0.479	0.012	0.459	0.027	**0.473**	**0.019**
C12:0	%	3.68	0.05			3.71	0.22	3.75	0.13	**3.712**	**0.075**
C14:0	%	1.52	0.01			1.53	0.05	1.51	0.05	**1.521**	**0.021**
C15:0	%	0.007	0.001			0.007	0.001			**0.0070**	**0.0003**
C16:0	%	2.53	0.05			2.59	0.13	2.39	0.08	**2.50**	**0.16**
C16:1 n–7		0.027	0.002			0.025	0.002	0.027	0.002	**0.0262**	**0.0016**
C17:0	%	0.015	0.001							0.015	0.001
C18:0	%	0.941	0.017			0.877	0.052	0.897	0.027	**0.905**	**0.056**
C18:1 n–9	%	11.26	0.38			10.35	0.65	10.29	0.51	**10.63**	**0.88**
C18:1 n–7	%	0.216	0.014			0.200	0.023	0.193	0.024	**0.203**	**0.021**
C18:2 n–6	%	5.99	0.15	6.05	0.08	5.99	0.26	6.05	0.21	**6.02**	**0.10**
20:0	%	0.098	0.002			0.094	0.003	0.095	0.003	**0.095**	0.003
C20:1 n–9	%	0.062	0.003							0.062	0.007
C18:3 n–3	%	0.542	0.019	0.590	0.010			0.553	0.019	**0.561**	**0.043**
C22:0	%	0.081	0.003							0.080	0.007
C20:3	%	0.020	0.002							0.020	0.004
C20:4 n–6	%	0.191	0.003	0.197	0.005	0.213	0.014	0.224	0.017	**0.206**	**0.022**
C24:0	%	0.042	0.001			0.038	0.002	0.038	0.002	**0.039**	**0.003**
C24:1 n–9	%	0.002								0.024	0.004
C22:6	%	0.068	0.002	0.072	0.003	0.064	0.004	0.064	0.003	**0.067**	**0.006**

[a] Each certified mass fraction value, expressed as a mass fraction for the material as received, is the mean from the combination of the mean from each set of results from analyses by NIST using GC/MS and GC-FID, the median of the mean results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. Each reference mass fraction value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the mean results provided by collaborating laboratories. The uncertainty in the certified and reference values, calculated according to the method described in the ISO Guide (12–14), is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one SD, 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 level for each analyte.

[b] Values shown as % represent mass fractions.

[c] MADe is the median absolute deviation, a robust estimate of the SD (25, 26).

[d] The reference value is assigned in units of kcal/100 g, the usual unit of energy used by the U.S. food industry. This unit is not recognized in the International System of Units (SI); the SI-acceptable equivalent is 2205 kcal/100 g with an expanded uncertainty of 17 kJ/100 g.

