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Milk and serum standard reference materials for monitoring organic contaminants in human samples

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Abstract

Four new Standard Reference Materials (SRMs) have been developed to assist in the quality assurance of chemical contaminant measurements required for human biomonitoring studies, SRM 1953 Organic Contaminants in Non-Fortified Human Milk, SRM 1954 Organic Contaminants in Fortified Human Milk, SRM 1957 Organic Contaminants in Non-Fortified Human Serum, and SRM 1958 Organic Contaminants in Fortified Human Serum. These materials were developed as part of a collaboration between the National Institute of Standards and Technology (NIST) and the Centers for Disease Control and Prevention (CDC) with both agencies contributing data used in the certification of mass fraction values for a wide range of organic contaminants including polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybrominated diphenyl ether (PBDE) congeners, and polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) congeners. The certified mass fractions of the organic contaminants in unfortified samples, SRM 1953 and SRM 1957, ranged from 12 ng/kg to 2200 ng/kg with the exception of 4,4′-DDE in SRM 1953 at 7400 ng/kg with expanded uncertainties generally <14 %. This agreement suggests that there were no significant biases existing among the multiple methods used for analysis.

Keywords

Human Serum; Human Milk; SRMs; PCBs; Pesticides PBDEs; PCDFs; PCDDs

Introduction

Data for contaminants in the human population from biomonitoring studies based on analysis of human serum, milk, adipose tissue, urine, and/or other tissues or fluids [1–8, to cite a few] abound in the literature. These representative studies have been done in the U.S.

[1, 2], Canada [3], Belgium [4], Japan [5], Korea [6], Sweden [7], and the Ukraine [8]. The published data are often used by risk assessors to evaluate potential biological effects from the amount of chemicals absorbed into the human body and to identify long-term trends in the population [9]. To compare the data among studies conducted both nationally and internationally, it is important that the accuracy of biomonitoring data be supported by appropriate quality control samples, including the use of reference materials and participation in interlaboratory comparison studies [10]. Risk assessors need to have confidence in the analytical methods that were used to generate the data as well as in the sampling strategy that was used to obtain the samples [10]. As part of the National Health and Nutrition Examination Survey (NHANES) and other biomonitoring programs, the Centers for Disease Control and Prevention (CDC) uses an extensive quality assurance program with as many as 60 % quality control samples included in their analyses [11].

Certified Reference Materials (CRMs), of which Standard Reference Materials (SRMs) are a subset produced by the National Institute of Standards and Technology (NIST), are valuable tools in developing and validating analytical methods to improve quality assurance standards. NIST developed the first serum-matrix SRM for the measurement of organic contaminants in 1985. This material, SRM 1589 Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum [12], was prepared by fortifying human serum with Aroclor 1260 prior to freeze-drying. In 2000 SRM 1589 was replaced with SRM 1589a PCBs, Pesticides and Dioxin/Furans in Human Serum which was prepared using a serum pool collected in 1996 [13] that had been prescreened and found to contain measurable levels of the contaminants of interest. SRM 1589a was not fortified, containing only natural levels of the organic contaminants with mass fractions value assigned for selected poly-chlorinated biphenyl (PCB) congeners, chlorinated pesticides, polychlorinated dibenzo-*p*-dioxin (PCDD) congeners, and polychlorinated dibenzofuran (PCDF) congeners. In 2006 the Certificate of Analysis for SRM 1589a was updated to increase the number of PCB congeners and chlorinated pesticides that were value assigned and also to add mass fraction values for selected polybrominated diphenyl ether (PBDE) congeners [14, 15]. This material found widespread use as a control material for human biomonitoring programs such as the NHANES and is no longer available.

Another matrix of interest in monitoring the exposure of the human population to environmental contaminants is human milk. Human milk is an important exposure route for organic contaminants to the nursing infant [5, 8, 16]. Human milk has approximately 10 times the lipid content compared to human serum; therefore, on a wet-mass basis, serum is not a good surrogate for milk. Some studies, such as Inoue et al. [5] and Hites [16], suggest that similar values of lipophilic contaminants are obtained from serum and breast milk if the measurements are normalized to lipid content. However, the calculation of total lipid varies among laboratories with gravimetric lipid determinations being the traditional method of choice, although some laboratories now use enzymatic analysis of lipid species in serum followed by summation [17]. The differences in lipid content and methods of lipid determinations suggest that quality control samples should match the matrix of interest.

