Certificate of Analysis

Standard Reference Material® 1954

Organic Contaminants in Fortified Human Milk

This Standard Reference Material (SRM) is intended for use in evaluating analytical methods for the determination of selected polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and polybrominated diphenyl ether (PBDE) congeners in human milk and similar matrices. Reference concentration values are provided for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), as well as some inorganic constituents. An information concentration value is provided for the PCB mixture Aroclor 1260. The compounds in Appendix A were spiked into the milk prior to bottling. A unit of SRM 1954 consists of five bottles of approximately 5 mL fortified human milk.

The development of SRM 1954 was a collaboration between the National Institute of Standards and Technology (NIST) and the Division of Laboratory Sciences, Organic Analytical Toxicology Branch, U.S. Centers for Disease Control and Prevention (CDC).

Certified Concentration Values: Certified values for concentrations, expressed as mass fractions, for PCB congeners, chlorinated pesticides, and PBDE congeners along with 1 polybrominated biphenyl congener are provided in Tables 1, 2, and 3, respectively. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values for the PCB congeners, chlorinated pesticides, and PBDE congeners are based on the agreement of results obtained at NIST using one or more analytical techniques and additional results from the CDC and from an interlaboratory study using different analytical techniques.

Reference Concentration Values: Reference concentration values, expressed as mass fractions, are provided in Table 4 for additional PCB, chlorinated pesticides, and PBDE congeners; in Table 5 for elements; and in Table 6 for PCDD and PCDF congeners. Reference values are noncertified values that are estimates of the true value; however, the values do not meet the 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 analytical methods [1].

Information Concentration Values: An information concentration value for Aroclor 1260 is provided in Table 7. An information value is considered to be a value that will be of use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1954** is valid, within the measurement uncertainty specified, until **31 August 2025**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of technical measurements leading to certification were performed by L.C. Sander and S.A. Wise of the NIST Chemical Sciences Division and M.M. Schantz formerly of NIST.

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Partial support for the development of SRM 1954 was provided by the Division of Laboratory Sciences, Organic Analytical Toxicology Branch, CDC, Atlanta, GA.

Preparation of the milk was performed by Aalto Scientific, Ltd., Carlsbad, CA.

Analytical measurements at NIST were performed by J.M. Keller, S.E. Long, M.M. Schantz, S.S. Vander Pol, and L.J. Wood of the NIST Chemical Sciences Division. Analytical measurements at CDC were performed by D. Patterson, A. Sjödin, and W. Turner of the CDC Organic Analytical Toxicology Branch. Laboratories participating in an interlaboratory study included Institut National de Santé Publique du Québec (Québec, Canada), Stockholm University (Stockholm, Sweden), and University of Liege (Liege, Belgium).

Statistical consultation was provided by S.D. Leigh of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

SRM 1954 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The milk was pasteurized prior to preparation of SRM 1954. However, no known test method can offer complete assurance that infectious agents are absent from this material. Accordingly, this human milk-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SPECIMEN in the CDC/National Institutes of Health (NIH) Manual [2].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The milk is frozen and should be stored in a freezer at –20 °C until use. It should not be exposed to sunlight or ultraviolet radiation. After thawing, the contents should be used immediately.

Use: Bring the vial to room temperature. Once the milk is thawed, mix contents by gently swirling. Do not shake vigorously because this will cause frothing. The recommended minimum sample size is 0.25 g.

PREPARATION AND ANALYSIS(1)

Source of Material: Milk was acquired from six milk banks located around the U.S: Florida (4 %), North Carolina (6 %), Iowa (6 %), Delaware (7 %), California (12 %), and Texas (65 %).

Preparation of Material: The milk was pooled (approximately 100 L total) and was stored at 4 °C. The pool was split into two for production of SRM 1953 Organic Contaminants in Non-Fortified Human Milk and SRM 1954. Before bottling, approximately 710 mL of methanol containing 169 compounds at varying concentrations (Appendix A) was added to 50 L of milk, which was then stirred for 4 h. Using a calibrated automatic pipetter, 5 mL aliquots of milk were dispensed into 10 mL amber glass vials.