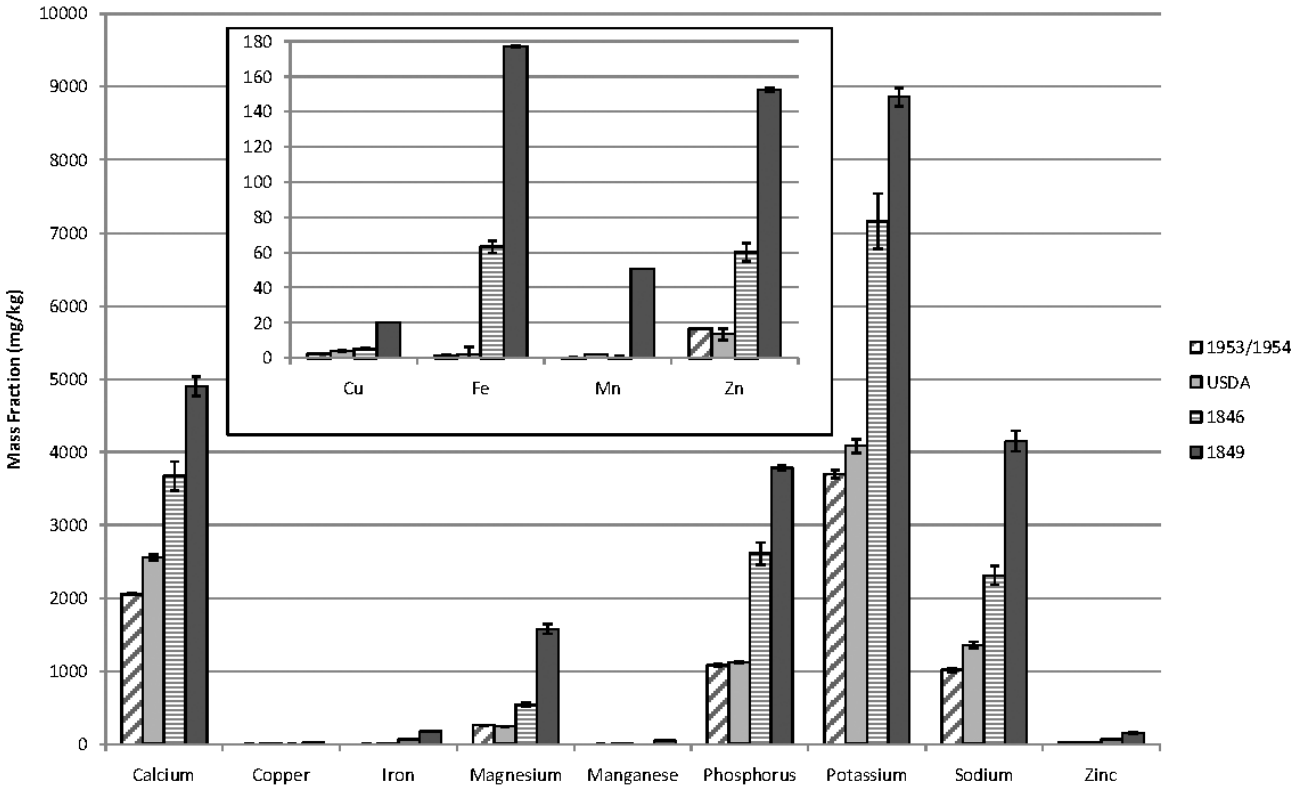

Figure 2. Comparison of element levels in SRMs 1849, 1846, 1953, and 1954 to values provided in the USDA food composition database. Mass fractions in SRMs 1953 and 1954 and the USDA values have been converted to a dry-mass basis using a conversion factor of 12.5% solids (ref. 29). Uncertainties on SRM values represent an expanded uncertainty at 95% confidence; uncertainties on the USDA values are two SDs. Error bars are not shown in instances where uncertainty information was unavailable (i.e., information values or *n* = 1).

measurements from NIST using two different analytical methods, collaborating laboratories, and the manufacturer, where available. NIST provided measurements by using instrumental neutron activation analysis (INAA; 11) and inductively coupled plasma-optical emission spectrometry (ICP-OES). Collaborating laboratories used their usual methods, most often ICP-OES, but also including direct current plasma atomic emission, flame atomic absorption, graphite furnace atomic absorption spectrometry, and ICP/MS.

(**d**) *Collaborating laboratories' analyses*.—The Grocery Manufacturers Association (GMA) Food Industry Analytical Chemistry Committee (FIACC) laboratories and a group of other laboratories, listed in Table 1, were asked to use their usual methods to make single measurements on test portions taken from each of two or three packets of SRM 1849, respectively. The median of the individual collaborating laboratory means was combined with the mean result from the manufacturer analyses (below) and means of NIST data for calculation of the certified values. Collaborating laboratories' data alone were used to assign reference and information values for proximates, amino acids, nucleotides, ascorbic acid, vitamin B_{12}, choline, inositol, and carnitine. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in the Certificate of Analysis for this material, available at http://www.nist.gov/srm.

(**e**) *Manufacturer's analyses*.—The manufacturer of SRM 1849 provided data for 57 analytes, measuring each from 30 to 240 times in the months following preparation. The mean of its data sets was averaged with the median of the individual collaborating laboratory means and the means of NIST data for calculation of certified values. Its mean results were combined with medians of the other collaborating laboratories' data to assign reference values.

(**f**) *Value assignment*.—The laboratories listed in Table 1 reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories

Table 4. Certified (bold) and reference (normal typeface) mass fractions (mg/kg) for selected elements[a]

Element	Collaborating laboratories' median	MADe[b]	Manufacturer's mean	SD	NIST ICP-OES mean	SD	NIST INAA mean	SD	Assigned value	*U*
Ca	4813	55	4920	42	4978	50			**4900**	**130**
Fe	175.5	3.4	179.0	2.1	176.7	0.6			**177.1**	**3.3**
Na	4090	53	4090	55	4133	7	4295	42	**4150**	**140**
K	8836	89	8790	103	8941	24			**8860**	**130**
P	3800	43	3780	42	3767	12			**3782**	**36**
Mg	1525	31	1610	17	1600	13			**1578**	**69**
Zn	149.5	3.6	152.0	1.8	155.3	0.5			**152.3**	**5.1**
Cu	20.49	0.36	20.00	0.27	20.39	0.32			**20.29**	**0.43**
Mn	51.13	0.68	51.00	0.45	50.86	0.31			**51.00**	**0.53**
Se	0.909	0.045	0.870	0.027					0.889	0.057
Cl	6210	53	6241	57			6382	78	**6280**	**140**
I	1.01	0.18					1.61	0.27	**1.37**	**0.41**
Cr	1.00	0.11			1.17	0.05			**1.09**	**0.21**
Mo	1.61	0.10			1.58	0.05			**1.62**	**0.15**

[a] Each certified mass fraction value, in units of mg/kg, expressed as a mass fraction for the material as received, is the mean from the combination of the mean of results from analyses by NIST, the median of the mean results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. Each reference mass fraction value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the mean results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the certified and reference mass fraction, calculated according to the method described in the ISO Guide (12–14), is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one SD, 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 level for each analyte.

[b] MADe is the median absolute deviation, a robust estimate of the SD (25, 26).

