National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1958

Organic Contaminants in Fortified Human Serum

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 serum and similar matrices. Reference values are provided for selected polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-ortho PCB congeners, perfluorinated compounds (PFCs), and serum lipid. Information values are provided for selected toxaphene congeners and hydroxylated compounds. A unit of SRM 1958 consists of five vials, each containing freeze-dried fortified human serum. Before use, the serum in each bottle must be reconstituted with 10.7 mL of distilled or HPLC-grade water.

The development of SRM 1958 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 Mass Fraction Values: Certified values, expressed as mass fractions, for PCB congeners, chlorinated pesticides, and PBDE congeners along with one polybrominated biphenyl congener are provided in Tables 1, 2, and 3, respectively [1]. 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 [2]. 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, additional results from CDC, and from an interlaboratory study using different analytical techniques. Values are reported based on the reconstituted serum in mass fraction units.

Reference Mass Fraction Values: Reference values, expressed as mass fractions, are provided in Table 4 for additional PCB congeners and chlorinated pesticides; Table 5 for selected PFC compounds; and Table 6 for selected PCDD and PCDF congeners and non-ortho PCB congeners [1]. Reference values for serum lipids are provided in Table 7. 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 [2]. Values are reported based on the reconstituted serum in mass fraction units.

Information Mass Fraction Values: Information values, expressed as mass fractions, for selected toxaphene congeners, additional compounds, and Aroclor 1260 are provided in Table 8 [2]. 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]. Values are reported based on the reconstituted serum in mass fraction units.

Expiration of Certification: The certification of **SRM 1958** is valid, within the measurement uncertainty specified, until **31 August 2015**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Stability, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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

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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.

Partial support for the development of SRM 1958 was provided by the Division of Laboratory Sciences, Organic Analytical Toxicology Branch, CDC, Atlanta, GA.

Analytical measurements at NIST were performed by R.M. Heltsley, J.M. Keller, M.M. Schantz, and S.S. Vander Pol of the NIST Chemical Sciences Division. Analytical measurements at CDC were performed by A. Calafat, D. Patterson, A. Sjödin, and W. Turner of the Organic Analytical Toxicology Branch, CDC. Laboratories participating in an interlaboratory study included 3M Corporation (Maplewood, MN), Axys Analytical (Sidney, B.C., Canada), 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 and N.A. Heckert 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 1958 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 by FDA-licensed tests 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 CDC/National Institutes of Health (NIH) Manual [3].

INSTRUCTIONS FOR STABILITY, STORAGE, AND USE

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.

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 10.7 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 various blood banks located around the U.S.: Wilmington and Greenville, NC; Jacksonville and Orlando, FL; Jonesboro, AR; Flagstaff, AZ; Gallup and Albuquerque, NM; Memphis, TN; Portland, ME; and Carbondale, IL.

Preparation of Material: Preparation of the serum was performed by Aalto Scientific, Ltd., Carlsbad, CA. Following the precipitation of fibrin and filtration, the serum was pooled (approximately 200 L total) and was stored at 4 °C. The pool was split into two for production of SRMs 1957 and 1958. Before bottling, approximately 710 mL of methanol containing 169 compounds at varying mass concentrations (Appendix A) was added to 100 L of serum, which was then stirred for 4 h. Using a calibrated automatic pipette, 10.7 mL aliquots of serum were

[(1)] Certain commercial equipment, instruments, 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.

dispensed into 30 mL amber glass vials. The samples were lyophilized. The samples were considered dry when a stable vacuum and temperature were achieved.