Analytical Methods Used at NIST: For NIST Method 1, the frozen milk in each of ten bottles was thawed. A known amount of internal standard solution (containing selected ^{13}C-labeled PCB congeners, selected ^{13}C-labeled pesticides, ^{13}C-labeled PBDE 209, fluorinated PBDE 47, PCB 103, and PCB 198) was added to each bottle, sonicated for 15 min and allowed to equilibrate overnight under refrigeration. After samples were removed from refrigeration and allowed to reach ambient temperature, 10 mL of formic acid was added, as a denaturation agent, followed immediately by 10 mL of a 1+1 (volume fraction) mixture of *n*-hexane:methyl-*tert*-butyl ether for extraction. The samples were vortexed and left to stand for 0.5 h with occasional stirring. After centrifugation to obtain a sharp phase boundary, the upper organic phase was transferred to a concentration vessel. The extraction was repeated two more times with 10 mL of *n*-hexane each time. The combined organic layers were concentrated to approximately 4 mL using an automated evaporation system. Approximately 2 mL of concentrated sulfuric acid was added to the concentration vessel with swirling. Following phase separation, the organic phase was removed, and the sulfuric acid phase was washed twice using 4 mL portions of *n*-hexane. The combined hexane phases were concentrated to approximately 0.5 mL for silica solid-phase extraction (SPE) clean-up. The fraction of interest was eluted with 15 mL of 10 % (volume fraction) dichloromethane in hexane. The concentrated samples were

(1) Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

analyzed using gas chromatography/mass spectrometry (GC/MS) operated in both the electron impact (EI) and negative ion chemical ionization (NICI) mode. A 0.25 mm × 60 m fused silica capillary column containing a non-polar proprietary phase (DB-XLB, Agilent Technologies, Wilmington, DE) 0.25 µm film thickness was used for the EI analysis (NIST Method 1a) while a 0.25 mm × 60 m fused silica capillary column containing a 50 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-17MS, Agilent Technologies) was used for the NICI analysis (NIST Method 1b). All injections were 1 µL using an on-column inlet.

For NIST Method 2, the frozen milk in each of six bottles was thawed. A known amount of internal standard solution (containing selected ^{13}C-labeled PCB congeners, selected ^{13}C-labeled pesticides, ^{13}C-labeled PBDE 209, and selected fluorinated PBDE congeners) was added to a 2.5 g milk test portion from each bottle, vortexed, and allowed to equilibrate overnight under refrigeration. After samples were removed from refrigeration and allowed to reach ambient temperature, 2 mL of formic acid was added followed by 3 mL of 20 % (volume fraction) dichloromethane in hexane. Samples were extracted using focused microwave extraction. Following extraction, samples were centrifuged, the organic phase was removed, and another 3 mL of 20 % (volume fraction) dichloromethane in hexane was added. The extraction was repeated, and the organic phases were combined. Following concentration with a solvent exchange to *iso*-octane, sulfuric acid-silica column clean-up was used, followed by alumina column (5 % deactivated) clean-up. The eluant from the clean-up columns was concentrated to 0.2 mL with a solvent change to *iso*-octane for analysis. The concentrated samples were analyzed using GC/MS in the EI mode (NIST Method 2a) with a 0.18 mm × 30 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS, Agilent Technologies) 0.18 µm film thickness. All injections were 20 µL using a programmable temperature vaporization (PTV) inlet. For NIST Method 2b, the same extracts were analyzed by GC/MS in the NICI mode using on-column injection into a 0.18 mm × 10 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS, Agilent Technologies), 0.18 µm film thickness.

For all of the NIST methods, multi-point calibration response curves for the compounds of interest relative to the internal standards were determined by processing gravimetrically diluted solutions of SRM 2261, SRM 2262, SRM 2274, and SRM 2275 plus gravimetrically prepared solutions of the additional analytes of interest with the internal standards added.

