National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1589a

PCBs, Pesticides, PBDEs, and Dioxins/Furans in Human Serum

This Standard Reference Material (SRM) 1589a 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 serum and similar matrices. Reference values are also provided for selected polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). All of the constituents for which certified and reference values are provided in SRM 1589a are naturally present in the freeze-dried human serum. A unit of SRM 1589a consists of five bottles of freeze-dried human serum. Before use, the serum in each bottle must be reconstituted with 10 mL of distilled or HPLC grade water.

Certified Concentration Values: Certified values for concentrations (expressed as mass fractions) for 27 PCB congeners (some in combination), 3 chlorinated pesticides, and 4 PBDE congeners 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 accounted for by NIST [1]. The certified values for the PCB congeners, chlorinated pesticides, and PBDE congeners are based on the agreement of results obtained at NIST using between one and three analytical techniques and additional results from the U.S. Centers for Disease Control and Prevention (CDC) using different analytical techniques.

Reference Concentration Values: Reference concentration values (expressed as mass fractions) are provided in Table 4 for 27 additional PCB congeners (some in combination), 6 additional chlorinated pesticides, and 3 additional PBDE congeners. Reference concentration values are provided in Table 5 for total cholesterol, triglycerides, "free" cholesterol, and phospholipids determined by the Lipid Standardization Laboratory at the CDC, and in Table 6 for PCDDs, PCDFs, and non-*ortho* PCB congeners also determined by the CDC. Reference values are noncertified values that are the best estimate 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].

Expiration of Certification: The certification of this SRM lot is valid until **31 March 2012**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Notice and Warnings to Users"). However, the certification is nullified if the SRM is damaged, contaminated, or 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) will facilitate notification.

The overall direction and coordination of technical measurements leading to certification were performed by M.M. Schantz and S.A. Wise of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by E. Dyremark, J.M. Keller, H.M. Stapleton, R. Swarthout, and M.M. Schantz of the NIST Analytical Chemistry Division.

Analytical measurements at CDC were performed by D. Patterson, A. Sjödin, and W. Turner of the CDC Toxicology Branch.

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See Certificate Revision History on Last Page

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

Preparation of the serum was performed by K. Fitzpatrick, M.A. Mildner, B.J. Porter, M.M. Schantz, and K.S. Sharpless of the NIST Analytical Chemistry Division. The serum was freeze-dried at the Natural Products Support Group at the Frederick Cancer Research and Development Center (Fort Detrick, Frederick, MD) under the direction of T. McCloud.

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

NOTICE AND WARNINGS TO USERS

SRM 1589a IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier has reported that each donor unit of plasma used in the preparation of this product was tested and found to be negative for human immunodeficiency virus (HIV), HIV-1 antigen, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control/National Institutes of Health (NIH) Manual [2].

Stability and Storage: The serum is freeze-dried and should be stored in a refrigerator at temperatures between 2 °C and 8 °C until ready for use. It should NOT be frozen or exposed to sunlight or ultraviolet radiation. After reconstitution, the contents should be used immediately or stored between 2 °C and 8 °C until ready for use, preferably within 4 h. Freezing of the reconstituted material is NOT recommended.

INSTRUCTIONS FOR USE

Bring the vial to room temperature, remove the metal closure, and lightly tap the bottom of the vial to dislodge any dried serum particles from the stopper. Carefully remove the stopper to avoid possible loss of serum particles. Use a dispenser of known accuracy to slowly add 10.0 mL of distilled or HPLC grade water at 20 °C to 25 °C to the sides of the vial while continually turning the vial. Replace the stopper, swirl the vial two or three times, and let stand for approximately 10 min. Mix contents by gently swirling, let stand for approximately 30 min, swirl again, let stand 10 min, and finally invert the vial several times. DO NOT shake vigorously because this will cause frothing. Total time for reconstitution is approximately 1 h. After reconstituting, use contents as soon as possible or store between 2 °C and 8 °C until analysis, preferably within 4 h.

PREPARATION AND ANALYSIS[1]

Source of Material: Plasma was acquired from Interstate Blood Bank, Inc. (IBB, Memphis, TN). IBB's Chicago branch shipped NIST approximately 5 mL of 50 plasma samples for screening for PCB levels. Samples were first selected for screening from donors who fished on the Great Lakes and ate their catches. Additional samples were selected from individuals who, in their judgement, ate large quantities of fish or the selections were made at random.

