National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1589a

PCBs, Pesticides, and Dioxins/Furans in Human Serum

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 total cholesterol 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 16 PCB congeners and 5 chlorinated pesticides are provided in Tables 1 and 2, respectively. The certified concentration for total cholesterol is provided in Table 3. 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. The certified values for the PCB congeners and chlorinated pesticides are based on the agreement of results obtained at NIST by one analytical technique and additional results from the U.S. Centers for Disease Control and Prevention (CDC) using a different analytical technique. The certified value for total cholesterol was determined at NIST using the isotope dilution/gas chromatography/mass spectrometry (ID/GC/MS) definitive method [1,2].

Reference Concentration Values: Reference concentration values, expressed as mass fractions, are provided in Table 4 for nine additional PCB congeners and five additional chlorinated pesticides. 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. Explanations in support of each reference value are given as notes in Tables 4, 5, and 6.

Expiration of Certification: The certification of this SRM lot is valid until **31 January 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. 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. Return of the attached registration card 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.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

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Consultation on the statistical design of the experimental work and evaluation of the data were provided by M.S. Levenson and L.M. Gill 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.

Analytical measurements at NIST were performed by E. Dyremark, M.M. Schantz, and L.T. Sniegoski of the NIST Analytical Chemistry Division.

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

NOTICE AND WARNING 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 [3].

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 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

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[1] 0.8-μm (pore size) 90-mm (diameter) polyethersulfone-based membrane filters (Gelman 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-mL 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, instruments, or materials are identified in this certificate in order 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 for the Chlorinated Analytes: The freeze-dried serum (total contents from each of six bottles) was reconstituted by adding 10.0 mL (weight known) of HPLC grade water, followed by 5 min ultra sonication and subsequent vortex mixing for 30 s. A known amount of internal standard solution (containing ^{13}C- labeled PCB 28, PCB 101, PCB 118, PCB 138, PCB 153, PCB 169, γ-HCH, 4,4'-DDE, and 4,4'-DDT) was added to each bottle, mixed well, and left in the refrigerator overnight to equilibrate.

The following day, 10 mL of formic acid, as a denaturation agent, was added to each sample, followed immediately by 10 mL of a 1:1 (v:v) mixture of *n*-hexane and 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 once with 20 mL of a mixture of 1:1 (v:v) *n*-hexane and methyl-*tert*-butyl ether, and once with only 10 mL of *n*-hexane.

The combined hexane layers were concentrated using an automated evaporation system to approximately 0.5 mL for size exclusion chromatography (SEC). SEC on a preparative-scale divinylbenzene-polystyrene column (10 µm particle size, 100 10 nm pore size, 2.5 cm i.d. x 60 cm, PL-Gel, Polymer Labs, Inc., Amherst, MA) was used to remove the majority of the lipid and biogenic material. Using a mobile phase of 100 % dichloromethane at 9.9 mL/min for SEC, the majority of the lipid and biogenic material elutes immediately after the void volume of the column while the PCBs and chlorinated pesticides are retained longer. The eluant (approximately 70 mL) was concentrated using an automated evaporation system to approximately 0.4 mL with a solvent change to hexane. The 0.4 mL portion was further cleaned using a silica solid phase extraction cartridge which was precleaned and the sample eluted using 15 mL of 10 % dichloromethane in *n*-hexane. The eluant was concentrated to approximately 0.25 mL for analysis.

The concentrated samples were analyzed using gas chromatography/mass spectrometry (GC/MS). A 0.25 mm x 30 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (HP-5MS, Hewlett Packard, Wilmington, DE) 0.25 µm film thickness was used. The column was held isothermally at 60 °C for 1 min, temperature programmed at 45 °C/min to 180 °C for 30 min, and then temperature programmed at 2 °C/min to 250 °C where it was held isothermally for 15 min. The transfer line was maintained at 280 °C, and the injection port was maintained at 250 °C. All injections were 2 µL in a pulsed splitless mode. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. Two forms of ionization were used: electron impact and negative chemical ionization with methane as the reagent gas.