Analytical Methods Used at CDC: Details of the analytical methods used at CDC can be found in Patterson and Turner [3] and Sjödin et al. [4]. In summary, the frozen milk was thawed and mixed. Sample extraction was performed using a C_{18} SPE method. After addition of the internal standard solution and formic acid, the sample was eluted through an SPE column using appropriate solvents. The eluant was then cleaned up using a Universal Prep system (Fluid Management Systems, Waltham, MA) containing an acid/neutral/base silica column, an alumina column, and a carbon column. Corresponding ^{13}C-labeled compounds were used as internal standards for the majority of the analytes.

Gas chromatography/high-resolution mass spectrometry (GC/HRMS) with mass resolution of 10 000 was used for the determination of the PCBs, chlorinated pesticides, PBDEs, PCDDs, and PCDFs. The GC column was a 0.25 mm × 30 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS, J&W Scientific, Folsom, CA), 0.25 µm film thickness. All injections were splitless with helium as the carrier gas.

Interlaboratory Study: The three laboratories participating in the interlaboratory study used their usual methods for these analyses. Not every laboratory reported data for every analyte. When more than one laboratory did report data for a particular analyte, the mean of the concentrations was used for combination with other data to assign the certified and reference concentration values.

Non-Volatile Extractable Mass Determination: The percent of non-volatile extractable material (or total extractable organics, TEO) was determined gravimetrically for the data sets described above. Non-volatile extractable material is 3.21 % ± 0.33 % (95% confidence interval for the mean).

Total Mercury: The concentration value for mercury is derived from isotope dilution cold vapor inductively coupled plasma mass spectrometry measurements performed at NIST [5–7].

Other Elements: The concentration values for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Test portions (10 g) were digested in nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used. Results from the ICP-OES measurement were corrected for spike recoveries.

Homogeneity Assessment: The homogeneity of PCBs, pesticides, and PBDEs was assessed at NIST by using Method 1. An analysis of variance did not show inhomogeneity for a 5 g sample. Other analytes were treated as though they were homogeneously distributed in the material although homogeneity was not assessed.

Table 1. Certified Concentration Values for Selected PCB Congeners[(a)] in SRM 1954