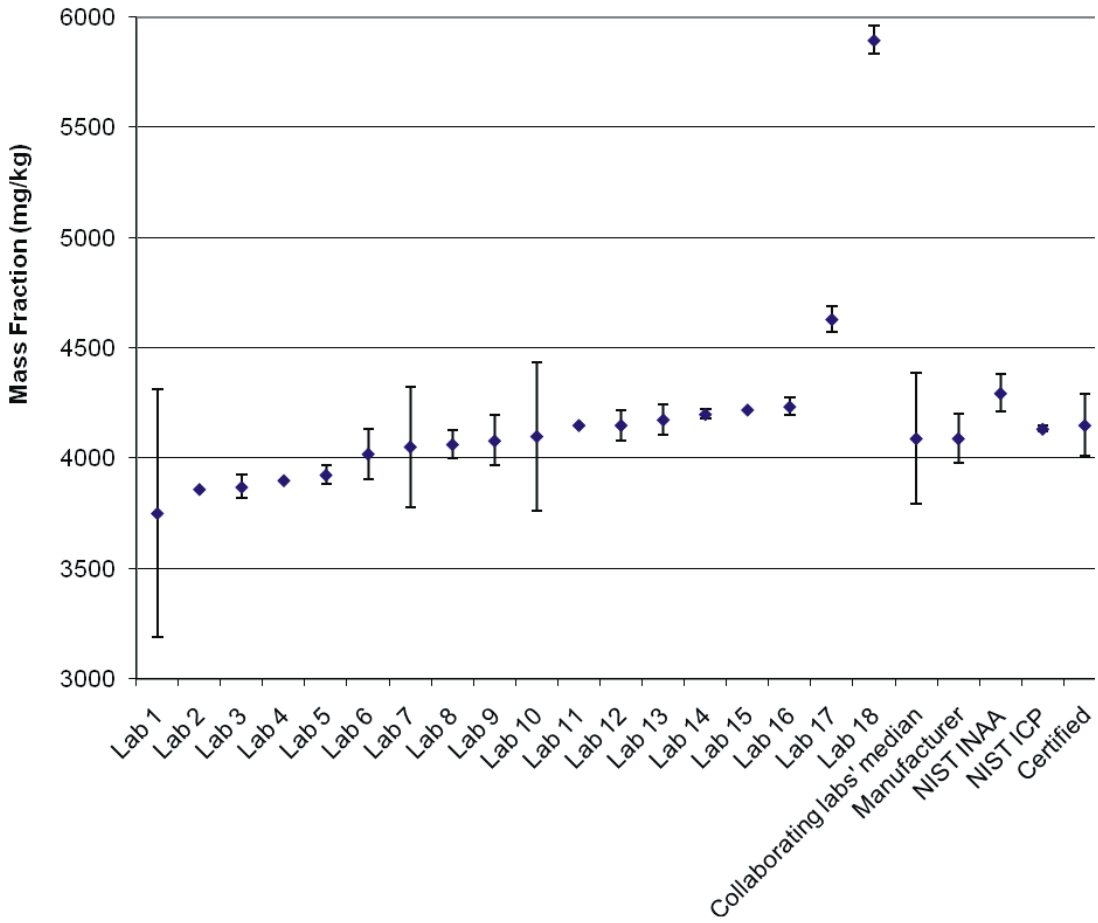

Figure 3. Comparison of data used to assign value for sodium in SRM 1849 Infant/Adult Nutrition Formula. Laboratories are not numbered in the order shown in Table 2. Error bars on collaborating laboratories', manufacturer, and NIST data represent two SDs. Error bars on the median represent two times the MADe. Error bars on the certified values represent the expanded uncertainty as described in footnote *a* in Table 4.

and/or the manufacturer, the median of the laboratory means and the mean of the manufacturer's data were averaged. For analytes that were also measured by NIST, the mean of individual sets of USDA data, the median of the individual collaborating laboratory means, the manufacturer's mean, and the mean of the individual sets of NIST data were averaged. None of the collected data was discarded as an outlier; the use of robust statistics (*see* below) make this possible.

Uncertainties in the assigned values were calculated according to the method described in the ISO Guide (12–14). The uncertainty of each value is expressed as an expanded uncertainty, U, calculated as:

$$U = ku_c$$

where u_c is intended to represent, at the level of one SD, the combined effect of between- 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 approximately 95% confidence for each analyte.

The material manufacturer recommended reconstitution of an entire packet of material (10 g) prior to removal of a test portion for analysis. A microhomogeneity assessment was performed for vitamin A, using test portions from 0.5 to 3.0 g, to determine the minimum test portion in which retinyl palmitate was homogeneously distributed. The homogeneity of elements, fatty acids, and vitamins was assessed at NIST using test portions less than 10 g (the contents of an entire packet): 0.5 g for fatty acid analysis, between 0.2 and 2 g for elemental analysis, and between 1.0 and 2.5 g for vitamin analysis. (The microheterogeneity study showed 2.0 g was the optimum test portion size for retinyl palmitate analysis.) Analysis of variance did not show statistically significant heterogeneity for test portion sizes employed; therefore, analytes have been treated as though they are homogeneously distributed in the material although the homogeneity of the other analytes was not assessed.