NIST and the CDC Division of Laboratory Sciences have collaborated to develop four new SRMs to meet the expanding needs for the measurement of organic contaminants in human

serum and milk. The timing was based not only on the impending depletion of the supply of SRM 1589a but also on the growing need for an SRM representative of more contemporary serum contaminant levels. The goals of the NIST/CDC collaboration were to produce human serum and milk SRMs at current contaminant levels and at elevated levels and to produce a sufficient supply to be available for 10 years. To accomplish these goals, collections of serum (total of 200 L) and milk (total of 100 L) were obtained from blood banks and milk banks, respectively, located around the U.S., combined, and mixed. For each matrix (serum and milk), one SRM is a natural level (SRM 1953 Organic Contaminants in Non-Fortified Human Milk [18] and SRM 1957 Organic Contaminants in Non-Fortified Human Serum [19]), and the other SRM (SRM 1954 Organic Contaminants in Fortified Human Milk [20] and SRM 1958 Organic Contaminants in Fortified Serum [21]) is from the same serum or milk pool to which 169 organic contaminants have been added (See Table S1). The fortified serum and milk SRMs were prepared to represent contaminant concentrations that are approximately ten times higher than the median concentrations found in the U.S. population during the 2003 NHANES study [2].

The preparation and characterization of these four SRMs for PCBs, chlorinated pesticides, PBDEs, PCDDs, and PCDFs are described in this paper. The assignment of mass fraction values for selected contaminants in these materials involved the combination of results from gas chromatography/mass spectrometry (GC/MS) analyses by NIST and CDC, as well as results from four laboratories, AXYS Analytical Services (Sidney, B.C., Canada), National Institute of Public Health of Québec (Québec, Canada), Stockholm University (Stockholm, Sweden), and University of Liege (Liege, Belgium), which participated in an interlaboratory study using the four SRMs as unknown samples with each laboratory using its normal methods of analysis.

Experimental

Source and preparation of serum and milk SRMs

Plasma used for SRM 1957 and SRM 1958 was acquired in 2004 from various blood banks located around the U.S.: Wilmington and Greenville, NC (10 %); Jacksonville and Orlando, FL (8 %); Jonesboro, AR (12 %); Flagstaff, AZ (15 %); Gallup and Albuquerque, NM (20 %); Memphis, TN (12 %); Portland, ME (16 %); and Carbondale, IL (7 %). Following the precipitation of fibrin and filtration to ensure better homogeneity of the sample, the serum was pooled (approximately 200 L total) and stored at 4 °C. The pool was split into two batches of 100 L each for production of SRM 1957 and SRM 1958. For SRM 1958 approximately 710 mL of a methanol solution containing 169 compounds (Table S1) at varying mass/volume (see below) was added to the 100 L batch of serum, which was then stirred for 4 h. Using a calibrated automatic pipetter, 10.7 mL aliquots of serum were dispensed into 30 mL amber glass vials, and the samples were lyophilized until a stable vacuum and temperature were achieved. Approximately 10,000 vials of each SRM were prepared, and a sales unit of SRM 1957 and SRM 1958 consists of 5 vials of freeze-dried serum. Before use, the serum in each vial must be reconstituted with 10.7 mL of distilled or HPLC-grade water.