A six point calibration response curve for the PCB congeners and a nine point calibration response curve for the pesticides relative to the internal standards were determined by processing gravimetrically-diluted solutions of SRM 2261, SRM 2262, SRM 2274, and SRM 2275 with the internal standards added.

Analytical Methods used at CDC for the Chlorinated Analytes: Details for the analytical methods used at CDC can be found in Patterson and Turner [4]. In summary, the freeze-dried serum (total contents from each of 12 bottles) 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 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} solid phase extraction (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 acid/neutral/base silica column, alumina column, and carbon column. As internal standards, corresponding ^{13}C-labeled compounds were used for all of the analytes except PCB 18, oxychlordane, *trans*-nonachlor, endrin, and 2,4'-DDT.

Gas chromatography/high resolution mass spectrometry (GC/HRMS) resolution of 10000 was used for the determination of the PCBs, chlorinated pesticides, PCDDs, and PCDFs. The GC column was a 0.25 mm x 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.

Total Cholesterol and Associated Analytes: For the determination of the certified concentration of cholesterol, a method considered to be "definitive" [1] for total serum cholesterol was used at NIST. The method uses isotope dilution/gas chromatography/mass spectrometry [2]. For the measurement of this SRM, a total of six bottles were analyzed in two sets. Each set consisted of a single weighed aliquot from each of three bottles of reconstituted serum. Each sample was spiked with a known amount of cholesterol-$^{13}C_3$ and hydrolyzed with KOH in ethanol. The free cholesterol was then extracted with hexane, dried, and converted to its trimethylsilyl ether for GC/MS analysis.

Measurements were performed using a GC column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (DB-5MS, J&W Scientific, Folsom, CA) and electron ionization with selected ion monitoring of the molecular ions at m/z 458 and 461. Calibration involved measurement of a set of standards prepared from SRM 911b Cholesterol and the same labeled cholesterol used to spike the samples.

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.

Most U.S. National Institutes of Health (NIH) funded epidemiological and interventional studies performed over the last 30 years have traced their total cholesterol values to the reference methods performed at the CDC Lipid Reference Laboratory. As shown in Table 5, there is a small bias in the CDC reference method value for total cholesterol (about 1.8 % high) compared to the NIST definitive method value presented in Table 3. The slight bias appears to be due to a small amount of nonspecificity of the CDC's reference method, which has been discussed in some detail previously [5,6]. The National Cholesterol Education Program (NCEP) recommends that total cholesterol measurements be traceable to the CDC reference method [7,8]. Thus, most manufacturers of total cholesterol reagents and calibrator materials, at least for distribution in the United States, will wish to use the CDC reference value (i.e., 161 mg/dL) and not the NIST definitive method value (157.76 mg/dL) at this time.

The total lipids in the serum are calculated from: TL = 1.677 x (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 elsewhere [9,10].

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

			Mass Fraction[b] ng/kg reconstituted serum
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl)[c,d]	121 ± 10
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)[c,d]	34 ± 9
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)[c,d,e]	29 ± 4
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)[c,d,e]	119 ± 9
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)[c,d,e]	483 ± 39
	163	(2,3,3',4',5,6-Hexachlorobiphenyl)	
	164	(2,3,3',4',5',6-Hexachlorobiphenyl)	
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl) [c,d,e]	56 ± 8
PCB	151	(2,2',3,5,5',6-Hexachlorobiphenyl)[d,e]	28 ± 4
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl)[c,d,e]	672 ± 35
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl [c,d,e]	66 ± 4
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)[c,d,e]	483 ± 29
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)[c,d,e]	65 ± 5
PCB	187	(2,2',3,4',5,5'6-Heptachlorobiphenyl)[c,d,e]	172 ± 25
	182	(2,2',3',4,4',5,6'-Heptachlorobiphenyl)	
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[d,e]	98 ± 14
PCB	195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl)[d,e]	22 ± 4
PCB	206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)[d,e]	40 ± 6
PCB	209	(Decachlorobiphenyl)[d,e]	25 ± 4

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [11] and later revised by Schulte and Malisch [12] to conform with 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] Each result is expressed as the certified value ± the expanded uncertainty. The certified value is the mean of the results obtained at NIST and CDC. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [13] and $k = 2$ is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of all the uncertainties, which includes variations within each laboratory and between the two laboratories.