Preparation of Material: Plasma samples were shipped from IBB on dry ice and were stored at –80 °C upon receipt at NIST. Samples were removed from the –80 °C freezer, allowed to thaw, and refrozen twice to cause fibrin to precipitate out of solution. After the second refreezing, the plasma was removed from the freezer, thawed at room temperature overnight, and vacuum-filtered the following day through Supor 800 membrane disc filters (hydrophilic polyethersulfone; 0.8-µm pore size, 90-mm diameter) (Pall Life Sciences, Ann Arbor, MI). Serum was pooled as it was filtered and was stored at 4 °C. Before bottling, the serum was poured through a double layer of cheesecloth. Using a calibrated automatic pipetter, two 5-milliliter aliquots of serum were dispensed into 30 mL amber glass vials. The samples were lyophilized at Frederick Cancer Research and Development Center for 4.5 days. Under vacuum, the starting condenser temperature was –50 °C, and the shelf temperature was –40 °C. The shelf temperature was slowly increased over the 4.5 days to 0 °C. The samples were considered dry when a stable vacuum and temperature were achieved.

[1]Certain commercial equipment, instrumentation, or materials are identified in this report 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.

Analytical Methods Used at NIST

NIST Method 1: For NIST Method 1, the freeze-dried serum (total contents from each of six bottles) was reconstituted by adding 10.0 mL (mass known) of HPLC grade water. A known amount of internal standard solution (containing selected ^{13}C-labeled PCB congeners, selected ^{13}C-labeled PBDE congeners, ^{13}C-*cis* chlordane, and perdueterated 4,4′-DDE, 4,4′-DDD, 4,4′-DDT, and endosulfan-I) was added to each bottle, sonicated for 15 min, and allowed to equilibrate for 2 h. Then, 10 mL of formic acid, as a denaturation agent, was added to each sample followed immediately by 20 mL of a 1:1 (volume fraction) mixture of *n*-hexane:methyl-*tert*-butyl ether for extraction. The samples were mixed well and left to stand for 1.25 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 20 mL of a mixture of 1:1 (volume fraction) *n*-hexane:methyl-*tert*-butyl ether each time. The combined hexane layers were concentrated using an automated evaporation system to approximately 0.5 mL for alumina clean-up using cartridges packed with approximately 1 g of alumina that had been baked at 800 °C overnight and mixed with 0.05 mL of water. After the extract was loaded on the columns, they were eluted with 9 mL of 35:65 (volume fraction) of methylene choride:hexane. The eluant was concentrated to approximately 0.1 mL followed by the addition of approximately 0.4 mL of methylene chloride. Size exclusion chromatography (SEC) on a semipreparative-scale divinylbenzene-polystyrene column was used to remove the remaining lipid and biogenic material. The eluant was concentrated to 0.1 mL with a solvent change to *iso*-octane for analysis. The concentrated samples were analyzed using gas chromatography/mass spectrometry (GC/MS). A 0.25 mm × 60 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS, Agilent Technologies, Wilmington, DE) 0.25 µm film thickness was used. All injections were 20 µL using a programmable temperature vaporization (PTV) inlet.

NIST Method 2: For NIST Method 2, the extraction and analysis methods were the same as for method 1 except that a mixture of 1:4 (volume fraction) methylene chloride:*n*-hexane was used as the extraction solvent instead of the 1:1 (volume fraction) *n*-hexane:methyl-*tert*-butyl ether mixture.

NIST Method 3: For NIST Method 3, microwave-assisted extraction (MAE) was used. The freeze-dried serum (total contents from each of five bottles) was reconstituted by adding 10.0 mL (mass known) of HPLC grade water. To 5 mL of serum, a known amount of internal standard solution (containing selected ^{13}C-labeled PCB congeners, selected ^{13}C- labeled PBDE congeners, ^{13}C-*cis* chlordane, and perdueterated 4,4′-DDE, 4,4′-DDD, 4,4′-DDT, and endosulfan-I) was added to each bottle, sonicated for 15 min, and allowed to equilibrate overnight in the refrigerator. Then, 5 mL of formic acid and 10 mL of 1:4 (volume fraction) methylene chloride:*n*-hexane was added. The MAE temperature conditions consisted of a ramp to 90 °C with a 10 min hold. The MAE was repeated two times with fresh solvent. The clean-up and analysis methods used for the extract were the same as those used for NIST Method 1.