The pasteurized milk used for SRM 1953 and SRM 1954 was acquired in 2006 from six milk banks located around the U.S: Florida (4 %), North Carolina (6 %), Iowa (6 %), Delaware (7 %), California (12 %), and Texas (65 %). The SRMs were prepared from milk that had either reached its expiration date or was considered “research milk” that could not be fed to babies. (Research milk includes milk that has too low a caloric content or tested positive for bacteria prior to pasteurization.) The milk was pooled (approximately 100 L total) and was stored at 4 °C. The pool was split into two batches of 50 L each for production of SRM 1953 and SRM 1954. For SRM 1954 approximately 355 mL of a methanol solution containing 169 compounds at varying concentrations (the same solution as added to SRM 1958, see below for more information) was added to the 50 L batch 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 which were then stored at −20 °C. Approximately 5,000 vials of each milk SRM were prepared, and a sales unit of SRM 1953 and SRM 1954 consists of 5 vials of frozen milk.

The methanol spiking solution was prepared by Cambridge Isotope Labs (Andover, MA) and contained 169 compounds at the indicated mass concentration: 38 non and mono *ortho*-PCBs (50 pg/mL to 500 pg/mL), 4 non-*ortho*-PCBs (0.4 pg/mL to 0.8 pg/mL), 22 chlorinated pesticides including 6 toxaphene congeners (500 pg/mL), 17 PBDEs (500 pg/mL), polybrominated biphenyl (PBB) 153 (500 pg/mL), hexabromocyclododecanes (500 pg/mL), 1,2-bis(2,4,6-tribromophenyloxy)ethane (500 pg/mL), hexabromobenzene (500 pg/mL), decabromodiphenylethane (500 pg/mL), 17 PCDDs/PCDFs (0.1 pg/mL to 2.4 pg/mL), 7 polybrominated dibenzodioxins, 10 polybrominated dibenzofurans, and 8 chlorobromo dibenzodioxins and dibenzofurans (0.05 pg/mL), 9 polychlorinated naphthalenes (PCNs) (1 pg/mL), 12 halogenated phenolic compounds, 5 hydroxylated PCBs (500 pg/mL), and 8 other persistent organochlorine compounds (500 pg/mL) including 4 chlorobenzenes, octachlorostyrene, pentachloronitrobenzene, hexachlorocyclopentadiene, and hexachloro-1,3-butadiene. The individual compounds in the spiking solution are listed in Table S1.

Analytical methods—The analytical methods used are described in detail in the supplementary information (see Electronic Supplementary Material Figure S1) with a summary here. Multiple methods of analysis were used at NIST for the determination of the PCBs, pesticides, and PBDEs in both the milk and serum, and an additional method was developed for the determination of the hydroxylated compounds in serum only (see Supplemental information). The first method was used for the homogeneity study, analyzing 10 bottles using a liquid-liquid extraction with *n*-hexane: methyl-*tert*-butyl ether, a sample clean-up using concentrated sulfuric acid followed by silica solid-phase extraction (SPE) fractionation. The final analysis used gas chromatography/mass spectrometry (GC/MS) operated both in the electron ionization (EI) and negative ion chemical ionization (NICI) mode with on-column injections. The GC column used for the EI analysis was a non-polar proprietary phase (Agilent Technologies, Wilmington, DE) while the column used for the NICI analysis was a 50 % (mole fraction) phenyl-substituted methylpolysiloxane phase.

The second method at NIST used focused microwave extraction with 3 mL of 20 % (volume fraction) dichloromethane in hexane as described by Keller et al. [22]. An acidified silica

column clean-up followed by an alumina column (5 % deactivated) clean-up was used with final analysis using GC/MS in the EI mode with a 5 % phenyl-substituted methylpolysiloxane phase. The injections used a programmable temperature vaporization (PTV) inlet. The extracts were also injected on-column using a shorter column (10 m compared to 30 m; note that the short column was used to reduce degradation of the higher brominated PBDEs on-column) connected to the NICI source in the GC/MS.

The methods used by CDC are described in more detail in Patterson and Turner [23] and Sjödin et al. [24, 25]. Gas chromatography/high resolution mass spectrometry (GC/HRMS) with mass resolution of 10,000 and equipped with a 5 % phenyl-substituted methylpolysiloxane phase was used.

The laboratories participating in the interlaboratory study employed the methods commonly used in their laboratories for the determination of these compounds in serum and milk. The methods used by University of Liege are described in more detail in Focant et al. [26].