[c] GC/MS analysis at NIST using electron impact ionization.

[d] GC/HRMS analysis at CDC.

[e] GC/MS analysis at NIST using negative chemical ionization; same serum extracts analyzed as for footnote c.

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

	Mass Fraction[a] ng/kg reconstituted serum
beta -HCH[b,c]	56 ± 13
trans-Nonachlor[b,c]	169 ± 29
Heptachlor Epoxide[b,c]	75 ± 9
Oxychlordane[b,c]	157 ± 14
4,4'-DDE[b,c]	6600 ± 1000

[a] Each result is expressed as the certified value ± the expanded uncertainty. The certified value is the mean of the results obtained at NIST and CDC. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [13] and $k = 2$ is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of all the uncertainties, which includes variations within each laboratory and between the two laboratories.

[b] GC/MS analysis at NIST using electron impact ionization.

[c] GC/HRMS analysis at CDC.

Table 3. Certified Concentration for Total Cholesterol in SRM 1589a by the NIST Definitive Method

Total Cholesterol	157.76 ± 0.37 mg/dL[a,b]

[a] The result is expressed as the certified value ± the expanded uncertainty. The uncertainty in the certified 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 [13]. 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, $k = 2.6$.

[b] The density of SRM 1589a was determined using the Lang-Levy pipet method [14] to be 1.021 g/mL at 21.6 °C.

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

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification. These reference values should be useful for comparison with results obtained using similar procedures.

	Mass Fraction ng/kg reconstituted serum
PCB 74 (2,4,4',5-Tetrachlorobiphenyl)[b,c]	226 ± 5
PCB 95 (2,2',3,5',6-Pentachlorobiphenyl)[d,e]	47 ± 4
PCB 146 (2,2',3,4',5,5'-Hexachlorobiphenyl)[b,c]	76 ± 5
PCB 170 (2,2',3,3',4,4',5-Heptachlorobiphenyl)[d,e,f]	186 ± 4
PCB 172 (2,2',3,3',4,5,5'-Heptachlorobiphenyl)[b,c]	26 ± 2
PCB 177 (2,2',3,3',4',5,6-Hepatchlorobiphenyl)[b,c]	58 ± 5
PCB 178 (2,2',3,3',5,5',6-Heptachlorobiphenyl)[b,c]	31 ± 2
PCB 196 (2,2',3,3',4,4',5,6'-Octachlorobiphenyl)[b,c]	92 ± 6
203 (2,2',3,4,4',5,5',6-Octachlorobiphenyl)[b,c]	
PCB 199 (2,2',3,3',4,5,5',6'-Octachlorobiphenyl)[a,b,c]	101 ± 2
Hexachlorobenzene[d,e]	49 ± 9
2,4'-DDE[d,e]	85 ± 5
4,4'-DDT[b,c]	85 ± 10
Dieldrin[b,c]	73 ± 7
Mirex[b,c]	43 ± 4

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [11] and later revised by Schulte and Malisch [12] to conform with IUPAC rules. For the specific congeners mentioned in this table, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch except for PCB 199. PCB 199 is PCB 201 in the Ballschmiter and Zell scheme. 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] Each result is expressed as the reference value ± the expanded uncertainty. The reference value is the mean of the results obtained at CDC. The expanded uncertainty in the reference value is equal to $U = ku_c$, where u_c is the combined standard uncertainity calculated according to the ISO Guide [13] and $k = 3.18$ is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the uncertainty in the reference value, which includes only variation observed at CDC and may not include some sources of bias.

[c] GC/HRMS analysis at CDC.