NIST Method 4: For NIST Method 4, the freeze-dried serum (total contents from each of 14 bottles) was reconstituted by adding 10.0 mL (mass known) of HPLC grade water. A known amount of internal standard solution (containing selected ^{13}C-labeled chlorinated diphenyl ether congeners and selected ^{13}C-labeled PBDE congeners) was added to each bottle, sonicated for 5 min, and allowed to equilibrate overnight in a refrigerator. Then, 10 mL of formic acid, as a denaturation agent, was added to each sample, followed immediately by 10 mL of a 1:1 (volume fraction) mixture of *n*-hexane:methyl-*tert*-butyl ether for extraction. The samples were mixed well and left to stand for 1.5 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 hexane layers were concentrated using an automated evaporation system to approximately 0.5 mL with a solvent change to methylene chloride. SEC on a preparative-scale divinylbenzene-polystyrene column was used to remove the remainder of the lipid and biogenic material. The eluant was concentrated to 0.5 mL with a solvent change to hexane and was further cleaned using a silica solid phase extraction (SPE) column, by eluting the fraction of interest with 20 mL of hexane. The concentrated samples were analyzed using GC/MS operated in negative chemical ionization mode. A 0.25 mm × 15 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS) 0.25 µm film thickness was used. All injections were 1 µL using an on-column inlet.

For all of the NIST methods, multi-point calibration response curves for the PCB congeners, chlorinated pesticides, and PBDE congeners, 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 for the analytical methods used at CDC can be found in Patterson and Turner [3] and Sjödin, et al [4]. In summary, the freeze-dried serum (total contents from each of 12 bottles for the dioxins and furans, 20 bottles for the PCBs, 85 bottles for the pesticides, and 5 bottles for the PBDEs) was reconstituted by adding 10.0 mL of HPLC grade water and mixing. The samples were stored overnight at 5 °C, warmed to room temperature, and then three random bottles were pooled together. From the pooled samples, one 1.5 mL vial was used for lipid determination, two 1.5 mL vials were used for *ortho* PCB and pesticide determinations, and the remaining serum was used for PCDD, PCDF, and non-*ortho* PCB determinations.

Sample extraction was done using a C_{18} SPE method. After addition of the internal standard solution and formic acid, the sample was eluted through a 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 all of the analytes except PCB 18, oxychlordane, *trans*-nonachlor, endrin, and 2,4'-DDT.

Gas chromatography/high resolution mass spectrometry (GC/HRMS) with resolution of 10 000 was used for the determination of the PCBs, chlorinated pesticides, 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), 0.25 μm film thickness. All injections were splitless with helium as the carrier gas.

Total Cholesterol and Associated Analytes: For the reference concentrations determined by the CDC, total cholesterol and triglycerides were determined using standard enzymatic methods. In the cholesterol analysis, the esters are first cleaved, and then the total serum cholesterol is measured by a cholesterol oxidase-peroxidase method. The absorbance of the resulting chromophore that absorbs at 540 nm is directly proportional to total cholesterol. In the triglyceride analysis, glycerides are hydrolyzed with a fungal lipase. The liberated glycerol is estimated from the rate of change in absorbance at 340 nm. No corrections are made for the free glycerol content of the serum. "Free" cholesterol is measured by an enzymatic method similar to that used for total cholesterol but in the absence of cholesterol esterase. Serum choline-containing phospholipids are also measured by an enzymatic method in which the phospholipids are hydrolyzed to free choline by phospholipase D.

The total lipids in the serum are calculated from $TL = 1.677 \times (TC - FC) + FC + TG + PL$, where TL is total lipids, TC is total cholesterol, FC is "free" cholesterol, TG is triglycerides, and PL is phospholipids. The constant 1.677 is based on fatty acid analysis of serum cholesterol esters and has been described in references 5 and 6.