ANALYTICAL METHODS USED AT NIST

For NIST Method 1, the freeze-dried serum in each of ten vials was reconstituted by adding 10.7 mL (mass known) of HPLC-grade water. A known amount of internal standard solution (containing selected ^{13C}-labeled PCB congeners, selected ^{13C}-labeled pesticides, ^{13C}-labeled PBDE 209, fluorinated PBDE 47, PCB 103, and PCB 198) was added to each bottle, which was 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 and methyl *tert*-butyl ether for extraction. The samples were vortexed and left to stand for 0.5 h with occasional mixing. After centrifugation to obtain a sharp phase boundary, the upper organic phase was transferred to a concentration vessel. The extraction was repeated twice with 10 mL of *n*-hexane each time. The combined hexane layers were concentrated using an automated evaporation system to approximately 4 mL. Approximately 2 mL of concentrated sulfuric acid was added to the concentration vessel with swirling. Following phase separation, the hexane 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 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 freeze-dried serum in each of six vials was reconstituted by adding 10.7 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 pesticides, ^{13}C-labeled PBDE 209, and selected fluorinated PBDE congeners) was added to a 2 g serum subsample 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, samples were cleaned-up on a sulfuric-acid silica column followed by clean-up on an alumina column (5 % deactivated). 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, same column, and PTV inlet were used with the GC/MS in the NICI mode. For NIST Method 2c, 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 NIST Method 3, the freeze-dried serum in each of six vials was reconstituted by adding 10.7 mL (mass known) of HPLC-grade water. A known amount of internal standard solution (containing selected ^{13}C-labeled hydroxylated compounds) was added to 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 and 0.5 mL of 6 mol/L hydrochloric acid (HCl) were added to the serum sample followed by 2 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. Potassium hydroxide (KOH) was then added to the organic phase. The samples were shaker-extracted for 15 min and centrifuged, and the KOH was removed. This step was repeated two times and the KOH phases combined. HCl (6 mol/L) was added to the KOH phases, followed by 2 mL 20 % (volume fraction) dichloromethane in hexane. The hexane phase was removed, and this step was repeated two times combining the hexane phases. A silica column was used for clean-up followed by analysis using liquid chromatography coupled to a triple quadrupole mass spectrometer (LC/MS/MS) using a C_{18} column (Agilent Eclipse Plus C_{18}, 3.0 mm × 150 mm × 3.5 µm, Agilent Technologies) with a methanol-water gradient.

For NIST Method 4, the freeze-dried serum in each of five vials was reconstituted by adding 10.7 mL (mass known) of HPLC-grade water. A known amount of internal standard solution (containing selected ^{13}C-labeled PFCs) was added to a 0.15 g serum subsample from each bottle, vortexed, and allowed to equilibrate overnight under refrigerated conditions. After samples were removed from refrigeration and allowed to reach ambient temperature, 0.6 mL of 50 % (volume fraction) formic acid in water was added, and the samples were loaded onto 60 mg Oasis WAX SPE columns (Waters, Milford, MA). Compounds of interest were eluted off the columns using methanol followed by 2 mL of 1 % (volume fraction) ammonium hydroxide in methanol. Following concentration, samples were analyzed using LC/MS/MS with a C_8 column (Thermo Betasil C_8, 100 mm × 2.1 mm × 5 µm, Thermo Fisher Scientific, Waltham, MA) and methanol-ammonium acetate in water gradient.

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 *Chlorinated Pesticides in Hexane (Nominal Concentration 2 µg/mL)*, SRM 2262 *Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane (Nominal Concentration 2 µg/mL)*, SRM 2274 *PCB Congener Solution-II in Isooctane*, and SRM 2275 *Chlorinated Pesticide Solution-II in Isooctane* plus gravimetrically prepared solutions of the additional analytes of interest with the internal standards added. SRM 1589a *PCBs, Pesticides, PBDEs, and Dioxins/Furans in Human Serum* was analyzed with each set of samples as a quality control material.

ANALYTICAL METHODS USED AT CDC

For the analytes other than PFCs, 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 was reconstituted by adding 10.7 mL of HPLC-grade water and mixing. The samples were stored overnight at 5 °C. 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.

For determination of the PFCs, the freeze-dried serum in each of three vials was reconstituted by adding 10.7 mL (mass known) of HPLC-grade water. A known amount of internal standard solution containing selected ^{13}C-labeled PFCs was added to two 0.2 mL serum subsamples from each bottle along with 0.5 mL of 0.1 mol/L formic acid. The samples were sonicated for 20 min and placed on an on-line SPE-HPLC system. The samples were loaded onto C_{18} SPE cartridges, and the compounds of interest were eluted off the cartridges directly into the LC/MS/MS with separation on a C_8 column (Thermo Betasil C_8, 50 mm × 3 mm × 5 µm, ThermoHypersil-Keystone, Bellefonte, PA) using a gradient of methanol and ammonium acetate in water.

