

Standard Reference Material[®] 1958
Organic Contaminants in Fortified Human Serum
(Freeze-Dried)

CERTIFICATE OF ANALYSIS

Purpose: 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. Non-certified values are provided for selected polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-ortho PCB congeners, per- and polyfluoroalkyl substances (PFAS), and serum lipid.

Description: 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.

Non-Certified Values: Non-certified values are provided in the Appendix A.

Additional Information: Values of selected toxaphene congeners and hydroxylated compounds provided in Appendix B. Additional information provided in Appendix C.

Period of Validity: The certified values delivered by **SRM 1958** are valid within the measurement uncertainty specified until **31 August 2025**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

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 ± 6 ^(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 [3] and later revised by Schulte and Malisch [4] 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 [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between method variance [7] following the ISO/JCGM Guides [8,9]. The measurand is the total mass fraction for each PCB congener in reconstituted serum listed in Table 1. Metrological traceable to the International System of Units (SI) derived unit for mass fraction (expressed as nanograms analyte per kilogram sample).

^(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 |
| <i>cis</i> -Chlordane ^(d,e,f,g) | 412 ± 25 |
| <i>trans</i> -Chlordane ^(d,e,g) | 412 ± 6 ^(h) |
| <i>cis</i> -Nonachlor ^(d,e,f,g) | 425 ± 23 |
| <i>trans</i> -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 |
| 2,4'-DDT ^(b,c,e) | 313 ± 43 |

^(a) The certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$ (unless otherwise noted), calculated by combining a pooled within method variance with a between method variance [7] following the ISO/JCGM Guides [8,9]. The measurand is the total mass fraction for each chlorinated pesticide in reconstituted serum listed in Table 2. Metrological traceable to the SI derived unit for mass fraction (expressed as nanograms analyte per kilogram sample).

^(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 [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$ (unless otherwise noted), calculated by combining a pooled within method variance with a between method variance [7] following the ISO/JCGM Guides [8,9]. The measurand is the total mass fraction for each PBDE congeners and for PBB 153 in reconstituted serum listed in Table 3. Metrological traceable to the SI derived unit for mass fraction (expressed as nanograms analyte per kilogram sample).

^(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 $k = 4$.

Safety: SRM 1958 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 [10].

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.

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Certificate Revision History: 24 May 2023 (Certified values for analytes 4,4'-DDD, and 4,4'-DDT from Table 2 and the non-certified value for oxychlorodane from Table A1 were removed due to instability; updated format; editorial changes); 11 October 2018 (Changes in values of PCB 180/193, PCB 196, and Phospholipids; editorial changes); 30 November 2015 (Editorial changes); 29 December 2014 (Change of expiration date; editorial changes); 17 December 2013 (Updated data from CDC; revised statistical methods used; editorial changes); 26 August 2010 (Corrected the units in Table 5 to $\mu\text{g}/\text{kg}$); 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).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

* * * * * End of Certificate of Analysis * * * * *

APPENDIX A

Non-Certified Values: Non-certified values, expressed as mass fractions, are provided in Table A1 for additional PCB congeners and chlorinated pesticides; Table A2 for selected PFAS; and Table A3 for selected PCDD and PCDF congeners and non-ortho PCB congeners [1]. Non-certified values for serum lipids are provided in Table A4. Non-certified values are 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.

Table A1. Non-Certified 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 ± 11 ⁽ⁱ⁾ |
| PBDEs | 28 and 31 | 2,4,4'-Tribromodiphenyl ether and 2, 4',5-Tribromodiphenyl ether | 484 ± 18 ^(j) |
| | | Pentachloropheno ^(g,k) | 2780 ± 550 |
| | | Pentabromopheno ^(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 |

^(a) PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [3] and later revised by Schulte and Malisch [4] to conform with IUPAC rules. PBDE congeners are numbered according to IUPAC rules.

^(b) The non-certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between- method- variance [7] following the ISO/JCGM Guides [8,9].

^(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 non-certified mass fraction value for PCB 153 with contribution from PCB 132.

⁽ⁱ⁾ Based on data from Methods 1b and CDC, a non-certified mass fraction value for PCB 180 with contribution from PCB 193.