			Mass Fraction (ng/kg)		
PCB	18	(2,2',5-Trichlorobiphenyl)[(b,c)]	355	±	45[(d)]
PCB	28	(2,4,4'-Trichlorobiphenyl)[(b,e,f)]	519	±	24[(d)]
PCB	44	(2,2',3,5'-Tetrachlorobiphenyl)[(b,c,e)]	415	±	35[(d)]
PCB	49	(2,2',4,5'-Tetrachlorobiphenyl)[(b,c,e)]	429	±	15[(d)]
PCB	52	(2,2',5,5'-Tetrachlorobiphenyl)[(b,c,e,f)]	425	±	40[(g)]
PCB	66	(2,3',4,4'-Tetrachlorobiphenyl)[(b,c,e)]	428	±	40[(d)]
PCB	74	(2,4,4',5-Tetrachlorobiphenyl)[(b,c,e)]	563	±	19[(d)]
PCB	87	(2,2',3,4,5'-Pentachlorobiphenyl)[(b,c,e)]	457	±	57[(d)]
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl)[(b,c,e,f,h)]	558	±	38[(d)]
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)[(b,c,e,f,h)]	442	±	26[(g)]
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)[(b,c,e,f,h)]	482	±	31[(d)]
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl)[(b,c,e,h)]	458	±	34[(g)]
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)[(b,e,f,h)]	597	±	35[(g)]
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl)[(b,c,e,f,h)]	400	±	26[(g)]
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)[(b,c,f)]	639	±	34[(d)]
PCB	146	(2,2',3,4',5,5'-Hexachlorobiphenyl)[(b,c,e,h)]	492	±	30[(g)]
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl)[(b,c,e,h)]	408	±	12[(d)]
PCB	151	(2,3,3',4,4'-Hexachlorobiphenyl)[(b,c,e,h)]	417	±	22[(d)]
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl)[(b,e,f)]	977	±	49[(d)]
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl)[(b,c,e,f,h)]	511	±	54[(d)]
PCB	157	(2,3,3',4,4',5'-Hexachlorobiphenyl)[(b,c,e,f,h)]	467	±	33[(g)]
PCB	158	(2,3,3',4,4',5'-Hexachlorobiphenyl)[(b,c,h)]	402	±	20[(d)]
PCB	167	(2,3',4,4',5,5'-Hexachlorobiphenyl)[(b,c,e,f,h)]	467	±	53[(g)]
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)[(b,c,e,f,h)]	507	±	63[(g)]
PCB	172	(2,2',3,3',4,5,5'-Heptachlorobiphenyl)[(b,c,e,h)]	443	±	29[(g)]
PCB	177	(2,2',3,3',4',5,6-Heptachlorobiphenyl)[(b,c,e,h)]	447	±	27[(d)]
PCB	178	(2,2',3,3',5,5',6-Heptachlorobiphenyl)[(b,c,e,h)]	449	±	12[(g)]
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)[(b,e,f)]	696	±	71[(d)]
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)[(b,c,e,f,h)]	445	±	19[(g)]
PCB	187	(2,2',3,4',5,5',6-Heptachlorobiphenyl)[(b,c,e,f,h)]	517	±	25[(g)]
PCB	189	(2,3,3',4,4',5,5'-Heptachlorobiphenyl)[(b,c,e,f,h)]	432	±	35[(g)]
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[(c,e,h)]	482	±	28[(d)]
PCB	195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl)[(c,e,h)]	461	±	40[(d)]
PCB	196	(2,2',3,3',4,4',5,6'-Octachlorobiphenyl)[(c,e)]	872	±	61[(d)]
	203	(2,2',3,4,4',5,5',6-Octachlorobiphenyl)			
PCB	199	(2,2',3,3',4,5,5',6'-Octachlorobiphenyl)[(c,e)]	469	±	35[(d)]
PCB	206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)[(c,e,h)]	465	±	30[(d)]
PCB	209	(Decachlorobiphenyl)[(c,e,h)]	436	±	36[(d)]

[(a)] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [8] and later revised by Schulte and Malisch [9] to conform with IUPAC rules. For the specific congeners mentioned in this table, PCB 199 is PCB 201 under the Ballschmiter and Zell scheme. When two congeners are known to coelute under the GC analysis conditions used, the PCB congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

[(b)] NIST Method 1a using liquid-liquid extraction followed by GC/MS in the EI mode.

[(c)] NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

[(d)] Certified values are unweighted means of the results from two to five analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [10] with a pooled, within-method variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected PCB congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

[(e)] CDC method using GC/HRMS.

[(f)] Results from interlaboratory study.

[g] Certified values are weighted means of the results from four or five analytical methods [12]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected PCB congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

[h] NIST Method 1b using liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).

Table 2. Certified Concentration Values for Selected Chlorinated Pesticides in SRM 1954

	Mass Fraction (ng/kg)		
Hexachlorobenzene[a,b,c,d]	672	±	41[e]
β-HCH[a,b,c,d]	829	±	38[f]
γ-HCH[a,c]	588	±	34[f]
Oxychlordane[b,c,d,g]	1050	±	130[e]
cis-Chlordane[d,g]	368	±	9[f]
trans-Chlordane[d,g]	377	±	9[f]
cis-Nonachlor[d,g]	496	±	11[f]
trans-Nonachlor[c,d,g]	1700	±	140[f]
Mirex[b,c,d,g]	515	±	23[f]
2,4'-DDE[a,b]	400	±	14[f]
4,4'-DDE[a,b,c,d]	8120	±	350[e]
2,4'-DDD[a,b]	427	±	16[f]
4,4'-DDD[a,d]	425	±	15[f]
2,4'-DDT[a,c]	444	±	21[f]
4,4'-DDT[a,b,c]	703	±	74[f]

[a] NIST Method 1a using liquid-liquid extraction followed by GC/MS in the EI mode.