INTERLABORATORY STUDY

The four 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 results was used for combination with other data to assign the certified and reference mass fraction values.

Total Cholesterol and Associated Analytes: The reference mass fractions total cholesterol and triglycerides were determined using standard enzymatic methods by the CDC and the Institut national de santé publique du Québec. In the cholesterol analysis, the esters were first cleaved (using cholesterol esterase), and then the total serum cholesterol was measured by a cholesterol oxidase - peroxidase method. The absorbance of the resulting chromophore at 540 nm is directly proportional to total cholesterol. "Free" cholesterol was measured by an enzymatic method similar the method for total cholesterol but did not include the cholesterol esterase. In the triglyceride analysis, glycerides were hydrolyzed with a fungal lipase and the liberated glycerol was estimated from the rate of change in absorbance at 340 nm. No corrections were made for the free glycerol content of the serum. Serum choline-containing phospholipids were also measured by an enzymatic method in which the phospholipids are hydrolyzed to free choline by phospholipase D.

Table 1. Certified Mass Fraction Values for PCB Congeners[a] in Reconstituted Serum SRM 1958

PCB Congeners			Mass Fraction[b] (ng/kg)
PCB	18	2,2',5-Trichlorobiphenyl[c,d,e]	407 ± 14
PCB	28	2,4,4'-Trichlorobiphenyl[c,e,f]	402 ± 12
PCB	44	2,2',3,5'-Tetrachlorobiphenyl[c,d,e]	408 ± 21
PCB	49	2,2',4,5'-Tetrachlorobiphenyl[c,d,e]	414 ± 15
PCB	52	2,2',5,5'-Tetrachlorobiphenyl[c,d,e,f]	401 ± 14
PCB	66	2,3',4,4'-Tetrachlorobiphenyl[c,d,e]	414 ± 6
PCB	74	2,4,4',5-Tetrachlorobiphenyl[c,d,e,f]	414 ± 46
PCB	87	2,2',3,4,5'-Pentachlorobiphenyl[c,d,e]	396 ± 23
PCB	99	2,2',4,4',5-Pentachlorobiphenyl[c,d,e,f,g]	385 ± 28
PCB	101	2,2',4,5,5'-Pentachlorobiphenyl[c,d,e,f,g]	409 ± 27
PCB	105	2,3,3',4,4'-Pentachlorobiphenyl[c,d,e,f,g]	419 ± 31
PCB	110	2,3,3',4',6-Pentachlorobiphenyl[c,d,e,g]	397 ± 26
PCB	118	2,3',4,4',5-Pentachlorobiphenyl[c,d,e,f,g]	412 ± 35
PCB	128	2,2',3,3',4,4'-Hexachlorobiphenyl[c,e,f,g]	420 ± 17
PCB	138	2,2',3,4,4',5'-Hexachlorobiphenyl[c,d,e]	473 ± 54
PCB	146	2,2',3,4',5,5'-Hexachlorobiphenyl[c,d,e,f,g]	378 ± 26
PCB	149	2,2',3,4',5',6-Hexachlorobiphenyl[c,d,e,g]	373 ± 22
PCB	151	2,3,3',4,4'-Hexachlorobiphenyl[c,e,f,g]	381 ± 18
PCB	153	2,2',4,4',5,5'-Hexachlorobiphenyl[c,e,f]	457 ± 36
PCB	156	2,3,3',4,4',5-Hexachlorobiphenyl[c,d,e,f,g]	418 ± 19
PCB	157	2,3,3',4,4',5'-Hexachlorobiphenyl[c,d,e,f,g]	420 ± 42
PCB	158	2,3,3',4,4',5'-Hexachlorobiphenyl[c,d,g]	365 ± 48
PCB	167	2,3',4,4',5,5'-Hexachlorobiphenyl[c,d,e,f,g]	403 ± 26
PCB	170	2,2',3,3',4,4',5-Heptachlorobiphenyl[c,d,e,f,g]	422 ± 23
PCB	172	2,2',3,3',4,5,5'-Heptachlorobiphenyl[c,d,e,f,g]	395 ± 28
PCB	177	2,2',3,3', 4',5,6-Heptachlorobiphenyl[c,d,e,f,g]	392 ± 9
PCB	178	2,2',3,3',5,5',6-Heptachlorobiphenyl[c,d,e,f,g]	386 ± 15
PCB	180	2,2',3,4, 4',5,5'-Heptachlorobiphenyl[c,e,f]	459 ± 49
PCB	183	2,2',3,4,4',5',6-Heptachlorobiphenyl[c,d,e,f,g]	407 ± 36
PCB	187	2,2',3,4',5,5',6-Heptachlorobiphenyl[c,d,e,f,g]	411 ± 38
PCB	189	2,3,3',4,4',5,5'-Heptachlorobiphenyl[c,d,e,f,g]	402 ± 31
PCB	194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl[d,e,f,g]	387 ± 20
PCB	195	2,2',3,3',4,4',5,6-Octachlorobiphenyl[d,e,f,g]	385 ± 25
PCB	196	2,2',3,3',4,4',5,6'-Octachlorobiphenyl[f,g]	397 ± 2[h]
PCB	199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl[d,e]	379 ± 21
PCB	203	2,2',3,4,4',5,5',6-Octachlorobiphenyl[f,g]	398 ± 49
PCB	206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl[d,e,f,g]	366 ± 8
PCB	209	Decachlorobiphenyl[d,e,f,g]	338 ± 16