Table 1. Certified Concentrations for Selected PCB Congeners[a] in SRM 1589a

PCB Congener			Mass Fraction (ng/kg reconstituted serum)		
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl)[b,c,d,e]	204	±	4[f]
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)[b,c,d,e]	58.3	±	3.2[f]
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)[b,c,d,e]	39.8	±	3.5[f]
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl)[b,c,d,e]	34.2	±	3.9[f]
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)[b,c,d,e]	168	±	8[f]
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl)[b,c,d,e]	10.8	±	1.4[f]
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)[b,c,d]	537	±	19[g]
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl)[b,c,d,e]	71.7	±	3.3[f]
PCB	151	(2,2',3,5,5',6-Hexachlorobiphenyl)[b,c,d,e]	37.8	±	4.2[f]
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl)[b,c,d,e]	936	±	45[f]
	132	(2, 2',3,3',4,6'-Hexachlorobiphenyl)			
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl[b,c,d,e]	74	±	12[f]
PCB	167	(2,3',4,4',5,5'-Hexachlorobiphenyl)[b,c,d,e]	25.9	±	2.6[f]
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)[b,c,d,e]	211	±	16[f]
PCB	172	(2,2',3,3',4,5,5'-Heptachlorobiphenyl)[b,c,d,e]	33.5	±	1.8[f]
PCB	174	(2,2',3,3',4,5,6'-Heptachlorobiphenyl)[b,c,d]	19.6	±	2.4[g]
PCB	177	(2,2',3,3',4',5,6-Heptachlorobiphenyl)[b,c,d,e]	67.1	±	6.1[f]
PCB	178	(2,2',3,3',5,5',6-Heptachlorobiphenyl)[b,c,d,e]	59.9	±	7.0[f]
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)[b,c,d,e]	523	±	67[f]
	193	(2,3,3',4',5,5',6-Heptachlorobiphenyl)			
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)[b,c,d,e]	90.0	±	7.5[f]
PCB	187	(2,2',3,4',5,5'6-Heptachlorobiphenyl)[b,c,d,e]	267	±	27[f]
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[b,c,d,e]	129	±	5[f]
PCB	195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl)[b,c,d,e]	31.3	±	2.4[f]
PCB	199	(2,2',3,3',4,5,5',6'-Octachlorobiphenyl)[b,c,d,e]	170	±	30[f]
PCB	206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)[b,c,d,e]	62.9	±	2.3[f]
PCB	209	(Decachlorobiphenyl)[b,c,d,e]	40.3	±	2.0[f]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [7] and later revised by Schulte and Malisch [8] to conform to the International Union for Pure and Applied Chemistry (IUPAC) rules. For the specific congeners mentioned in this table, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch. When two or more 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 1 using GC/MS following liquid-liquid extraction.

[c] NIST Method 2 using GC/MS following liquid-liquid extraction.

[d] NIST Method 3 using GC/MS following MAE.

[e] GC/HRMS analysis at CDC.

[f] Certified values are weighted means of the results from four analytical methods [9]. 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 Guide to the Expression of Uncertainty in Measurements* [10].

[g] Certified values are unweighted means of the results from 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 [11] with a pooled, within-method variance following the *ISO Guide to the Expression of Uncertainty in Measurement* [10].

Table 2. Certified Concentrations for Selected Chlorinated Pesticides in SRM 1589a

Chlorinated Pesticide	Mass Fraction[a] (ng/kg reconstituted serum)
Oxychlordane[b,c,d,e]	125 ± 15
4,4'-DDE[b,c,d,e]	11510 ± 610
4,4'-DDT[b,c,d,e]	100 ± 7

[a] Certified values are weighted means of the results from four analytical methods [9]. 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 Guide to the Expression of Uncertainty in Measurements* [10].
[b] NIST Method 1 using GC/MS following liquid-liquid extraction.
[c] NIST Method 2 using GC/MS following liquid-liquid extraction.
[d] NIST Method 3 using GC/MS following MAE.
[e] GC/HRMS analysis at CDC.