Results and discussion

Since the development of the first natural-matrix SRMs for organic contaminants at NIST over 30 years ago, the assignment of certified mass fraction values have been based on the combination of results from two or more independent methods [28]. Currently NIST SRMs for chemical composition are assigned mass fraction values designated certified, reference, or information values based on the number and independence of the analytical methods used, source of the results, and degree of confidence in the accuracy of the assigned value [27, 28].

As described above, results from two to five methods of analysis were used to assign certified and reference mass fraction values for the milk and serum materials. For the PCBs, chlorinated pesticides, and PBDEs, the assigned values were based on results from two or three NIST methods, the CDC method, and the interlaboratory study. The various methods included different extraction procedures, clean up procedures, and GC/MS or GC/HRMS with different stationary phase columns. The PCDD/PCDF congeners were assigned reference values using results from only the CDC method and the interlaboratory study of results from two laboratories. Similarly the reference values for the hydroxylated compounds were assigned based on results from LC/MS/MS (NIST method 3, see Supplemental information) and the interlaboratory study.

The results from each of the five methods are summarized in Electronic Supplementary Material Tables S2 through S5 for SRM 1953, SRM 1954, SRM 1957, and SRM 1958, respectively. The results of the different methods were combined as weighted means [29] to assign the certified and reference mass fraction values. The resulting certified, reference, and information mass fraction values are summarized in Tables 1, 2, 3, and 4 for the PCB congeners, pesticides, PBDE congeners, and PCDD and PCDF congeners, respectively.

For each of the materials, the homogeneity of the PCBs, pesticides, and PBDEs was assessed at NIST using method 1 described above. An analysis of variance did not show inhomogeneity for a 2.5 g sample of milk or for a 5 g sample of serum [18–21]. Other

analytes were treated as though they were homogeneously distributed in the material although homogeneity was not assessed. The long-term stability of the freeze-dried serum materials has been demonstrated for SRM 1589a in which the PCBs and pesticides have been shown to be stable over an 8 year period [15]. Stability assessments are conducted regularly on the freeze-dried serum and frozen milk samples primarily through their use as control samples for other analyses.

For the non-fortified serum and milk, the certified mass fractions of the organic contaminants ranged from 12 ng/kg to 2200 ng/kg (excluding 4,4′-DDE at 7400 ng/kg) with expanded relative uncertainties from approximately 1 % to 14 %, with the exception of PCB 138 in SRM 1957 for which the expanded relative uncertainty is 24 %. Of the 46 certified values in the non-fortified milk and serum SRMs, 26 had relative uncertainties less than 5 % and only 8 had relative uncertainties greater than 10 %. For the fortified serum and milk, the certified mass fractions ranged from 400 ng/kg to 2600 ng/kg (excluding 4,4′-DDE at 8200 ng/kg) with expanded relative uncertainties from approximately 1 % to 15 %. For the 128 certified values in the fortified milk and serum SRMs, 111 had relative uncertainties less than 10 % with 65 having relative uncertainties less than 5 %. The agreement of the results from the different methods for both the non-fortified and the fortified serum and milk, based on the uncertainties associated with the certified values, are consistent over a wide range in mass fractions (two orders of magnitude) suggesting that no significant biases exist among the methods using different extraction, isolation, and GC/MS columns.