Table 5. Certified (bold) and reference (normal typeface) mass fractions (mg/kg) for selected vitamins[a]

Vitamin	Collaborating laboratories' median	MADe[b]	Manufacturer's mean	SD	USDA LC/abs and FL (FL for B_6 and B_2)	SD	USDA LC/MS	SD	NIST LC/abs means	SD	NIST LC/MS means	SD	NIST LC/FL mean	SD	Assigned value	*U*
Retinol[c]	16.5	0.4	16.6	0.6					16.3	0.4	17.8	0.5			**16.4**	**1.3**
											14.8	1.1				
Vitamin D_3[d]	0.231	0.006	0.260	0.013			0.262	0.002			0.251	0.036			**0.251**	**0.027**
-Tocopherol[e]	379	8							361	4			368	13	**369**	**16**
-Tocopherol	5.35	0.36											6.19	0.19	**5.77**	**0.79**
-Tocopherol	191.6	0.4							198	5			177	6	**189**	**13**
-Tocopherol									81.2	1.1			77.2	0.8	**79.2**	**2.4**
Vitamin K[f]	2.20	0.02	2.3	0.1							2.1	0.2			**2.20**	**0.18**
Ascorbic acid[g]	1057	28	1070	14											1060	30
Thiamine Cl[h]	14.3	0.9	15.9	0.5	18.1	0.1	15.9	0.2	15.2	0.2	15.2	0.7			**15.8**	**1.3**
Riboflavin[h]	17.0	0.6	18.0	0.3	18.6	0.4	16.1	0.6	18.1	0.7	16.6	0.9			**17.4**	**1.0**
Niacinamide[h]	100.2	2.7	99.0	1.6	98.0	1.7	94.5	0.4	93.7	1.2	98.4	4.2			**97.5**	**2.3**
									98.8	2.8						
Pantothenic acid[h]	74.2	5.8	65.0	1.3			63.3	1.0			66.0	2.0			**64.8**	**2.2**
Pyridoxine HCl[h]	13.9	0.3	12.4	0.2	16.4	0.4	15.2	0.1	13.3	0.4	14.0	0.7			**14.2**	**1.5**
Vitamin B_{12}	0.050	0.004	0.035	0.002											0.040	0.008
Folic acid[h]	2.19	0.11	2.09	0.07			2.24	0.14			1.97	0.06			**2.11**	**0.13**
											2.05	0.04				
Biotin	1.68	0.18	1.77	0.07			1.89	0.08			2.11	0.01			**1.92**	**0.25**
											2.15	0.09				
Choline ion	888	74	876	43											882	88
Myo-inositol	384	12	413	12											398	26

[a] Each certified mass fraction value, in units of mg/kg, expressed as a mass fraction for the material as received, is the mean from the combination of the mean results from each set of analyses by NIST, the median of the mean results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. Each reference mass fraction value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the mean results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the certified and reference mass fraction, calculated according to the method described in the ISO Guide (12–14), is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one SD, the combined effect of between- 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 level for each analyte.

[b] MADe is the median absolute deviation, a robust estimate of the SD (25, 26).

[c] Retinol was added to SRM 1849 as retinyl palmitate. The certified value is expressed as retinol equivalents, and represents total (*cis* + *trans*) retinol.

[d] Some of the collaborating laboratories included previtamin D in their vitamin D measurements, however results were indistinguishable from results for just vitamin D_3. Therefore, results were combined for value assignment.

[e] -Tocopherol was added to SRM 1849 as *RRR*- -tocopheryl acetate. The certified value is expressed as -tocopherol equivalents and includes "naturally occurring" -tocopherol as well as the acetate. The -tocopherol content, excluding -tocopheryl acetate, is about 50 mg/kg.

[f] The manufacturer reported a value for *trans*-vitamin K; results were indistinguishable from the range of results reported for total vitamin K, and results were combined for value assignment.

[g] Does not include dehydroascorbic acid.