[b] NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

[c] CDC method using GC/HRMS.

[d] Results from interlaboratory study.

[e] Certified values are weighted means of the results from four or five analytical methods [12]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected chlorinated pesticides. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

[f] Certified values are unweighted means of the results from two or three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [10] with a pooled, within-method variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected chlorinated pesticides. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

[g] NIST Method 1b using liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).

Table 3. Certified Concentration Values for Selected PBDE Congeners and for PBB 153[(a)] in SRM 1954

			Mass Fraction (ng/kg)
PBDE	17	(2,2',4-Tribromodiphenyl ether)[(b,c,d,e)]	401 ± 44[(f)]
PBDE	28	(2,4,4'-Tribromodiphenyl ether)[(b,c)]	579 ± 21[(g)]
	33	(2',3,4-Tribromodiphenyl ether)	
PBDE	47	(2,2',4,4'-Tetrabromodiphenyl ether)[(b,d,e,h)]	2570 ± 190[(g)]
PBDE	66	(2,3',4,4'-Tetrabromodiphenyl ether)[(b,c,d,e,h)]	411 ± 24[(g)]
PBDE	85	(2,2',3,4,4'-Pentabromodiphenyl ether)[(b,c,d,e,h)]	473 ± 7[(f)]
PBDE	99	(2,2',4,4',5-Pentabromodiphenyl ether)[(b,c,d,e,h)]	739 ± 41[(f)]
PBDE	100	(2,2',4,4',6-Pentabromodiphenyl ether)[(b,c,d,e,h)]	1280 ± 90[(g)]
PBDE	153	(2,2',4,4',5,5'-Hexabromodiphenyl ether)[(b,c,d,e,h)]	1440 ± 90[(f)]
PBDE	154	(2,2',4,4',5,6'-Hexabromodiphenyl ether)[(b,d,h)]	464 ± 30[(g)]
PBDE	183	(2,2',3,4,4',5',6-Heptabromodiphenyl ether)[(b,c,d,e)]	511 ± 34[(f)]
PBDE	209	(Decabromodiphenyl ether)[(b,c,e)]	423 ± 24[(g)]
PBB	153	(2,2',4,4',5,5'-Hexabromobiphenyl)[(b,e)]	474 ± 25[(g)]

(a) PBDE congeners and PBB 153 are numbered according to IUPAC rules. When two congeners are known to coelute under the GC analysis conditions used, the PBDE congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

(b) NIST Method 1b using liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).

(c) NIST Method 2b using focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).

(d) CDC method using GC/HRMS

(e) Results from interlaboratory study

(f) Certified values are weighted means of the results from four or five analytical methods [12]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected PBDE congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

(g) Certified values are the unweighted means of the results from two to five analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [10] with a pooled, within-method variance following the ISO/JCGM Guide [11]. The measurands are the total mass fractions of selected PBDE congeners and for PBB 153. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

(h) NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

Table 4. Reference Concentration Values for Selected PCB Congeners[a], Chlorinated Pesticides, and PBDE Congeners in SRM 1954