[d] Each result is expressed as the reference value ± the expanded uncertainty. The reference value is the mean of the results obtained at NIST. The expanded uncertainty in the reference value is equal to $U = ku_c$, where u_c is the combined standard uncertainity calculated according to the ISO Guide [13] and $k = 2.6$ is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the uncertainty in the reference value which includes only variation observed at NIST and may not include some sources of bias.

[e] GC/MS analysis at NIST using electron impact ionization.

[f] GC/MS analysis at NIST using negative chemical ionization; same serum extracts analyzed as for footnote e.

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

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification. These reference values should be useful for comparison with results obtained using similar procedures.

	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] Each result is expressed as the reference value ± the expanded uncertainty. 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 [13]. 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, $k = 3.2$.

[b] The total lipids in the serum are calculated from: TL = 1.677 x (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 elsewhere [9,10]. 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 [13]. 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, and Dibenzofuran Congeners in SRM 1589a

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification. These reference values should be useful for comparison with results obtained using similar procedures.

	Mass Fraction[b,c] pg/kg reconstituted serum
PCB 77 (3,3',4,4'-Tetrachlorobiphenyl)	790 ± 100
PCB 81 (3,4,4',5-Tetrachlorobiphenyl)	51 ± 12
PCB 126 (3,3',4,4',5-Pentachlorobiphenyl)	150 ± 14
PCB 169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	128 ± 8
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	19 ± 10
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	36 ± 2
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	30 ± 2
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	289 ± 28
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	33 ± 5
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	260 ± 20
Octachlorodibenzo-*p*-dioxin	2673 ± 95
2,3,7,8-Tetrachlorodibenzofuran	3.8 ± 1.5
2,3,4,7,8-Pentachlorodibenzofuran	34 ± 4
1,2,3,4,7,8-Hexachlorodibenzofuran	39 ± 4
1,2,3,6,7,8-Hexachlorodibenzofuran	29 ± 3
2,3,4,6,7,8-Hexachlorodibenzofuran	5.6 ± 0.9
1,2,3,4,6,7,8-Heptachlorodibenzofuran	70 ± 10
1,2,3,4,7,8,9-Heptachlorodibenzofuran	3.6 ± 0.3
Octachlorodibenzofuran	11 ± 3

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [11] and later revised by Schulte and Malisch [12] to conform with IUPAC rules. For the specific congeners mentioned in this table, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch.

[b] Each result is expressed as the reference value ± the expanded uncertainty. The reference value is the mean of the results obtained at CDC. The expanded uncertainty in the reference value is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [13] and $k = 3.18$ is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the uncertainty in the reference value. It includes only variation observed at CDC and may not include some sources of bias.

[c] GC/HRMS analysis at CDC.

[1] "Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline," NCCLS Publication NRSCL 1-A, National Committee for Clinical Laboratory Standards, Wayne, PA, (1991).

[2] Ellerbe, P., Meiselman, S., Sniegoski, L.T., Welch, M.J., and White V, E., "Determination of Serum Cholesterol by a Modification of the Isotope Dilution Mass Spectrometric Definitive Method," Anal. Chem. **61**, pp. 1718-1723, (1989).

[3] U.S. Department of Health and Human Services, "Biosafety in Microbiological and Biomedical Laboratories," U.S. Government Printing Office, Washington, DC (1988).

[4] Patterson, D.G. Jr. and 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 Preventation, Atlanta, GA, pp. 1-253, (1997).

[5] Bernert, J.T., Aikns, J.R., Cooper, G.R., Poulose, A.K., Myers, G.L., and Sampson, E.R., "Factors Influencing the Accuracy of the National Reference System Total Cholesterol Reference Method," Clin. Chem. **37**, pp. 2053-2063, (1991).

[6] Ellerbe, P., Myers, G.L., Cooper, G.R., Hertz, H.S., Sniegoski, L.T., and Welch, M.J., "A Comparison of Results for Cholesterol in Human Serum Obtained by the Reference Method and by the Definitive Method of the National Reference System for Cholesterol," Clin. Chem. **36**, pp. 370-375, (1990).

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