

Standard Reference Material[®] 1954

Organic Contaminants in Fortified Human Milk

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for evaluating analytical methods for the determination of selected polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and polybrominated diphenyl ether (PBDE) congeners in human milk and similar matrices.

Description: A unit of SRM 1954 consists of five bottles of approximately 5 mL fortified human milk. The compounds in Appendix C Table C1 were spiked into the milk prior to bottling.

Certified Values: Certified values for concentrations, expressed as mass fractions, for PCB congeners, chlorinated pesticides, and PBDE congeners along with 1 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.

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

Additional Information: A value of potential interest to users is provided in Appendix B. Additional information is provided in Appendix C.

Period of Validity: The certified values delivered by **SRM 1954** are valid within the measurement uncertainty specified until **31 May 2040**. 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>). 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>).

Safety: This is a human source material. SRM 1954 is a Biosafety Level 2 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization. The milk was pasteurized prior to preparation of SRM 1954. However, no known test method can offer complete assurance that infectious agents are absent from this material.

Storage: The milk is frozen and should be stored in a freezer at -20°C until use. It should not be exposed to sunlight or ultraviolet radiation. After thawing, the contents should be used immediately.

Use: SRM 1954 IS INTENDED FOR RESEARCH USE. Bring the vial to room temperature. Once the milk is thawed, mix contents by gently swirling. Do not shake vigorously because this will cause frothing. The recommended minimum sample size is 0.25 g.

Table 1. Certified Concentration Values for Selected PCB Congeners^(a) in SRM 1954

			Mass Fraction (ng/kg)
PCB	18	(2,2',5-Trichlorobiphenyl) ^(b,c)	355 ± 45 ^(d)
PCB	28	(2,4,4'-Trichlorobiphenyl) ^(b,e,f)	519 ± 24 ^(d)
PCB	44	(2,2',3,5'-Tetrachlorobiphenyl) ^(b,c,e)	416 ± 35 ^(d)
PCB	49	(2,2',4,5'-Tetrachlorobiphenyl) ^(b,c,e)	429 ± 15 ^(d)
PCB	52	(2,2',5,5'-Tetrachlorobiphenyl) ^(b,c,e,f)	425 ± 40 ^(g)
PCB	66	(2,3',4,4'-Tetrachlorobiphenyl) ^(b,c,e)	428 ± 40 ^(d)
PCB	74	(2,4,4',5-Tetrachlorobiphenyl) ^(b,c,e)	563 ± 19 ^(d)
PCB	87	(2,2',3,4,5'-Pentachlorobiphenyl) ^(b,c,e)	457 ± 57 ^(d)
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl) ^(b,c,e,f,h)	558 ± 38 ^(d)
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl) ^(b,c,e,f,h)	442 ± 26 ^(g)
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl) ^(b,c,e,f,h)	482 ± 31 ^(d)
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl) ^(b,c,e,h)	458 ± 34 ^(g)
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl) ^(b,e,f,h)	597 ± 35 ^(g)
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl) ^(b,c,e,f,h)	400 ± 26 ^(g)
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl) ^(b,c,f)	639 ± 34 ^(d)
PCB	146	(2,2',3,4',5,5'-Hexachlorobiphenyl) ^(b,c,e,h)	492 ± 30 ^(g)
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl) ^(b,c,e,h)	408 ± 12 ^(d)
PCB	151	(2,3,3',4,4'-Hexachlorobiphenyl) ^(b,c,e,h)	417 ± 22 ^(d)
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl) ^(b,e,f)	977 ± 49 ^(d)
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl) ^(b,c,e,f,h)	511 ± 54 ^(d)
PCB	157	(2,3,3',4,4',5'-Hexachlorobiphenyl) ^(b,c,e,f,h)	467 ± 33 ^(g)
PCB	158	(2,3,3',4,4',5'-Hexachlorobiphenyl) ^(b,c,h)	402 ± 20 ^(d)
PCB	167	(2,3',4,4',5,5'-Hexachlorobiphenyl) ^(b,c,e,f,h)	467 ± 53 ^(g)
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl) ^(b,c,e,f,h)	507 ± 63 ^(g)
PCB	172	(2,2',3,3',4,5,5'-Heptachlorobiphenyl) ^(b,c,e,h)	443 ± 29 ^(g)
PCB	177	(2,2',3,3',4',5,6-Heptachlorobiphenyl) ^(b,c,e,h)	447 ± 27 ^(d)
PCB	178	(2,2',3,3',5,5',6-Heptachlorobiphenyl) ^(b,c,e,h)	449 ± 12 ^(g)
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl) ^(b,e,f)	696 ± 71 ^(d)
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl) ^(b,c,e,f,h)	445 ± 19 ^(g)
PCB	187	(2,2',3,4',5,5',6-Heptachlorobiphenyl) ^(b,c,e,f,h)	517 ± 25 ^(g)
PCB	189	(2,3,3',4,4',5,5'-Heptachlorobiphenyl) ^(b,c,e,f,h)	432 ± 35 ^(g)
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl) ^(c,e,h)	482 ± 28 ^(d)
PCB	195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl) ^(c,e,h)	461 ± 40 ^(d)
PCB	196	(2,2',3,3',4,4',5,6'-Octachlorobiphenyl) ^(c,e)	872 ± 61 ^(d)
	203	(2,2',3,4,4',5,5',6-Octachlorobiphenyl)	
PCB	199	(2,2',3,3',4,5,5',6'-Octachlorobiphenyl) ^(c,e)	469 ± 35 ^(d)
PCB	206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl) ^(c,e,h)	465 ± 30 ^(d)
PCB	209	(Decachlorobiphenyl) ^(c,e,h)	436 ± 36 ^(d)