Table 3. Certified Concentrations for Selected PBDE Congeners[a] in SRM 1589a

PBDE Congener	Mass Fraction (ng/kg reconstituted serum)
PBDE 47 (2,2',4,4'-Tetrabromodiphenyl Ether)[b,c,d]	172 ± 10[e]
PBDE 99 (2,2',4,4',5-Pentabromodiphenyl Ether)[b,c,d,f]	39.9 ± 5.2[g]
PBDE 100 (2,2',4,4',6-Pentabromodiphenyl Ether)[b,c,d,f]	25.0 ± 3.2[g]
PBDE 183 (2,2',3,4,4',5',6-Heptabromodiphenyl Ether)[d,e]	4.65 ± 0.26[e]

[a] PBDE congeners are numbered according to IUPAC rules.
[b] NIST Method 2 using GC/MS following liquid-liquid extraction.
[c] NIST Method 4 using GC/NCI-MS following liquid-liquid extraction.
[d] GC/HRMS analysis at CDC.
[e] 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 [11] with a pooled, within-method variance following the *ISO Guide to the Expression of Uncertainty in Measurement* [10].
[f] NIST Method 1 using GC/MS following liquid-liquid extraction.
[g] Certified values are weighted means of the results from four analytical methods [9]. 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 Guide to the Expression of Uncertainty in Measurements* [10].

Table 4. Reference Concentrations for Selected PCB Congeners[a], Chlorinated Pesticides, and PBDE Congeners[a] in SRM 1589a

PCB Congener, Chlorinated Pesticide, or PBDE Congener	Mass Fraction (ng/kg reconstituted serum)
PCB 28 (2,4,4'-Trichlorobiphenyl)[b,c,d]	40.8 ± 7.1[e]
31 (2,4',5-Trichlorobiphenyl)	
PCB 44 (2,2',3,5'-Tetrachlorobiphenyl)[b,c,d,f]	30.7 ± 9.3[g]
PCB 49 (2,2',4,5'-Tetrachlorobiphenyl)[d,f]	24 ± 14[e]
PCB 52 (2,2',5,5'-Tetrachlorobiphenyl)[b,c,d,f]	45 ± 10[g]
PCB 66 (2,3',4,4'-Tetrachlorobiphenyl)[b,c,d,f]	30.2 ± 10.5[g]
PCB 70 (2,3',4',5-Tetrachlorobiphenyl)[b,c]	18.8 ± 3.6[e]
PCB 74 (2,4,4',5-Tetrachlorobiphenyl)[b,c,d,f]	169 ± 40[g]
PCB 87 (2,2',3,4,5'-Pentachlorobiphenyl)[b,c,d,f]	18.3 ± 6.1[g]
PCB 92 (2,2',3,5,5'-Pentachlorobiphenyl)[b,c,d]	13.8 ± 3.6[e]
PCB 95 (2,2',3,5',6-Pentachlorobiphenyl)[b,c,d]	51 ± 15[e]
121 (2,3′,4,5′,6-Pentachlorobiphenyl)	
PCB 114 (2,3,4,4′,5-Pentachlorobiphenyl)[b,c,d]	16.2 ± 3.6[e]
PCB 130 (2,2′,3,3′,4,5'-Hexachlorobiphenyl)[b,c,d]	20.4 ± 3.1[e]
PCB 137 (2,2',3,4,4',5-Hexachlorobiphenyl)[b,c,d]	34.3 ± 6.4[e]
PCB 146 (2,2',3,4',5,5'-Hexachlorobiphenyl)[b,c,d,f]	95 ± 13[g]
PCB 157 (2,3,3′,4,4′,5′-Hexachlorobiphenyl)[b,c,d,f]	18.9 ± 6.3[g]
PCB 158 (2,3,3',4,4',6-Hexachlorobiphenyl)[b,c,d]	15.3 ± 5.4[e]
PCB 163 (2,3,3',4',5,6-Hexachlorobiphenyl)[b,c,d]	123 ± 23[e]
PCB 189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)[b,c,d]	7.8 ± 1.9[e]
PCB 191 (2,3,3',4,4',5',6-Heptachlorobiphenyl)[b,c,d]	8.4 ± 1.4[e]
PCB 196 (2,2',3,3',4,4',5,6'-Octachlorobiphenyl)[b,c,d,f]	154 ± 31[g]
203 (2,2',3,4,4',5,5',6-Octachlorobiphenyl)	
PCB 201 (2,2',3,3',4,5',6,6'-Octachlorobiphenyl)[b,c,d]	7.8 ± 4.3[e]
PCB 202 (2,2',3,3′,5,5',6,6′-Octachlorobiphenyl)[b,c,d]	61 ± 23[e]
PCB 207 (2,2',3,3′,4,4′,5,6,6′-Nonachlorobiphenyl)[c,d]	9.4 ± 1.6[e]
PCB 208 (2,2',3,3′,4,5,5',6,6′-Nonachlorobiphenyl)[b,c,d]	41.4 ± 7.8[e]
Hexachlorobenzene[b,c,d]	76 ± 28[e]
Dieldrin[c,d,f]	107 ± 37[e]
Mirex[b,c,d,f]	112 ± 35[g]
β-HCH[f]	86.2 ± 3.3[h]
Heptachlor Epoxide[f]	96.6 ± 2.9[h]
trans-Nonachlor[f]	318 ± 11[h]
PBDE 28 (2,4,4'-Tribromodiphenyl Ether)[c,f,i]	11.9 ± 6.7[e]
PBDE 153 (2,2',4,4',5,5'-Hexabromodiphenyl Ether)[c,f,i]	18.5 ± 4.3[e]
PBDE 154 (2,2',4,4',5,6'-Hexabromodiphenyl Ether)[i]	62.8 ± 7.5[h]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [7] and later revised by Schulte and Malisch [8] to conform to IUPAC rules. When two or more 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. PBDE congeners are numbered according to IUPAC rules.