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

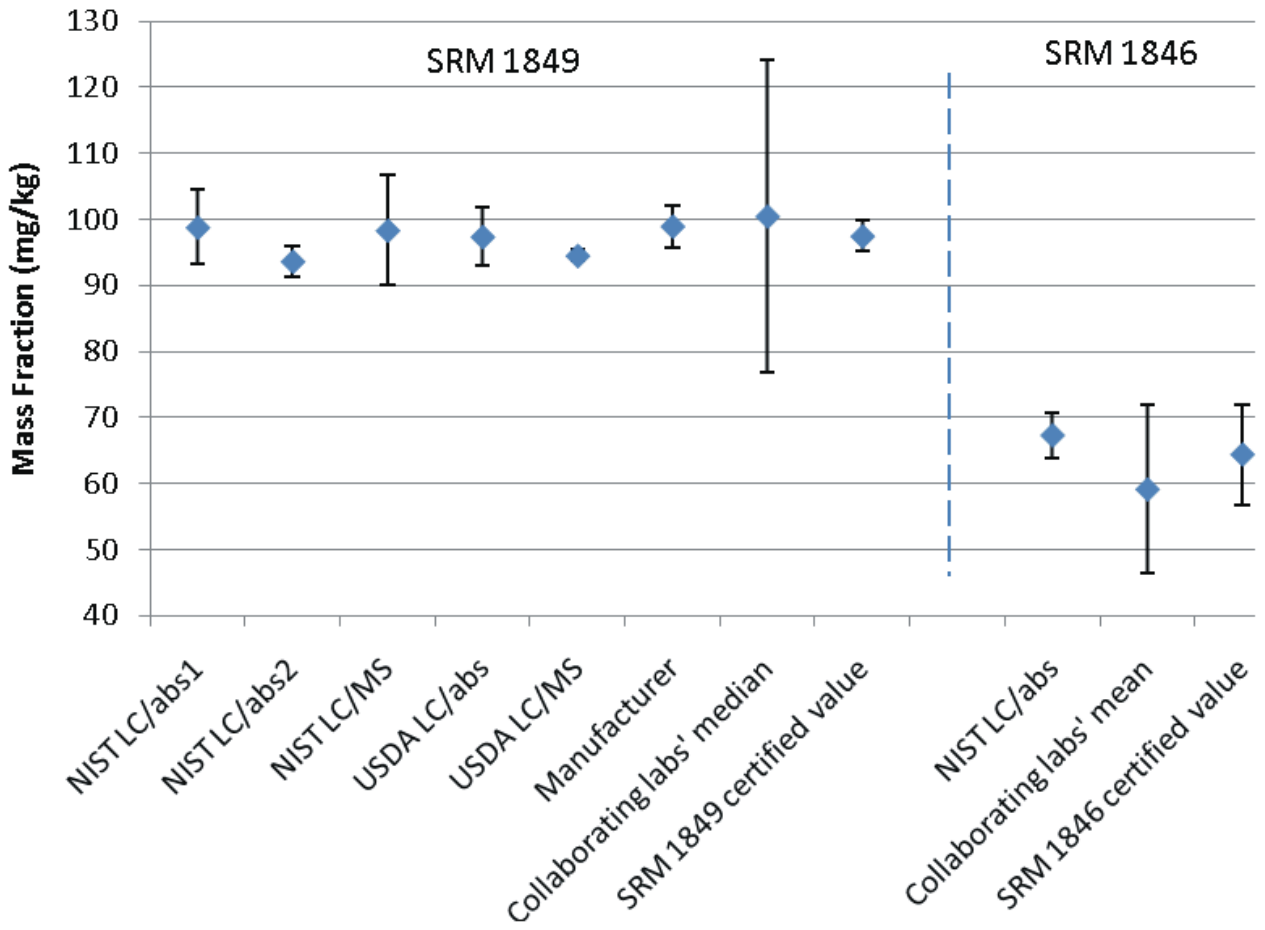

Figure 4. Comparison of data used to assign value for nuacinamide in SRM 1849 Infant/Adult Nutritional Formula compared to data used for value assignment of SRM 1846 Infant Formula. Error bars on collaborating laboratories, manufacturer, and NIST data represent two SDs. Error bars on the median represent two times the MADe. Error bars on the certified values represent the expanded uncertainty, as described in footnote *a* in Table 5.

Results and Discussion

NIST has a number of food-matrix reference materials with values assigned for constituents of nutritional interest. The effort to produce these materials was driven largely by the requirements of the Nutrition Labeling and Education Act of 1990 (15). As shown in Figure 1, SRMs have been developed for a wide range of compositions (16–22). Foods are positioned in this AOAC-developed triangle based on their fat, protein, and carbohydrate content. One or two foods within each sector are expected to be representative of—and useful as control materials for analysis of—other foods within that sector (23, 24). SRM 1849 is located in sector 6, as was SRM 1846. These two materials are somewhat different with respect to their proximate composition: SRM 1846 contained 27.1% fat, 11.10% protein, 2.91% ash, and 57.2% carbohydrate, whereas SRM 1849 contains 31.0, 13.3, 4.52, and 50.2%, respectively. Certified and reference values for proximates, fatty acids, lactose, cholesterol, and calories in SRM 1849 are provided in Table 3, along with the data used to assign the values. The median of the collaborating laboratory means and the mean of the manufacturer's results were combined with the mean of USDA and NIST data, where available. [The sometimes very wide variations of the round-robin results indicate that it is more appropriate to use the median rather than the mean of the laboratory means. The SD of the median for the collaborating laboratories' data was calculated as a robust estimate based on the median absolute deviation, the MADe (25, 26).] The use of the median allows the outliers to be neglected without necessitating outlier removal.

The elemental composition of SRMs 1846 and 1849 is compared in Figure 2. Also plotted is the elemental composition of SRMs 1953 and 1954 Organic Contaminants in Human Milk, Unfortified and Fortified (with contaminants), respectively (27, 28), and that of human milk according to the USDA food composition database (29). In the case of the human milk SRMs and the USDA data, values

Table 6. Reference mass fractions for amino acids, taurine, and nucleotides and information value for carnitine[a]

Compound	Units[b]	Collaborating laboratories' median	MADe[c]	Manufacturer's mean	SD	Assigned value	*U*
Alanine	%	0.485	0.009	0.490	0.006	0.488	0.011
Arginine	%	0.395	0.010	0.426	0.016	0.411	0.024
Aspartic acid	%	1.09	0.03	1.14	0.02	1.11	0.05
Cystine	%	0.142	0.010	0.144	0.005	0.143	0.012
Glutamic acid	%	2.72	0.10	2.84	0.04	2.78	0.14
Glycine	%	0.245	0.003	0.254	0.004	0.250	0.007
Histidine	%	0.320	0.012	0.312	0.006	0.316	0.015
Isoleucine	%	0.690	0.037	0.687	0.015	0.688	0.043
Leucine	%	1.28	0.02	1.35	0.02	1.31	0.06
Lysine	%	1.04	0.02	1.07	0.02	1.05	0.03
Methionine	%	0.410	0.028	0.504	0.010	0.457	0.070
Phenylalanine	%	0.583	0.010	0.594	0.007	0.589	0.013
Proline	%	1.18	0.04	1.240	0.022	1.21	0.06
Serine	%	0.700	0.037	0.753	0.012	0.726	0.054
Taurine	%	0.034	0.006	0.036	0.001	0.035	0.007
Threonine	%	0.615	0.016	0.658	0.009	0.636	0.036
Tryptophan	%	0.180	0.009	0.195	0.003	0.188	0.015
Tyrosine	%	0.475	0.034	0.556	0.015	0.516	0.071
Valine	%	0.798	0.036	0.799	0.018	0.798	0.041
Carnitine	mg/kg			84.60	3.00	85	
CMP[d]	mg/kg	302	2	308	13	305	5
GMP[d]	mg/kg	150	17	144	6	147	38
UMP[d]	mg/kg	149	3	148	6	148	8
AMP[d]	mg/kg	108	3	103	4	106	5

