National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material® 1957

### Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried)

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), perflourinated compounds (PFCs), and serum lipid. Information values are provided for selected hydroxylated compounds. All of the constituents for which values are provided in SRM 1957 are naturally present in the freeze-dried human serum. A unit of SRM 1957 consists of five 30 mL vials each containing freeze-dried non-fortified human serum. Before use, the serum in each vial must be reconstituted with 10.7 mL of distilled or HPLC-grade water.

The development of SRM 1957 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 selected PCB congeners, chlorinated pesticides, and PBDE congeners along with one polybrominated biphenyl congener are provided in Tables 1, 2, and 3, respectively. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values for the PCB congeners, chlorinated pesticides, and PBDE congeners are based on the agreement of results obtained at NIST using one or more analytical techniques and additional results from the CDC and from an interlaboratory study using different analytical techniques. Values are reported based on the reconstituted serum in mass fraction units [2].

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

**Information Mass Fraction Values:** Information values for mass fractions of selected hydroxylated compounds and Aroclor 1260 are provided in Table 8. An information value is considered to be a value that will be of interest 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 [2]. Information values cannot be used to assess metrological traceability.

**Expiration of Certification:** The certification of **SRM 1957** is valid, within the measurement uncertainty specified, until **31 August 2025**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for 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.

Carlos A. Gonzalez, Chief
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Certificate Issue Date: 26 January 2016
*Certificate Revision History on Last Page*

Steven J. Choquette, Acting Director
Office of Reference Materials

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

Partial support for the development of SRM 1957 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 CDC Organic Analytical Toxicology Branch. Laboratories participating in an interlaboratory study included 3M Corporation (Maplewood, MN), Environment Canada (Toronto, ON, Canada), U.S. Environmental Protection Agency (Research Triangle Park, NC), Institut national de santé publique du Québec (Montréal, QC, Canada), Stockholm University (Stockholm, Sweden), University of Liege (Liege, Belgium), and University of Toronto (Toronto, ON, Canada).

Statistical consultation was provided by S.D. Leigh and A.L. Pintar 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 1957 IS INTENDED FOR RESEARCH USE. 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. Using a calibrated automatic pipette, 10.7 mL aliquots of serum were dispensed into 30 mL amber glass vials. The samples were lyophilized and were considered dry when a stable vacuum and temperature were achieved.

[(1)] Certain commercial equipment, instruments, or materials are identified in this report to specify adequately 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.

**ANALTYICAL 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 vial, 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 *C*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 vial, 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 bottles 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 vial, 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 C18, 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 bottles 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 vial, 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 Mass Concentration 2 µg/mL)*, SRM 2262 *Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane (Nominal Mass 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.

**ANALTYICAL 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 C18 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 bottles 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 vial 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 C18 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 C8, 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 SRM 1957

| | | | Mass Fraction[b] (ng/kg) |
|---|---|---|---|
| PCB | 118 | 2,3',4,4',5-Pentachlorobiphenyl[c,d,e] | 18.9 ± 1.2 |
| PCB | 138 | 2,2',3,4,4',5'-Hexachlorobiphenyl[e,f] | 36.9 ± 9.0 |
| PCB | 153 | 2,2',4,4',5,5'-Hexachlorobiphenyl[e,f] | 58.2 ± 0.9 |
| PCB | 170 | 2,2',3,3',4,4',5-Heptachlorobiphenyl[d,e,f] | 16.2 ± 2.0 |
| PCBs | 180 and 193 | 2,2',3,4,4',5,5'-Heptachlorobiphenyl[e,f] and 2,3,3',4',5,5',6-Heptachlorobiphenyl | 54.5 ± 0.5[g] |
| PCB | 187 | 2,2',3,4',5,5'6-Heptachlorobiphenyl[d,e,f] | 15.5 ± 0.5 |
| PCB | 194 | 2,2',3,3',4,4',5,5'-Octachlorobiphenyl[d,e,f] | 11.9 ± 0.3 |