^(a) PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [2] and later revised by Schulte and Malisch [3] to conform with IUPAC rules. For the specific congeners mentioned in this table, PCB 199 is PCB 201 under the Ballschmiter and Zell scheme. When two congeners are known to coelute under the GC analysis conditions used, the PCB congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

^(b) NIST Method 1a using liquid-liquid extraction followed by GC/MS in the EI mode.

^(c) NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

^(d) Certified values are unweighted means of the results from two to five analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected

PCB congeners. The certified values are metrologically traceable to the International System of Units (SI) unit for mass, expressed as nanograms per kilogram.

(e) CDC method using GC/HRMS.

(f) Results from interlaboratory study.

(g) Certified values are weighted means of the results from four or five analytical methods [6]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected PCB congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

(h) NIST Method 1b using liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).

Table 2. Certified Concentration Values for Selected Chlorinated Pesticides in SRM 1954

	Mass Fraction (ng/kg)
Hexachlorobenzene ^(a,b,c,d)	672 ± 41 ^(e)
β-HCH ^(a,b,c,d)	829 ± 38 ^(f)
γ-HCH ^(a,c)	588 ± 34 ^(f)
Oxychlordane ^(b,c,d,g)	1 050 ± 130 ^(e)
<i>cis</i> -Chlordane ^(d,g)	368 ± 9 ^(f)
<i>trans</i> -Chlordane ^(d,g)	377 ± 9 ^(f)
<i>cis</i> -Nonachlor ^(d,g)	496 ± 11 ^(f)
<i>trans</i> -Nonachlor ^(c,d,g)	1 700 ± 140 ^(f)
Mirex ^(b,c,d,g)	515 ± 23 ^(f)
2,4'-DDE ^(a,b)	400 ± 14 ^(f)
4,4'-DDE ^(a,b,c,d)	8 120 ± 350 ^(e)
2,4'-DDD ^(a,b)	427 ± 16 ^(f)
4,4'-DDD ^(a,d)	425 ± 15 ^(f)
2,4'-DDT ^(a,c)	444 ± 21 ^(f)
4,4'-DDT ^(a,b,c)	703 ± 74 ^(f)

(a) NIST Method 1a using liquid-liquid extraction followed by GC/MS in the EI mode.