[a] Each certified mass fraction value, in units of mg/kg, expressed as a mass fraction for the material as received, is the mean from the combination of the mean results from each set of analyses by NIST, the median of the mean results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. Each reference mass fraction value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the mean results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the certified and reference mass fraction, calculated according to the method described in the ISO Guide (12–14), is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one SD, 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 level for each analyte. An uncertainty is not provided on the information value for carnitine because insufficient information was available to assess the uncertainty.

[b] Values shown as % represent mass fractions.

[c] MADe is the median absolute deviation, a robust estimate of the SD (25, 26).

[d] CMP = Cytidine monophosphate; GMP = guanosine monophosphate; UMP = uridine monophosphate; AMP = adenosine monophosphate.

have been converted to a dry-mass basis using a value of 87.5% moisture (12.5% solids; 29). As expected, levels present in the human milk SRMs are most similar to levels provided in the USDA tables. Levels in SRM 1849 are higher than those of human milk and SRM 1846 in all cases, emphasizing further that SRM 1849 is not an infant formula.

Certified and reference values for elements in SRM 1849 are provided in Table 4, along with the sets of data used to assign these values. A plot of sodium values reported by collaborating laboratories and the manufacturer, and those obtained using the two NIST methods (ICP-OES and INAA) are provided in Figure 3. The median of the individual collaborating laboratory means was combined with the manufacturer's mean, the INAA mean, and the ICP-OES mean to calculate the certified value. Note that sodium contamination may be a problem for some laboratories, as has been previously observed in low-sodium materials, such as SRM 2384 Baking Chocolate (20).

Certified and reference values for vitamins in SRM 1849 are provided in Table 5 along with the data sets used to