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [6] and later revised by Schulte and Malisch [7] to conform with IUPAC rules.
[b] The certified mass fraction value, unless otherwise noted, is a weighted mean of the mass fractions 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 Guides [11,12]. The measurand is the total mass fraction of the constituent listed. Metrological traceability is to the SI derived unit for mass fraction (expressed as nanogram analyte per kilogram sample in reconstituted serum).
[c] NIST Method 1a, liquid-liquid extraction followed by GC/MS in the EI mode.
[d] CDC method, GC/HRMS.
[e] Results from interlaboratory study.
[f] NIST Method 1b, liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).
[g] PCBs 180 and 193 coelute under the GC analysis conditions used; PCB 180 is the major component and PCB 193 may be present as a minor component. The quantitative results are based on the response of the congener listed first. See Table 4 for reference value of PCB 180 without contribution from PCB 193. The coverage factor used for PCBs 180 and 193 was $k = 4$.

Table 2. Certified Mass Fraction Values for Chlorinated Pesticides in Reconstituted SRM 1957

| | Mass Fraction[a] (ng/kg) |
|---|---|
| Hexachlorobenzene[b,c,d,e,f] | 29.7 ± 3.5 |
| *trans*-Nonachlor[d,e,f,g] | 58.3 ± 1.9 |
| 4,4'-DDE[b,c,e,f] | 921 ± 76 |

[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, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guides [11,12]. The measurand is the total mass fraction of the constituent listed. Metrological traceability is to the SI derived unit for mass fraction (expressed as 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 using 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).

Table 3. Certified Mass Fraction Values for PBDE Congeners and for PBB 153[a] in Reconstituted SRM 1957

| | | | Mass Fraction[b] (ng/kg) | | |
|---|---|---|---|---|---|
| PBDE | 47 | 2,2',4,4'-Tetrabromodiphenyl ether[c,d,e,f,g] | 268 | ± | 14 |
| PBDE | 99 | 2,2',4,4',5-Pentabromodiphenyl ether[c,d,e,f,g] | 76.0 | ± | 3.8 |
| PBDE | 100 | 2,2',4,4',6-Pentabromodiphenyl ether[c,d,e,f,g] | 49.7 | ± | 2.7 |
| PBDE | 153 | 2,2',4,4',5,5'-Hexabromodiphenyl ether[c,d,e,f,g] | 61.0 | ± | 3.2 |
| PBB | 153 | 2,2',4,4',5,5'-Hexabromobiphenyl[c,f,g] | 15.5 | ± | 0.1 |

[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, $k = 2$, calculated by combining a pooled within-method variance with a between-method variance [10] following the ISO/JCGM Guides [11,12]. The measurand is the total mass fraction of the constituent listed. Metrological traceability is to the SI derived unit for mass fraction (expressed as 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 2a, focused microwave extraction followed by GC/MS in the EI mode.

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

[f] CDC method, GC/HRMS

[g] Results from interlaboratory study

Table 4. Reference Mass Fraction Values for PCB Congeners[a], Chlorinated Pesticides, and PBDE Congeners[a] in Reconstituted SRM 1957