For the natural (non-fortified) serum and milk SRMs, the wet-mass based contaminant levels are higher in the milk compared to the serum due to the higher lipid content of the milk. The milk sample contains a mass fraction of 3.21 %± 0.33 % total extractable lipid as determined by a gravimetric drying step following extraction. Despite using research milk (milk that has too low a caloric content to be fed to infants), the lipid content of the SRM is similar to that found in breast milk from biomonitoring studies [8, 25]. The serum sample contains 0.471 % ±0.065 % lipid for SRM 1957 and 0.406 %±0.036 % lipid for SRM 1958 as determined by three methods: a gravimetric drying, the Bligh and Dyer method, and an enzymatic analysis [17]. Comparing the contaminant levels on a lipid-basis, the non-fortified milk sample (SRM 1953) has higher values of the tetra-, penta-, and some hexachlorinated PCB congeners while the non-fortified serum (SRM 1957) has equivalent or slightly higher mass fractions of the higher chlorinated PCB congeners, particularly PCB 180 (see Electronic Supplementary Material Figure S2). PCB 153/132 is the predominant PCB congener in both samples contributing almost 30 % of the total PCB composition (see Figure S2). PCB 153 and PCB 132 are separable on a number of GC columns; however, since a number of the methods for the certification of these SRMS relied on a 5 % phenyl methylpolysiloxane phase which does not separate PCB 132 from PCB 153, the certified value is for the combined PCB 153/132. On a lipid basis the non-fortified milk sample has higher mass fractions for most of the chlorinated pesticides and some of the PBDE congeners, particularly PBDE 100 and PBDE 153. For the PBDE congeners, PBDE 47 is the most abundant in both the milk and serum samples, contributing approximately 50 % of the total PBDE content (see Electronic Supplementary Material Figure S3). In comparing these samples, however, note that the milk and serum pools were not collected from the same individuals.

The goal in preparing the fortified serum SRM was to provide a material in which compounds not detectable in the average natural level found in the US would be present at detectable levels. These compounds may be present at higher concentrations in a sub-segment of the population with higher exposure; therefore reference materials with detectable levels are needed. Based on the certified values for the two serum SRMs, the PCB, pesticide, and PBDE mass fractions in the fortified material range from approximately 10 to 30 times the mass fractions in the non-fortified (natural) material with the exception of the highest level contaminants such as 4,4′-DDE and PBDE 47 which are only 1 to 3 times greater in SRM 1958. The two milk SRMs differ by only a factor of 2 to 4 in mass fraction levels for many of the pesticides and PBDEs (the predominant compounds), whereas the PCBs generally differ by a factor of 10 to 20. The fortified materials, SRM 1954 and SRM 1958, were both spiked with the same spiking solution (see Table S1). When the measured mass fraction differences between the fortified and non-fortified milk samples are compared to the difference between the fortified and non-fortified serum samples, the differences are similar and consistent. Based on the homogeneity analyses, the spiking solution was well mixed within the samples prior to bottling.

In addition to the compounds characterized in this study, SRM 1954 and SRM 1958 were fortified with compounds anticipated to be of interest in future monitoring studies including additional brominated flame retardants, polybrominated dibenzodioxins, polybrominated dibenzofurans, mixed chlorinated and brominated dibenzodioxins and dibenzofurans, polychlorinated naphthalenes, and hydroxylated PCBs (see Table S1). As new flame retardants are introduced into products, they will need to be monitored in human samples, as well as the combustion products from the incineration of the products, which produce the brominated and mixed chlorinated/brominated dioxins and furans. Hydroxylated metabolites of the contaminants, such as the hydroxylated PCBs are toxicologically more active than their parent compounds, although found at lower levels in the serum and milk. With these additional compounds added to the serum and milk pools, these SRMs will be valuable for future method development. The serum materials have also been characterized for selected perfluorinated compounds (PFCs) as described in Keller et al. [30]. (PFCs were not included in the spiking solution.) Nutrient elements, including calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium, and zinc, and the toxic metal mercury, were determined in the milk samples, and the results are provided on the Certificates on Analysis as reference mass fractions [18, 20, 31].

SRM 1957 was developed in part to address the need for a serum material representing more contemporary contaminant levels in the U.S. population than the previous material, SRM 1589a. Using biomonitoring data, Sjödin et al. [32] demonstrated the decrease in legacy chemicals (no longer in use in the U.S.) such as PCBs and the increase in more recently used chemicals such as PBDEs in serum collected from 1984 to 2002. Comparison of the mass fractions of contaminants in SRM 1589a (collected in 1996) and SRM 1957 (collected in 2004) indicates similar trends as shown in Fig. 1. For example, PCB congener values in SRM 1957 are generally 10 to 15 times lower than in SRM 1589a. Similarly the mass fraction of 4,4′-DDE was 12 times lower in SRM 1957 compared to SRM 1589a. For the PCDD/PCDF congeners (not shown in Fig. 1), the mass fractions were typically a factor of four lower in SRM 1957 compared to SRM 1589a, e.g., 1,2,3,6,7,8-hexaCDD (416 pg/kg