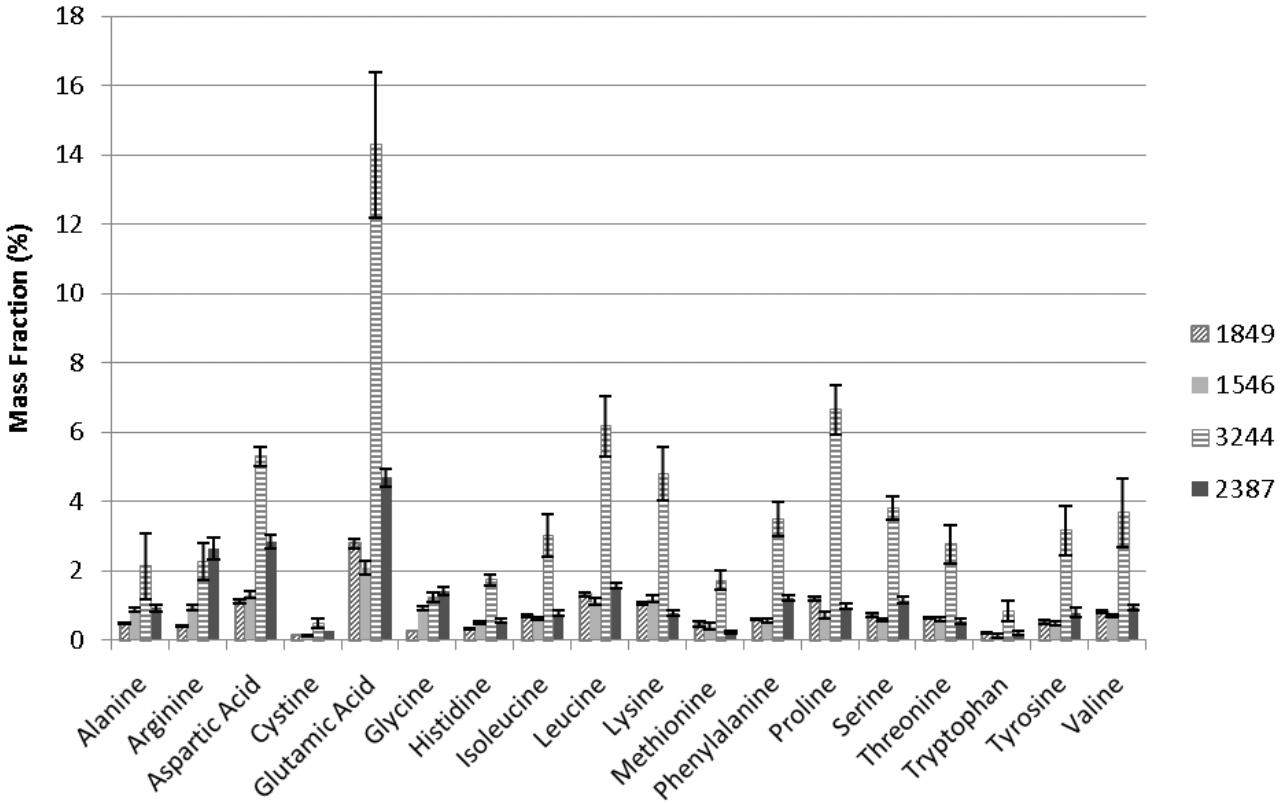

Figure 5. Comparison of reference values for amino acids in SRM 1849 Infant/Adult Nutritional Formula, 1546 Meat Homogenate, 3244 Ephedra-Containing Protein Powder, and 2387 Peanut Butter. Error bars represent the 95% confidence interval described in Table 6.

produce them. Most NIST results were generated using two methods, one of which typically involved LC/MS or LC/MS/MS with a stable-isotope labeled internal standard (5). Collaborating laboratories reported measuring free and total forms of the water-soluble vitamins. Results were indistinguishable, and data were combined to calculate median values. Because the SRM was fortified with vitamins, the naturally occurring levels of vitamins are "lost" in the variability of the measurements. NIST is currently working on characterizing SRM 3233 Fortified Breakfast Cereal, and will then begin characterizing food-matrix SRMs that have not been vitamin-fortified (e.g., candidate SRM 3234 Soy Flour and SRM 2383a Baby Food Composite).

The data used for value assignment and the certified values for niacinamide in SRMs 1846 and 1849 are compared in Figure 4. In 1991, for SRM 1846, the value was generated by combining collaborating laboratory data (relative uncertainty of 22%) with NIST LC/abs data (with a relative uncertainty of 5.0%) to result in a final value with 12% relative expanded uncertainty. The value assignment data and their uncertainties overlapped, but there was a 13% difference between the two values. By contrast, for SRM 1849 the lowest (NIST LC/abs) and highest (median of collaborating laboratory means) of the seven values used to assign the certified value differ by only 7%. The large number of different methods (seven) and their general agreement are reflected in the certified value for niacinamide in SRM 1849 having a relative expanded uncertainty of only 2.4%.

Commercial infant formulas have evolved over time in an effort to mimic the composition or performance of human milk. In 1984, taurine fortification began (30), although the role of taurine in a baby's diet is still not fully understood (31). In the late 1990s, nucleotide fortification began; nucleotides may act as growth factors and affect the immune system (30). And in the early 2000s, long-chain polyunsaturated fatty acids were added with the expectation of improving babies' visual and cognitive development (30). These compounds were not present in SRM 1846, but they were added to SRM 1849; certified (fatty acids) and reference values (nucleotides and taurine; Table 6) are assigned. Collaborating laboratories' and the manufacturer's data were used to assign reference values for amino acids, taurine, carnitine, and nucleotides. This is the only NIST SRM with values assigned for nucleotides.

The USDA has been including amino acid values in their nutrient databases for a number of years; until the introduction of SRM 2387 Peanut Butter in 2003, NIST had no food-matrix SRMs available with values assigned for amino acids to provide QA for these measurements. Three other SRMs with values assigned for amino acids are now available: SRM 1849, SRM 1546 Meat Homogenate, and SRM 3244 Ephedra-Containing Protein Powder. Amino acid levels are compared in Figure 5, and reference values for amino acids in SRM 1849 are provided in Table 6, along with the averages from the two data sets (collaborating laboratories and manufacturer). With the release of SRM 1849, SRMs with values assigned for amino acids are now available in sectors 3, 4, 6, and 9 of the AOAC triangle. (Candidate SRM 3234 Soy Flour, which is in sector 7, is also expected to have values assigned for amino acids.)

With 43 certified and 43 reference values assigned, SRM 1849 is the most characterized food-matrix reference material available from NIST. Methods developed for certification of this material will be employed and adapted for other SRMs currently in preparation, including candidate SRMs 1549a Whole Milk Powder, 1845a Whole Egg Powder, 3233 Fortified Breakfast Cereal, and 3234 Soy Flour. We plan to characterize a similarly large number of nutrients in these materials.

Acknowledgments

We thank the members of GMA's Food Industry Analytical Chemists Committee; the USDA's Food Composition and Methods Development Laboratory in Beltsville, MD; the manufacturer; and the other collaborating laboratories for assisting with characterization of this SRM. We recognize and appreciate the time and effort they contributed in assisting in value assignment.