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [5] and later revised by Schulte and Malisch [6] to conform with IUPAC rules. PCB 199 is PCB 201 under the Ballschmiter and Zell scheme in this table.

[b] The certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed and the values listed are metrologically traceable to the SI unit of mass fraction in nanogram analyte per kilogram sample in reconstituted serum.

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

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

[e] CDC method, GC/HRMS.

[f] Results from interlaboratory study.

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

[h] The coverage factor used for PCB 196 was $k = 4$.

Table 2. Certified Mass Fraction Values for Chlorinated Pesticides in Reconstituted Serum SRM 1958

	Mass Fraction[a] (ng/kg)
Hexachlorobenzene[b,c,d,e,f]	442 ± 46
cis-Chlordane[d,e,f,g]	412 ± 25
trans-Chlordane[d,e,g]	412 ± 6[h]
cis-Nonachlor[d,e,f,g]	425 ± 23
trans-Nonachlor[d,e,f,g]	469 ± 6
Mirex[c,d,e,f,g]	384 ± 59
Octachlorostyrene[c,e,f]	359 ± 19
2,4'-DDE[b,c,e]	450 ± 20
4,4'-DDE[b,c,e,f]	1250 ± 130
2,4'-DDD[b,c]	347 ± 46
4,4'-DDD[b,c]	416 ± 17
2,4'-DDT[b,c,e]	313 ± 43
4,4'-DDT[b,c,e]	293 ± 12

[a] The certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$ (unless otherwise noted), calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed and the values listed are metrologically traceable to the SI unit of mass fraction in nanogram analyte per kilogram sample in reconstituted serum.
[b] NIST Method 1a, liquid-liquid extraction followed by GC/MS in the EI mode.
[c] NIST Method 2a, focused microwave extraction followed by GC/MS in the EI mode.
[d] NIST Method 2b, focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).
[e] CDC method, GC/HRMS.
[f] Results from interlaboratory study.
[g] NIST Method 1b, liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).
[h] The coverage factor used for *trans*-chlordane was $k = 4$.

Table 3. Certified Mass Fraction Values for PBDE Congeners and for PBB 153[a] in Reconstituted Serum SRM 1958

			Mass Fraction[b] (ng/kg)
PBDE	17	2,2',4-Tribromodiphenyl ether[c,d,e,f]	458 ± 32
PBDE	28	2,4,4'-Tribromodiphenyl ether[e,f,,g]	462 ± 19
PBDE	47	2,2',4,4'-Tetrabromodiphenyl ether[c,d,e,f,g]	651 ± 29
PBDE	66	2,3',4,4'-Tetrabromodiphenyl ether[c,d,e,f,g]	440 ± 41
PBDE	85	2,2',3,4,4'-Pentabromodiphenyl ether[c,d,e,f,g]	475 ± 39
PBDE	99	2,2',4,4',5-Pentabromodiphenyl ether[c,d,e,f,g]	492 ± 15
PBDE	100	2,2',4,4',6-Pentabromodiphenyl ether[c,d,e,f,g]	475 ± 27
PBDE	153	2,2',4,4',5,5'-Hexabromodiphenyl ether[c,d,e,f,g]	455 ± 54
PBDE	154	2,2',4,4',5,6'-Hexabromodiphenyl ether[c,e,g]	441 ± 39
PBDE	183	2,2',3,4,4',5',6-Heptabromodiphenyl ether[c,d,e,f]	453 ± 42
PBDE	206	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether[c,d]	426 ± 4[h]
PBDE	209	Decabromodiphenyl ether[c,d]	417 ± 5
PBB	153	2,2',4,4',5,5'-Hexabromobiphenyl[c,e,f]	421 ± 13