| | | | Mass Fraction (ng/kg) | | |
|---|---|---|---|---|---|
| PCB | 18 | 2,2',5-Trichlorobiphenyl[b] | 4.5 | ± | 0.8[c] |
| PCB | 28 | 2,4,4'-Trichlorobiphenyl[b] | 8.6 | ± | 1.1[c] |
| PCB | 66 | 2,3',4,4'-Tetrachlorobiphenyl[b] | 5.6 | ± | 1.7[c] |
| PCB | 74 | 2,4,4',5-Tetrachlorobiphenyl[b,d] | 13.8 | ± | 0.1[e] |
| PCB | 99 | 2,2',4,4',5-Pentachlorobiphenyl[b,d] | 11.6 | ± | 0.6[e] |
| PCB | 146 | 2,2',3,4',5,5'-Hexachlorobiphenyl[b] | 7.17 | ± | 0.26[c] |
| PCB | 156 | 2,3,3',4,4',5-Hexachlorobipheny)[b,d] | 8.24 | ± | 0.57[e] |
| PCB | 178 | 2,2',3,3',5,5',6-Heptachlorobiphenyl[b] | 3.61 | ± | 0.32[c] |
| PCB | 183 | 2,2',3,4,4',5',6-Heptachlorobiphenyl[b] | 5.77 | ± | 0.35[c] |
| PCBs | 196 and 203 | 2,2',3,3',4,4',5,6'-Octachlorobiphenyl[b] and 2,2',3,4,4',5,5',6-Octachlorobiphenyl | 10.9 | ± | 0.7[c,h] |
| PCB | 199 | 2,2',3,3',4,5,5',6'-Octachlorobiphenyl[b] | 11.2 | ± | 0.8[c] |
| PCB | 206 | 2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl[b] | 6.98 | ± | 0.37[c] |
| PCB | 209 | Decachlorobiphenyl[b] | 3.33 | ± | 0.59[c] |
| | | Pentachlorophenol[d,f] | 2710 | ± | 880[e] |
| | | β-HCH[b,d,g] | 31.3 | ± | 6.0[e] |
| PBDE | 17 | 2,2',4-Tribromodiphenyl ether[b,d] | 4.2 | ± | 1.5[e] |
| PBDE | 28 | 2,4,4'-Tribromodiphenyl ether[b,d] | 20.0 | ± | 2.4[e] |
| PDBEs | 28 and 33 | 2,4,4'-Tribromodiphenyl ether and 2',3,4-Tribromodiphenyl ether | 25.1 | ± | 0.1[e,i] |
| PBDE | 66 | 2,3',4,4'-Tetrabromodiphenyl ether[b,d] | 6.70 | ± | 0.13[e] |
| PBDE | 85 | 2,2',3,4,4'-Pentabromodiphenyl ether[b,d] | 8.2 | ± | 1.9[e] |
| PBDE | 154 | 2,2',4,4',5,6'-Hexabromodiphenyl ether[b] | 7.0 | ± | 1.0[e] |

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [6] and later revised by Schulte and Malisch [7] to conform with IUPAC rules. PBDE congeners are numbered according to IUPAC rules.

[b] CDC method, GC/HRMS

[c] The reference values are the means of the mass fraction results using one analytical technique. The expanded uncertainty, $U$, is calculated as $U = ku_c$, where $u_c$ is one standard deviation of the analyte mean, and the coverage factor, $k$, is determined from the Student's $t$-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The measurand is the total mass fraction of the constituent listed as determined by the method indicted. Metrological traceability is to the SI derived unit for mass fraction (expressed as nanogram analyte per kilogram sample in reconstituted serum).

[d] Results from interlaboratory study.

[e] 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 Guides [11,12]. The measurand is the total mass fraction of the constituent listed as determined by the methods indicted. Metrological traceability to the SI derived unit for mass fraction (nanogram analyte per kilogram sample in reconstituted serum).

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

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

[h] PCBs 196 and 203 coelute under the GC analysis conditions used; PCB 196 is the major component and PCB 203 may be present as a minor component. The quantitative results are based on the response of the congener listed first.

[i] PDBEs 28 and 33 coelute under the GC analysis conditions used; PDBE 28 is the major component and PDBE 33 may be present as a minor component. The quantitative results are based on the response of the congener listed first. Based on data from NIST Method 1b and Method 2b, the reference mass fraction value for PBDE 28 with contribution from PBDE 33. The coverage factor used for PBDEs 28 and 33 was $k = 4$.