±12 pg/kg vs. 84.7 pg/kg±8.1 pg/kg). For the flame retardants such as PBDEs, the values in SRM 1957 have increased typically by a factor of two compared to SRM 1589a. Likewise for PFCs, another group of compounds used recently, mass fractions were a factor of 5 and 10 higher in SRM 1957 compared to SRM 1589a for the predominant PFCs [30]. In making the comparison of SRM 1589a to SRM 1957, however, it should be noted that SRM 1589a was prepared from serum collected from donor units that had been prescreened at NIST for the PCB content, and the units with high PCB content were selected (all coming from the Chicago area, preferably from donors who ate fish caught in the Great Lakes), whereas SRM 1957 was prepared from random donor units (i.e., no prescreening) from multiple collection sites.

Conclusion

These new human serum and milk SRMs represent human samples commonly used for the monitoring of environmental contaminants. SRMs 1953 and 1954 are the first human milk certified reference materials available worldwide that have been characterized for organic contaminants. It should be noted that although there are some environmental contaminants present in human milk, the nutritional benefits of breastfeeding to the infant likely outweigh any harmful effects (for example, see http://www.cdc.gov/breastfeeding/). SRM 1957 represents the most extensively characterized natural human serum certified reference material for persistent organic contaminants available from any source worldwide. The fortified milk and serum SRMs, with certified and reference values for 91 and 92 PCBs, pesticides, PBDEs, and dioxins/furans, respectively, are valuable reference materials for contaminants currently monitored, as well as for contaminant classes that may be monitored extensively in the future. These materials will find extensive use in the biomonitoring community and will aid in the comparison of data among biomonitoring studies performed in different years and by different laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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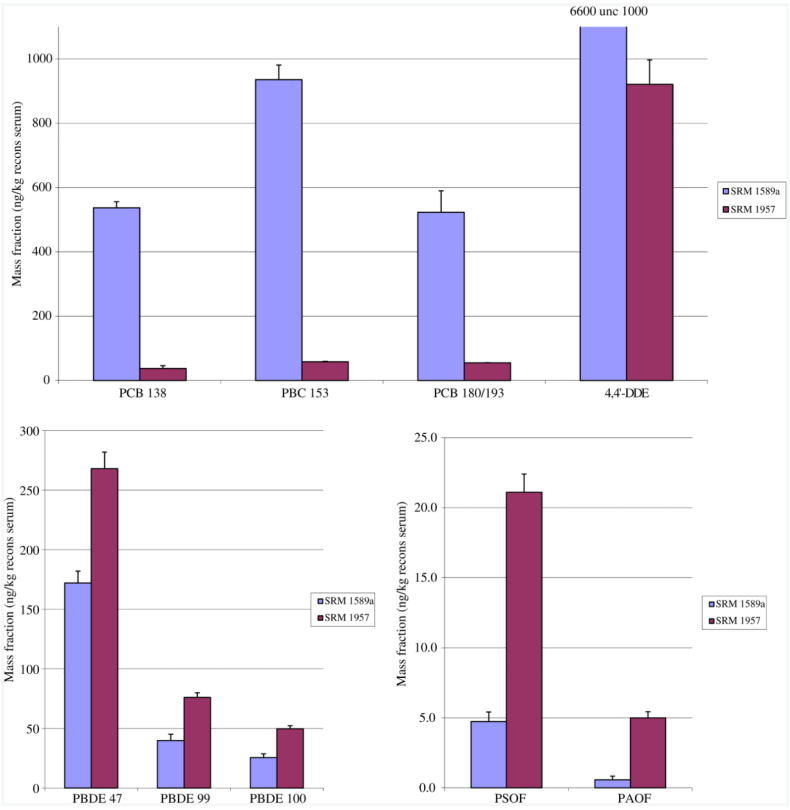

Fig. 1.