(b) NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

(c) CDC method using GC/HRMS.

(d) Results from interlaboratory study.

(e) Certified values are weighted means of the results from four or five analytical methods [6]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected chlorinated pesticides. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

(f) Certified values are unweighted means of the results from two or three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected chlorinated pesticides. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

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

Table 3. Certified Concentration Values for Selected PBDE Congeners and for PBB 153^(a) in SRM 1954

			Mass Fraction (ng/kg)	
PBDE	17	(2,2',4-Tribromodiphenyl ether) ^(b,c,d,e)	401	± 44 ^(f)
PBDE	28	(2,4,4'-Tribromodiphenyl ether) ^(b,c)	579	± 21 ^(g)
	33	(2',3,4-Tribromodiphenyl ether)		
PBDE	47	(2,2',4,4'-Tetrabromodiphenyl ether) ^(b,d,e,h)	2 570	± 190 ^(g)
PBDE	66	(2,3',4,4'-Tetrabromodiphenyl ether) ^(b,c,d,e,h)	411	± 24 ^(g)
PBDE	85	(2,2',3,4,4'-Pentabromodiphenyl ether) ^(b,c,d,e,h)	473	± 7 ^(f)
PBDE	99	(2,2',4,4',5-Pentabromodiphenyl ether) ^(b,c,d,e,h)	739	± 41 ^(f)
PBDE	100	(2,2',4,4',6-Pentabromodiphenyl ether) ^(b,c,d,e,h)	1 280	± 90 ^(g)
PBDE	153	(2,2',4,4',5,5'-Hexabromodiphenyl ether) ^(b,c,d,e,h)	1 440	± 90 ^(f)
PBDE	154	(2,2',4,4',5,6'-Hexabromodiphenyl ether) ^(b,d,h)	464	± 30 ^(g)
PBDE	183	(2,2',3,4,4',5',6-Heptabromodiphenyl ether) ^(b,c,d,e)	511	± 34 ^(f)
PBDE	209	(Decabromodiphenyl ether) ^(b,c,e)	423	± 24 ^(g)
PBB	153	(2,2',4,4',5,5'-Hexabromobiphenyl) ^(b,e)	474	± 25 ^(g)

^(a) PBDE congeners and PBB 153 are numbered according to IUPAC rules. When two congeners are known to coelute under the GC analysis conditions used, the PBDE congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

^(b) NIST Method 1b using liquid-liquid extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 1a).

^(c) NIST Method 2b using focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).

^(d) CDC method using GC/HRMS

^(e) Results from interlaboratory study

^(f) Certified values are weighted means of the results from four or five analytical methods [6]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled within-source variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected PBDE congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

^(g) Certified values are the unweighted means of the results from two to five analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [5]. The measurands are the total mass fractions of selected PBDE congeners and for PBB 153. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

^(h) NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

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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: A NIST non-certified value does not meet the NIST criteria for certification and is provided with an associated uncertainty that may not include all sources of uncertainty [1]. Non-certified concentration values, expressed as mass fractions, are provided in Table A1 for additional PCB, chlorinated pesticides, and PBDE congeners; in Table A2 for elements; and in Table A3 for PCDD and PCDF congeners.

Table A1. Non-Certified Concentration Values for Selected PCB Congeners^(a), Chlorinated Pesticides, and PBDE Congeners in SRM 1954