References

(1) Infant Formula Act of 1980 (September 26, 1980) Public Law 96-359 H.R. 6940

(2) 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. (1997) *J. AOAC Int.* **80**, 611–621

(3) Certificate of Analysis (September 30, 2007) Standard Reference Material 1846 Infant Formula, NIST, Gaithersburg, MD, http://www.nist.gov/srm

(4) May, W., Parris, R., Beck, C., Fassett, J., Greenberg, R., Guenther, F., Kramer, G., Wise, S., Gills, T., Colbert, J., Gettings, R., & MacDonald, B. (2000) *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements, National Institute of Standards and Technology, NIST Special Publication 260-136*, U.S. Government Printing Office, Gaithersburg, MD, http://www.cstl.nist.gov/nist839/ NIST_special_publications.htm

(5) Phinney, K.W., Rimmer, C.A., Thomas, J.B., Sander, L.C., Sharpless, K.E., & Wise, S.A. (2010) *Anal. Chem.* (submitted)

(6) Nelson, B.C., Sharpless, K.E., & Sander, L.C. (2006) *J. Chromatogr. A* **1135**, 203–211

(7) Nelson, B.C., Sharpless, K.E., & Sander, L.C. (2006) *J. Agric. Food Chem.* **54**, 8710–8715

(8) Goldschmidt, R.J., & Wolf, W.R. (2010) *Anal. Bioanal. Chem.* **397**, 471–481

(9) Chen, P., & Wolf, W.R. (2007) *Anal. Bioanal. Chem.* **387**, 2441–2448

(10) Byrdwell, W.C. (2009) *J. Agric. Food Chem.* **57**, 2135–2146

(11) Greenberg, R.R., Fleming, R.F., & Zeisler, R. (1984) *Environ. Int.* **10**, 129–136

(12) ISO Guide to the Expression of Uncertainty in Measurement (1993) 1st Ed., ISBN 92-67-10188-9, International Organization for Standardization (ISO), Geneva, Switzerland

(13) Taylor, B.N., & Kuyatt, C.E. (1994) *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results,* NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC, http://www.physics.nist.gov/Pubs/ contents.html

(14) Levenson, M.S., Banks, D.L., Eberhardt, K.R., Gill, L.M., Guthrie, W.F., Liu, H.K., Vangel, M.G., Yen, J.H., & Zhang, N.F. (2000) *J. Res. Nat. Inst. Stand. Technol.* **105**, 571–579

(15) Nutrition Labeling and Education Act (November 8, 1990) Public Law 101-535 H.R. 3562

(16) Sharpless, K.E., Thomas, J.B., Christopher, S.J., Greenberg, R.R., Sander, L.C., Schantz, M.M., Welch, M.J., & Wise, S.A. (2007) *Anal. Bioanal. Chem.* **389**, 171–178

(17) Sander, L., Schantz, M., Sharpless, K., & Wise, S. (2009) *Lipid Technol.* **21**, 7–12

(18) Welch, M.J., Colbert, J.C., Gill, L.M., Phinney, C.S., Sharpless, K.E., Sniegoski, L.T., & Wood, L.J. (2001) *Fresenius' J. Anal. Chem.* **370**, 42–47

(19) Brown Thomas, J., Kline, M.C., Gill, L.M., Yen, J.H., Duewer, D.L., Sniegoski, L.T., & Sharpless, K.E. (2001) *Clin. Chim. Acta* **305**, 141–155

(20) Sharpless, K.E., Brown, T.J., Nelson, B.C., Phinney, C.S., Sieber, J.R., Wood, L.J., Yen, J.H., & Howell, D.W. (2002) *J. Agric. Food Chem.* **50**, 7069–7075

(21) Sharpless, K.E., Phinney, C.S., Wood, L.J., Yen, J.H., & Howell, D.W. (2003) *J. Agric. Food Chem.* **51**, 6745–6751

(22) Sharpless, K.E., Anderson, D.L., Betz, J.M., Butler, T.A., Capar, S.G., Cheng, J., Fraser, C., Gay, M., Gardner, G., Howell, D., Ihara, T., Lam, J., Long, S., McCooeye, M., Mackey, E.A., Mindak, W., Mitvalsky, S., Murphy, K., Phinney, K.W., NguyenPho, A., Porter, B.J., Roman, M., Sander, L.C., Satterfield, M., Scriver, C., Sturgeon, R.E., Brown Thomas, J., Vocke, R., Wise, S.A., Wood, L.J., Yang, L., Yen, J.H., & Ziobro, G. (2006) *J. AOAC Int.* **89**, 1483–1495

(23) Wolf, W.R. (1993) in *Methods of Analysis for Nutrition Labeling,* D.R. Sullivan & D.E. Carpenter (Eds), AOAC INTERNATIONAL, Gaithersburg, MD, pp 111–122

(24) Wolf, W.R., & Andrews, K.W. (1995) *Fresenius' J. Anal. Chem.* **352**, 73–76

(25) Analytical Methods Committee of the RSC (1989) *Analyst* **114**, 1693–1698

(26) Analytical Methods Committee of the RSC (1989) *Analyst* **114**, 1699–1702

(27) Certificate of Analysis, Standard Reference Material 1953 Organic Contaminants in Human Milk (Unfortified), NIST, Gaithersburg, MD, http://www.nist.gov/srm

(28) Certificate of Analysis, Standard Reference Material 1954 Organic Contaminants in Human Milk (Fortified), NIST, Gaithersburg, MD, http://www.nist.gov/srm

(29) http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl

(30) Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, Food and Nutrition Board, Institute of Medicine of the National Academies (2004) Infant Formula: Evaluating the Safety of New Ingredients, Washington, DC, The National Academies Press, http://www.nap.edu/catalog.php?record_id=10935

(31) Agostoni, C., Carratù, B., Boniglia, C., Riva, E., & Sanzini, E. (2000) *J. Am. Coll. Nutr.* **19**, 434–438