[a] PBDE congeners and PBB 153 are numbered according to IUPAC rules.
[b] The certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$ (unless otherwise noted), calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed and the values listed are metrologically traceable to the SI unit of mass fraction in nanogram analyte per kilogram sample in reconstituted serum.
[c] NIST Method 1b, liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).
[d] NIST Method 2c, focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).
[e] CDC method GC/HRMS
[f] Results from interlaboratory study
[g] NIST Method 2a, focused microwave extraction followed by GC/MS in the NICI mode.
[h] The coverage factor used for PBDE 206 was $k = 4$.

Table 4. Reference Mass Fraction Values for PCB Congeners[a] , PBDE Congeners[a], and Chlorinated Pesticides in Reconstituted Serum SRM 1958

			Mass Fraction[b] (ng/kg)		
PCB	114	2,3,4,4',5-Pentachlorobiphenyl[c,d,e,f,g]	46.6	±	8.4
PCB	123	2',3,4,4',5-Pentachlorobiphenyl[e,g]	52.5	±	4.8
PCBs	153 and 132	2,2',4,4',5,5' Hexachlorobiphenyl and 2,2',3,3',4,6' Hexachlorobiphenyl	490	±	23[h]
PCBs	180 and 193	2,2',3,4, 4',5,5' Heptachlorobiphenyl and 2,3,3', 4',5,5',6 Heptachlorobiphenyl	495	±	1[i]
PBDEs	28 and 31	2,4,4' Tribromodiphenyl ether and 2, 4',5-Tribromodiphenyl ether	484	±	18[j]
		Pentachlorophenol[g,,k]	2780	±	550
		Pentabromophenol[g,,k]	445	±	93
		α-HCH[c,e,g,l]	260	±	44
		β-HCH[c,e,g,l]	278	±	47
		γ-HCH[c,e,g,l]	315	±	43
		Oxychlordane[d,e,f,g,l]	226	±	43

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [5] and later revised by Schulte and Malisch [6] to conform with IUPAC rules. PBDE congeners are numbered according to IUPAC rules.

[b] The reference mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed as determined by the methods indicted and the values listed are metrologically traceable to the SI unit of mass fraction in nanogram analyte per kilogram sample in reconstituted serum.

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

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

[e] CDC method GC/HRMS.

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

[g] Results from interlaboratory study.

[h] Based on data from Method 1b, Method 2a, and the interlaboratory study, a reference mass fraction value for PCB 153 with contribution from PCB 132.

[i] Based on data from Methods 1b and CDC, a reference mass fraction value for PCB 180 with contribution from PCB 193.

[j] Based on data from Method 1b and Method 2b, a reference mass fraction value for PBDE 28 with contribution from PBDE 31.

[k] NIST Method 3, focused microwave extraction followed by LC/MS/MS analysis.

[l] NIST Method 2b, focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).

Table 5. Reference Mass Fraction Values for PFCs in Reconstituted Serum SRM 1958 (Reported as Linear Plus Branched Isomers)

	Mass Fraction[a] (µg/kg)		
Pentadecafluorooctanoic acid (PFOA)[b,c]	4.11	±	0.17
Heptadecafluorononanoic acid (PFNA)[b,c]	0.66	±	0.13
Perfluorohexanesulfonic acid (PFHxS)[b,c]	2.66	±	0.07
Perfluorooctanesulfonic acid (PFOS)[b,c]	16.6	±	0.9

[a] The reference mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed as determined by the methods indicted and the values listed are metrologically traceable to the SI unit of mass fraction in microgram analyte per kilogram sample in reconstituted serum.