			Mass Fraction (ng/kg)	
PCB	77	(3,3',4,4'-Tetrchlorobiphenyl) ^(b)	2.71	± 0.14 ^(c)
PCB	81	(3,4,4',5-Tetrchlorobiphenyl) ^(b)	0.630	± 0.028 ^(c)
PCB	114	(2,3,4,4',5-Pentachlorobiphenyl) ^(d)	90.5	± 7.4 ^(c)
PCB	123	(2',3,4,4',5-Pentachlorobiphenyl) ^(d)	67.9	± 3.1 ^(c)
PCB	126	(3,3',4,4',5-Pentachlorobiphenyl) ^(b,d)	10.4	± 1.5 ^(c)
PCB	169	(3,3',4,4',5,5'-Hexachlorobiphenyl) ^(b,d)	9.3	± 1.2 ^(c)
Pentachlorobenzene ^(f)			362	± 24 ^(c)
Octachlorostyrene ^(f)			374	± 28 ^(c)
PBDE	203	(2,2',3,4,4',5,5',6-Octabromodiphenyl ether) ^(g)	494	± 44 ^(c)
PBDE	206	(2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether) ^(d,g)	816	± 170 ^(c)

^(a) PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [2] and later revised by Schulte and Malisch [3] to conform with IUPAC rules.

^(b) CDC method using GC/HRMS.

^(c) Non-certified values are the means of 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 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method.

^(d) Results from interlaboratory study.

^(e) Non-certified values are unweighted means of the results from two analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [5].

^(f) NIST Method 2a using focused microwave extraction followed by GC/MS in the EI mode.

^(g) NIST Method 2b using focused microwave extraction followed by GC/MS in the NICI mode (same extracts as NIST Method 2a).

Table A2. Non-Certified Concentration Values for Selected Elements in SRM 1954

	Mass Fraction (mg/kg) ^(a)	
Calcium ^(b)	257	± 2
Copper ^(b)	0.268	± 0.003
Iron ^(b)	0.194	± 0.007
Magnesium ^(b)	32.4	± 0.2
Manganese ^(b)	0.040	± 0.002
Phosphorus ^(b)	135	± 2
Potassium ^(b)	462	± 7
Sodium ^(b)	127	± 3
	Mass Fraction (µg/kg) ^(a)	
Mercury ^(c)	0.101	± 0.033

^(a) The non-certified values are the means of results obtained using one analytical technique. The expanded uncertainty, U , is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k , is determined from the Student's t -distribution corresponding to the associated degrees of freedom and 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method.

^(b) Results from ICP-OES measurements performed at NIST.

^(c) Results from isotope dilution cold vapor inductively coupled plasma mass spectrometry measurements performed at NIST.

Table A3. Non-Certified Concentration Values for Selected Dibenzo-*p*-dioxin and Dibenzofuran Congeners in SRM 1954

	Mass Fraction (pg/kg) ^(a)	
2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin ^(b,c)	162	± 20
1,2,3,7,8-Pentachlorodibenzo- <i>p</i> -dioxin ^(b,c)	240	± 17
1,2,3,4,7,8-Hexachlorodibenzo- <i>p</i> -dioxin ^(b,c)	182	± 16
1,2,3,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin ^(b,c)	890	± 140
1,2,3,7,8,9-Hexachlorodibenzo- <i>p</i> -dioxin ^(b,c)	207	± 20
1,2,3,4,6,7,8-Heptachlorodibenzo- <i>p</i> -dioxin ^(b,c)	1 080	± 240
Octachlorodibenzo- <i>p</i> -dioxin ^(b,c)	4 890	± 850
2,3,7,8-Tetachlorodibenzofuran ^(b,c)	125	± 10
1,2,3,7,8-Pentachlorodibenzofuran ^(b,c)	132	± 18
2,3,4,7,8-Pentachlorodibenzofuran ^(b,c)	347	± 25
1,2,3,4,7,8-Hexachlorodibenzofuran ^(b,c)	171	± 15
1,2,3,6,7,8-Hexachlorodibenzofuran ^(b,c)	186	± 17
1,2,3,7,8,9-Hexachlorodibenzofuran ^(b,c)	129	± 15
2,3,4,6,7,8-Hexachlorodibenzofuran ^(b,c)	1 090	± 100
1,2,3,4,6,7,8-Heptachlorodibenzofuran ^(b,c)	407	± 45
1,2,3,4,7,8,9-Heptachlorodibenzofuran ^(b,c)	160	± 100
Octachlorodibenzofuran ^(b)	94.3	± 12.7

^(a) Non-certified values are unweighted means of the results from two analytical methods except for octachlorodibenzofuran. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [5]. The non-certified value for octachlorodibenzofuran is the mean of 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 95 % confidence level for each analyte. The measurand is the mass fraction as determined by the indicated method.