Table 5. Reference Mass Fraction Values for PFCs in Reconstituted SRM 1957
(Reported as Linear plus Branched Isomers)

| | Mass Fraction[a] (µg/kg) | | |
|---|---|---|---|
| Tridecafluoroheptanoic acid (PFHpA)[b,d] | 0.305 | ± | 0.051 |
| Pentadecafluorooctanoic acid (PFOA)[b,c,d] | 5.00 | ± | 0.44 |
| Heptadecafluorononanoic acid (PFNA)[b,c,d] | 0.878 | ± | 0.077 |
| Nonadecafluordecanoic acid (PFDA)[b,c,d] | 0.39 | ± | 0.12 |
| Perfluoroundecanoic acid (PFUnA)[b,c,d] | 0.172 | ± | 0.036 |
| Perfluorohexanesulfonic acid (PFHxS)[b,c,d] | 4.00 | ± | 0.83 |
| Perfluorooctanesulfonic acid (PFOS)[b,c,d] | 21.1 | ± | 1.3 |

[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 Guides [11,12]. The measurands are the mass fractions of selected PFC congeners in the reconstituted serum as determined by the methods indicated. Metrological traceability is to the SI unit for mass fraction (expressed as microgram analyte per kilogram sample in reconstituted serum).
[c] CDC method using LC/MS/MS
[d] Results from interlaboratory study

Table 6. Reference Mass Fraction Values for Dibenzo-*p*-Dioxin and Dibenzofuran Congeners in Reconstituted SRM 1957

| | Mass Fraction[a] (pg/kg) | | |
|---|---|---|---|
| 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin | 17.3 | ± | 2.9 |
| 1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin | 11.8 | ± | 1.7 |
| 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin | 82 | ± | 18 |
| 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin | 18.7 | ± | 0.3 |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin | 104 | ± | 36 |
| Octachlorodibenzo-*p*-dioxin | 716 | ± | 81 |
| 2,3,4,7,8-Pentachlorodibenzofuran | 16.4 | ± | 2.5 |
| 1,2,3,4,7,8-Hexachlorodibenzofuran | 17.0 | ± | 3.1 |
| 1,2,3,6,7,8-Hexachlorodibenzofuran | 14.1 | ± | 2.5 |
| 1,2,3,4,6,7,8-Heptachlorodibenzofuran | 39.8 | ± | 6.2 |

[a] The reference mass fraction value is a weighted mean of the mass fractions determined by two methods (CDC and University of Liege) [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 Guides [11,12]. The measurands are the mass fractions of selected congeners in the reconstituted serum as determined by the CDC methods as described in the text. Metrological traceability to the SI derived unit for mass fraction (expressed as picogram analyte per kilogram sample in reconstituted serum).

Table 7. Reference Mass Fraction Values for Serum Lipids in reconstituted serum SRM 1957[a]

| | Mass Fraction[b] (mg/dL) |
|---|---|
| Total Cholesterol (TC) | 153 ± 5 |
| Free Cholesterol (FC) | 34.4 ± 0.5 |
| Phospholipids (PL) | 181 ± 3 |
| Triglycerides (TG) | 119 ± 2 |

[a] The total lipids in the serum may be 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 elsewhere [13,14]. Based on this calculation, the mass fraction of total lipids in SRM 1957 is 533 mg/dL.

[b] 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 Guides [11,12]. The measurand is the mass fraction of the selected free fatty acid in reconstituted serum as determined by the methods indicated. Metrological traceability to the SI derived unit for mass fraction (expressed as milligram analyte per deciliter sample in reconstituted serum).

Table 8. Information Mass Fraction Values for PCB 180, Hydroxylated Compounds, and for Aroclor 1260 in reconstituted serum SRM 1957

| | Mass Fraction (ng/kg) |
|---|---|
| 2,2',3,4,4',5,5' Heptachlorobiphenyl[a] | 46 |
| 2,3,4,6-Tetrabromophenol[b] | 120 |
| 2,3,4,6-Tetrachlorophenol[b] | 99 |
| Aroclor 1260 | 540 |

[a] CDC method, GC/HRMS

[b] Mass fractions determined by Institut national de santé publique du Québec.

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