[b] NIST Method 1 using GC/MS following liquid-liquid extraction.

[c] NIST Method 2 using GC/MS following liquid-liquid extraction.

[d] NIST Method 3 using GC/MS following MAE.

[e] Reference 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 [11] with a pooled, within-method variance following the *ISO Guide to the Expression of Uncertainty in Measurement* [10].

[f] GC/HRMS analysis at CDC.

[g] Reference values are weighted means of the results from four analytical methods [9]. 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 Guide to the Expression of Uncertainty in Measurements* [10].

[h] The reference values are the means of results obtained by CDC 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.

[i] NIST Method 4 using GC/NCI-MS following liquid-liquid extraction.

Table 5. Reference Concentrations for Serum Lipid in SRM 1589a by the CDC Reference Method

Serum Lipid	Concentration (mg/dL reconstituted serum)
Total Cholesterol (TC)[a]	161 ± 2
"Free" Cholesterol (FC)[a]	32 ± 2
Phospholipids (PL)[a]	192 ± 3
Triglycerides (TG)[a]	164 ± 7
Total Lipids (TL)[b]	603 ± 7

[a] The reference values are the means of results obtained by CDC 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.

[b] The total lipids in the serum are calculated from TL = 1.677 × (TC – FC) + FC + TG + PL, where TL is total lipids, TC is total cholesterol, FC is "free" cholesterol, TG is triglycerides, and PL is phospholipids. The constant 1.677 is based on fatty acid analysis of serum cholesterol esters and has been described in references 5 and 6. The uncertainty in the reference value 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 [10]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the measurement error. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence. For this analysis, $k = 2.4$. The true uncertainty is likely to be larger.

Table 6. Reference Concentrations for Selected Non-*ortho* PCB[a], Dibenzo-*p*-dioxin (PCDD), and Dibenzofuran (PCDF) Congeners in SRM 1589a

Non-*ortho* PCB, PCDD, or PCDF Congener	Mass Fraction[b,c] (pg/kg reconstituted serum)
PCB 77 (3,3',4,4'-Tetrachlorobiphenyl)	1870 ± 90
PCB 81 (3,4,4',5-Tetrachlorobiphenyl)	118 ± 6
PCB 126 (3,3',4,4',5-Pentachlorobiphenyl)	252 ± 7
PCB 169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	184 ± 6
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	18 ± 1
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	49 ± 2
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	39 ± 4
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	416 ± 12
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	46 ± 3
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	357 ± 12
Octachlorodibenzo-*p*-dioxin	4130 ± 100
2,3,4,7,8-Pentachlorodibenzofuran	47 ± 2
1,2,3,4,7,8-Hexachlorodibenzofuran	55 ± 3
1,2,3,6,7,8-Hexachlorodibenzofuran	41 ± 2
2,3,4,6,7,8-Hexachlorodibenzofuran	7.6 ± 1.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	124 ± 4
1,2,3,4,7,8,9-Heptachlorodibenzofuran	6.0 ± 0.7
Octachlorodibenzofuran	8.2 ± 3.0

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [7] and later revised by Schulte and Malisch [7] to conform to IUPAC rules.

[b] The reference values are the means of results obtained by CDC 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.

[c] GC/HRMS analysis at CDC.

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