^(b) CDC method using GC/HRMS.

^(c) Results from interlaboratory study.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **31 May 2040**. 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 Certificate of Analysis. 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

Value of Potential Interest: A NIST value of potential interest to users is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [1]. A value of potential interest to users is provided below.

Mass Fraction Value of Potential Interest for Aroclor 1260 in SRM 1954^(a)

	Mass Fraction (ng/kg)
Aroclor 1260	8 065

^(a) Concentrations determined by Institut national de santé publique du Québec (Québec, Canada).

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

APPENDIX C

Preparation and Analysis of SRM 1954

Table C1. Details of Spiking Solution used for SRM 1954

Compound Class	Number of Compounds	Concentration Range Added
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-tribromophenoxy)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: Milk was acquired from six milk banks located around the U.S: Florida (4 %), North Carolina (6 %), Iowa (6 %), Delaware (7 %), California (12 %), and Texas (65 %).

Preparation of Material: The milk was pooled (approximately 100 L total) and was stored at 4 °C. The pool was split into two for production of SRM 1953 Organic Contaminants in Non-Fortified Human Milk and SRM 1954. Before bottling, approximately 710 mL of methanol containing 169 compounds at varying concentrations (Appendix A) was added to 50 L of milk, which was then stirred for 4 h. Using a calibrated automatic pipetter, 5 mL aliquots of milk were dispensed into 10 mL amber glass vials.

Analytical Methods Used at NIST: For NIST Method 1, the frozen milk in each of ten bottles was thawed. 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, 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:methyl-*tert*-butyl ether for extraction. The samples were vortexed and left to stand for 0.5 h with occasional stirring. After centrifugation to obtain a sharp phase boundary, the upper organic phase was transferred to a concentration vessel. The extraction was repeated two more times with 10 mL of *n*-hexane each time. The combined organic layers were concentrated to approximately 4 mL using an automated evaporation system. Approximately 2 mL of concentrated sulfuric acid was added to the concentration vessel with swirling. Following phase separation, the organic 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 frozen milk in each of six bottles was thawed. A known amount of internal standard solution (containing selected ¹³C-labeled PCB congeners, selected ¹³C-labeled pesticides, ¹³C-labeled PBDE 209, and selected fluorinated PBDE congeners) was added to a 2.5 g milk test portion 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 SRM 1954

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, sulfuric acid-silica column clean-up was used, followed by alumina column (5 % deactivated) clean-up. 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 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 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, SRM 2262, SRM 2274, and SRM 2275 plus gravimetrically prepared solutions of the additional analytes of interest with the internal standards added.

Analytical Methods Used at CDC: Details of the analytical methods used at CDC can be found in Patterson and Turner [7] and Sjödin et al. [8]. In summary, the frozen milk was thawed and mixed. 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 ¹³C-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.

Interlaboratory Study: The three 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 concentrations was used for combination with other data to assign the certified and reference concentration values.

Non-Volatile Extractable Mass Determination: The percent of non-volatile extractable material (or total extractable organics, TEO) was determined gravimetrically for the data sets described above. Non-volatile extractable material is 3.21 % ± 0.33 % (95 % confidence interval for the mean).

Total Mercury: The concentration value for mercury is derived from isotope dilution cold vapor inductively coupled plasma mass spectrometry measurements performed at NIST [9–11].

Other Elements: The concentration values for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Test portions (10 g) were digested in nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used. Results from the ICP-OES measurement were corrected for spike recoveries.

Homogeneity Assessment: The homogeneity of PCBs, pesticides, and PBDEs was assessed at NIST by using Method 1. An analysis of variance did not show inhomogeneity for a 5 g sample. Other analytes were treated as though they were homogeneously distributed in the material although homogeneity was not assessed.

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