^(j) Based on data from Method 1b and Method 2b, a non-certified 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 A2. Non-Certified Mass Fraction Values for PFAS 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 non-certified mass fraction value is a weighted mean of the mass fractions determined by the methods indicated for each analyte [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between- method- variance [7] following the ISO/JCGM Guides [8,9].

^(b) NIST Method 4, SPE followed by LC/MS/MS analysis.

^(c) CDC method, on-line SPE with LC/MS/MS analysis.

Table A3. Non-Certified 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- <i>p</i> -dioxin | 94.2 ± 7.8 |
| 1,2,3,7,8-Pentachlorodibenzo- <i>p</i> -dioxin | 106 ± 12 |
| 1,2,3,4,7,8-Hexachlorodibenzo- <i>p</i> -dioxin | 95.3 ± 9.9 |
| 1,2,3,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin | 340 ± 60 |
| 1,2,3,7,8,9-Hexachlorodibenzo- <i>p</i> -dioxin | 99.6 ± 7.2 |
| 1,2,3,4,6,7,8-Heptachlorodibenzo- <i>p</i> -dioxin | 565 ± 90 |
| Octachlorodibenzo- <i>p</i> -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 non-certified 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 Université de Liège) for the nonortho- PCB congeners [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between- method- variance [7] following the ISO/JCGM Guides [8,9].

Table A4. Non-Certified 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 |
| Phospholipids (PL) | 150.0 ± 0.2 |
| Triglycerides (TG) | 95.6 ± 5.4 |

^(a) The non-certified mass fraction value is a weighted mean of the mass fractions determined by two methods (CDC and Institut national de santé publique du Québec) [5]. The uncertainty listed with each value is an expanded uncertainty about the mean [5,6], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between- method- variance [7] following the ISO/JCGM Guides [8,9].

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **31 August 2025**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

***** End of Appendix A *****

APPENDIX B

Table B1. Mass Fraction Values of Potential Interest 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: $TL = 1.677 \times (TC - FC) + FC + TG + PL$, where TL is total lipids, TC is total cholesterol, FC is free cholesterol, TG is triglycerides, and PL is phospholipids. The constant 1.677 is based on fatty acid analysis of serum cholesterol esters and has been described elsewhere [11,12].

***** End of Appendix B *****

APPENDIX C

Table C1. Details of Spiking Solution Used for SRM 1958

| Compound Class | Number of Compounds | Mass Concentration Range of Spike |
|---|---------------------|-----------------------------------|
| <i>ortho</i> PCBs | 38 | 50 pg/mL to 500 pg/mL |
| non- <i>ortho</i> 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, 1,2bis (2,4,6tribromophenoxy) 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 |

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 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 (DB17MS, Agilent Technologies) was used for the NICI analysis (NIST Method 1b). All injections were 1 μ L 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 *isooctane*, 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 *isooctane* for analysis. The concentrated samples were analyzed using GC/MS in the EI mode (NIST Method 2a) with a 0.18 mm \times 30 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane- phase (DB5MS, Agilent Technologies) 0.18 μ m film thickness. All injections were 20 μ L 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 \times 10 m fused silica capillary column containing a 5 % (mole fraction) phenyl-substituted methylpolysiloxane- phase (DB-5MS, Agilent Technologies), 0.18 μ m 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 \times 150 mm \times 3.5 μ m, 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 PFAS) 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 \times 2.1 mm \times 5 μ m, Thermo Fisher Scientific, Waltham, MA) and methanol-ammonium acetate in water gradient.

For all of the NIST methods, multipoint 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 μ g/mL)*, SRM 2262 *Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane (Nominal Concentration 2 μ g/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 PFAS, details for the analytical methods used at CDC can be found in Patterson and Turner [10] and Sjödin et al. [13]. 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 $^{\circ}\text{C}$. 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 ¹³C-labeled compounds were used as internal standards for most 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 (DB5MS, J&W Scientific, Folsom, CA), 0.25 µm film thickness. All injections were splitless with helium as the carrier gas.

For determination of the PFAS, 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 ¹³C-labeled PFAS 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 online SPEHPLC 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 C₈ 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 non-certified mass fraction values.

Total Cholesterol and Associated Analytes: The non-certified 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.

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* * * * * End of Appendix C * * * * *