	Mass Fraction (ng/kg)
PCB 77 (3,3',4,4'-Tetrchlorobiphenyl)[b]	2.71 ± 0.14[c]
PCB 81 (3,4,4',5-Tetrchlorobiphenyl)[b]	0.630 ± 0.028[c]
PCB 114 (2,3,4,4',5-Pentachlorobiphenyl)[d]	90.5 ± 7.4[c]
PCB 123 (2',3,4,4',5-Pentachlorobiphenyl)[d]	67.9 ± 3.1[c]
PCB 126 (3,3',4,4',5-Pentachlorobiphenyl)[b,d]	10.4 ± 1.5[e]
PCB 169 (3,3',4,4',5,5'-Hexachlorobiphenyl)[b,d]	9.3 ± 1.2[e]
Pentachlorobenzene[f]	362 ± 24[c]
Octachlorostyrene[f]	374 ± 28[c]
PBDE 203 (2,2',3,4,4',5,5',6-Octabromodiphenyl ether)[g]	494 ± 44[c]
PBDE 206 (2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether)[d,g]	816 ± 170[e]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [8] and later revised by Schulte and Malisch [9] to conform with IUPAC rules.
[b] CDC method using GC/HRMS.
[c] Reference values are the means of results using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method. The reference values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.
[d] Results from interlaboratory study.
[e] Reference values are unweighted means of the results from two analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [10] with a pooled, within-method variance following the ISO/JCGM Guide [11].
[f] NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.
[g] NIST Method 2b using focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).

Table 5. Reference Concentration Values for Selected Elements in SRM 1954

	Mass Fraction (mg/kg)[a]
Calcium[b]	257 ± 2
Copper[b]	0.268 ± 0.003
Iron[b]	0.194 ± 0.007
Magnesium[b]	32.4 ± 0.2
Manganese[b]	0.040 ± 0.002
Phosphorus[b]	135 ± 2
Potassium[b]	462 ± 7
Sodium[b]	127 ± 3
	Mass Fraction µg/kg[a]
Mercury[c]	0.101 ± 0.033

[a] The reference values are the means of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method. The reference values are metrologically traceable to the SI unit for mass, expressed as milligrams per kilogram or micrograms per kilogram.
[b] Results from ICP-OES measurements performed at NIST.
[c] Results from isotope dilution cold vapor inductively coupled plasma mass spectrometry measurements performed at NIST.

Table 6. Reference Concentration Values for Selected Dibenzo-*p*-dioxin and Dibenzofuran Congeners in SRM 1954

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	Mass Fraction (pg/kg) [a]		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin[b,c]	162	±	20
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin[b,c]	240	±	17
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin[b,c]	182	±	16
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin[b,c]	890	±	140
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin[b,c]	207	±	20
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin[b,c]	1080	±	240
Octachlorodibenzo-*p*-dioxin[b,c]	4890	±	850
2,3,7,8-Tetachlorodibenzofuran[b,c]	125	±	10
1,2,3,7,8-Pentachlorodibenzofuran[b,c]	132	±	18
2,3,4,7,8-Pentachlorodibenzofuran[b,c]	347	±	25
1,2,3,4,7,8-Hexachlorodibenzofuran[b,c]	171	±	15
1,2,3,6,7,8-Hexachlorodibenzofuran[b,c]	186	±	17
1,2,3,7,8,9-Hexachlorodibenzofuran[b,c]	129	±	15
2,3,4,6,7,8-Hexachlorodibenzofuran[b,c]	1090	±	100
1,2,3,4,6,7,8-Heptachlorodibenzofuran[b,c]	407	±	45
1,2,3,4,7,8,9-Heptachlorodibenzofuran[b,c]	160	±	100
Octachlorodibenzofuran[b]	94.3	±	12.7

[a] Reference values are unweighted means of the results from two analytical methods except for octachlorodibenzofuran. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [10] with a pooled, within-method variance following the ISO/JCGM Guide [11]. The reference value for octachlorodibenzofuran is the mean of results using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method. The reference values are metrologically traceable to the SI unit for mass, expressed as pictograms per kilogram.

[b] CDC method using GC/HRMS.

[c] Results from interlaboratory study.

Table 7. Information Concentration Value for Aroclor 1260 in SRM 1954[a]

	Mass Fraction (ng/kg)
Aroclor 1260	8065

[a] Concentrations determined by Institut National de santé publique du Québec (Québec, Canada).

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at: http://www.nist.gov/srm/publications.cfm (accessed Feb 2016).