[b] NIST Method 4, SPE followed by LC/MS/MS analysis.

[c] CDC method, on-line SPE with LC/MS/MS analysis.

Table 6. Reference Mass Fraction Values for Dibenzo-*p*-dioxin and Dibenzofuran Congeners and Two Non-Ortho PCB Congeners in Reconstituted Serum SRM 1958

	Mass Fraction[(a)] (pg/kg)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	94.2 ± 7.8
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	106 ± 12
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	95.3 ± 9.9
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	340 ± 60
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	99.6 ± 7.2
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	565 ± 90
Octachlorodibenzo-*p*-dioxin	2570 ± 280
2,3,7,8-Tetrachlorodibenzofuran	104 ± 3
1,2,3,7,8-Pentachlorodibenzofuran	98 ± 15
2,3,4,7,8-Pentachlorodibenzofuran	199 ± 30
1,2,3,4,7,8-Hexachlorodibenzofuran	95.9 ± 9.3
1,2,3,6,7,8-Hexachlorodibenzofuran	102 ± 11
1,2,3,7,8,9-Hexachlorodibenzofuran	94 ± 11
2,3,4,6,7,8-Hexachlorodibenzofuran	900 ± 110
1,2,3,4,6,7,8-Heptachlorodibenzofuran	289 ± 9
1,2,3,4,7,8,9-Heptachlorodibenzofuran	84 ± 12
Octachlorodibenzofuran	83.3 ± 1.7
PCB 126 3,4,5,3',4'-Pentachlorobiphenyl	7780 ± 1140
PCB 169 3,4,5,3',4',5'-Hexachlorobiphenyl	8100 ± 330

[(a)] The reference mass fraction value is a weighted mean of the mass fractions determined by three methods (CDC, Axys Analytical, and University of Liege) for the dioxins and furans and two methods (CDC and Univeristy of Liege) for the non-ortho PCB congeners [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed as determined by the methods indicted and the values listed are metrologically traceable to the SI unit of mass fraction in picogram analyte per kilogram sample in reconstituted serum.

Table 7. Reference Mass Fraction Values for Serum Lipids in Reconstituted Serum SRM 1958

	Mass Fraction[(a)] mg/dL
Total Cholesterol (TC)	129 ± 2
Free Cholesterol (FC)	31.3 ± 0.7
Phopholipids (PL)	150 ± 1
Triglycerides (TG)	95.6 ± 5.4

[(a)] The reference mass fraction value is a weighted mean of the mass fractions determined by two methods (CDC and Institut national de santé publique du Québec) [8]. The uncertainty listed with each value is an expanded uncertainty about the mean [8,9], with coverage factor, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guide [11,12]. The measurand is the total mass fraction of the constituent listed as determined by the methods indicted and the values listed are metrologically traceable to the SI unit of mass fraction in milligram analyte per deciliter sample in reconstituted serum.

Information Values

Table 8. Information Mass Fraction Values for Additional Compounds, Aroclor 1260, and Total Lipids in Reconstituted Serum SRM 1958

	Mass Fraction (ng/kg)
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (Parlar 26)[a,b,c]	340
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (Parlar 50)[a,b,c]	230
2,2,5,5,8,9,9,10,10-nonachlorobornane (Parlar 62)[a,c]	320
Pentachloronitrobenzene[d]	480
2,3,4,6-Tetrabromophenol[d]	150
2,3,4,6-Tetrachlorophenol[d]	98
Aroclor 1260[d]	5730
	(mg/dL)
Total Lipids[e]	441

[a] CDC method using GC/HRMS.
[b] Results from interlaboratory study.
[c] NIST Method 2c using focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).
[d] Mass fractions determined by Institut national de santé publique du Québec.
[e] The total lipids in the serum may be 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 [13,14].

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Certificate Revision History: 17 December 2013 (Updated data from CDC; revised statistical methods used; editorial changes); 26 August 2010 (Corrected the units in Table 5 to µg/kg); 05 August 2009 (Corrected the substituent location numbers for 1,2,3,4,7,8,9-Heptachlorodibenzofuran in Table 6); 13 May 2009 (Original certificate date).

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 1958

Compound Class	Number of Compounds	Mass Concentration Range of Spike
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