[2] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 5th ed*.; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, CDC and National Institutes of Health; US Government Printing Office: Washington, D.C. (2007); available at http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf. (accessed Feb 2016).

[3] Patterson, D.G. Jr; Turner, W.E.; *Analysis of Serum, Adipose Tissue, and Breast Milk for PCDDs, PCDFs, cPCBs, PCB Congeners, Chlorinated Pesticides by High Resolution Gas Chromatography Isotope-Dilution High Resolution Mass Spectrometry*; CLIA Document, Environmental Health Laboratory, Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, pp. 1–253 (1997).

[4] Sjödin, A.; Jones, R.S.; Lapeza, C.R.; Focant, J.-F.; McGahee, E.E. III; Patterson, D.G. Jr; *Semiautomated High-Throughput Extraction and Cleanup Method for the Measurement of Polybrominated Diphenyl Ethers, Polybominated Biphenyls, and Polychlorinated Biphenyls in Human Serum*; Anal. Chem.; Vol. 76, pp. 1921–1927 (2004).

[5] Christopher, S.J.; Long, S.E.; Rearick, M.S.; Fassett, J.D.; *Development of High Accuracy Vapor Generation Inductively Coupled Plasma Mass Spectrometry and its Application to the Certification of Mercury in Standard Reference Materials;* Anal. Chem.; Vol. 73, pp. 2190–2199 (2001).

[6] Long, S.E.; Kelly, W.R.; *Accurate Determination of Mercury in Coal by Isotope Dilution Cold-Vapor Generation Inductively Coupled Plasma Mass Spectrometry*; Anal. Chem.; Vol. 74, pp. 1477–1483 (2002).

[7] Long, S.E.; Davis, W.C.; Day, R.; Christopher, S.J.; Mann, J.L.; Kelly, W.R.; *Improved Certified Values for Total Mercury and Speciated Mercury in NIST Standard Reference Materials Using Isotope Dilution ICP-MS;* Am. Lab; Vol. 39, pp. 26–27 (2007).

[8] Ballschmiter, K.; Zell, M.; *Analysis of Polychlorinated Biphenyls (PCB) by Glass Capillary Gas Chromatography - Composition of Technical Aroclor- and Clophen-PCB Mixtures*; Fresenius Z. Anal. Chem., Vol. 302, pp. 20–31 (1980).

[9] Schulte, E.; Malisch, R.; *Calculation of the Real PCB Content in Environmental Samples. I. Investigation of the Composition of Two Technical PCB Mixtures*; Fresenius Z. Anal. Chem., Vol. 314, pp. 545–551 (1983).

[10] 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.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

[11] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Feb 2016).

[12] Ruhkin, A.L.; Vangel, M.G.; *Estimation of a Common Mean and Weighted Means Statistics*; J. Am. Statist. Assoc.; Vol. 93, pp. 303–308 (1998).

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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

Details of Spiking Solution used for SRM 1954

Compound Class	Number of Compounds	Concentration Range Added
ortho-PCBs	38	50 pg/mL to 500 pg/mL
non-ortho PCBs	4	0.4 pg/mL to 0.8 pg/mL
Chlorinated pesticides	22	500 pg/mL
Toxaphene congeners	6	500 pg/mL
Chlorobenzenes and octachlorostyrene	8	500 pg/mL
PBDEs and PBB 153	18	500 pg/mL
Hexabromocyclododecane, 1,2-bis(2,4,6-tribromophenyoxy)ethane, hexabromobenzene, and decabromodiphenylethane	4	500 pg/mL
PCDD/PCDF	18	0.1 pg/mL to 2.4 pg/mL
Brominated dioxins and furans	17	0.05 pg/mL
Chloro-bromo dioxins and furans	8	0.05 pg/mL
PCNs	9	1 pg/mL
Halogenated phenolic compounds	12	500 pg/mL
Hydroxylated PCBs	5	500 pg/mL