Comparison of mass fractions for legacy chemicals (PCBs and 4,4′-DDE) and chemicals used more recently (PBDEs and PFCs) in serum collected in 1996 (SRM 1589a) and serum collected in 2004 (SRM 1957). *Error bars* represent the expanded uncertainties

Table 1

Certified, Reference, and *Information* mass fractions (ng/kg milk or reconstituted serum) of selected PCB congeners in SRM 1953 organic contaminants in non-fortified human milk, SRM 1954 organic contaminants in fortified human milk, SRM 1957 organic contaminants in non-fortified human serum, and SRM 1958 organic contaminants in fortified human serum

PCB congener[a]	SRM 1953	SRM 1954	SRM 1957	SRM 1958
PCB 18		355±76[b]	4.5±0.8[c]	**407±14**[b]
PCB 28	**62.8±1.2**[b]	**519±23**[b]	8.6±1.1[c]	**402±12**[b]
PCB 44		**417±31**[b]		**408±21**[b]
PCB 49		**426±8**[b]		**414±15**[b]
PCB 52	7.65±0.23[c]	**424±46**[b]		**401±14**[b]
PCB 66	**32.8±1.1**[b]	**429±39**[b]	5.6±1.7[c]	**414±6**[b]
PCB 74	**149±2**[b]	**562±8**[b]	13.8±0.1[b]	**414±46**[b]
PCB 77	0.673±0.047[c]	2.71±0.14[c]		
PCB 81	0.092±0.006[c]	0.630±0.028[c]		
PCB 87		**455±52**[b]		**396±23**[b]
PCB 99	**139±5**[b]	**552±8**[b]	11.6±0.6[c]	**385±28**[b]
PCB 101		**441±29**[b]		**409±27**[b]
PCB 105	**47.5±6.7**[b]	**478±6**[b]		**419±31**[b]
PCB 110	**12.3±0.4**[b]	**459±36**[b]		**397±26**[b]
PCB 114		90.5±7.4[c]		46.6±8.4[b]
PCB 118	**213±4**	**594±34**	**18.9±1.2**	**412±35**[b]
PCB 123		67.9±3.1[c]		52.5±4.8[b]
PCB 126	0.79±0.15[b]	10.4±2.5[b]		7.8±1.1[b]
PCB 128		**400±29**[b]		**420±17**[b]
PCB 138	**319±27**[b]	**646±17**[b]	**36.9±9.0**[b]	**473±54**[b]
PCB 146	**55.6±0.8**[b]	**495±25**[b]	7.17±0.26[c]	**378±26**[b]
PCB 149	7.40±0.60[c]	**409±3**[b]		**373±22**[b]
PCB 151		**418±11**[b]		**381±18**[b]
PCB 153	**478±6**[b]	**980±6**[b]	**58.2±0.9**[b]	**457±36**[b]
PCB 156	**60.7±4.0**[b]	**505±7**[b]	8.24±0.57[b]	**418±19**[b]
PCB 157	**14.7±0.6**[b]	**467±37**[b]		**420±42**[b]
PCB 158		**408±8**[b]		**365±48**[b]
PCB 167	**15.9±2.2**[b]	**467±59**[b]		**403±26**[b]
PCB 169	0.216±0.020[b]	9.3±2.0[b]		8.10±0.33[b]
PCB 170	100±18[b]	**505±69**[b]	**16.2±2.0**[b]	**422±23**[b]

PCB congener[a]	SRM 1953	SRM 1954	SRM 1957	SRM 1958
PCB 172	**14.8±0.6**[b]	**443±34**[b]		**395±28**[b]
PCB 177	**23.7±1.2**[b]	**450±6**[b]		**392±9**[b]
PCB 178	**22.2±0.5**[b]	**449±15**[b]	3.61±0.32[c]	**386±15**[b]
PCB 180	**234±8**[b]	**678±58**[b]		**459±49**[b]
PCB 180/193			**54.5±0.5**[b]	
PCB 183	**37.1±5.2**[b]	**446±22**[b]	5.77±0.35[c]	**407±36**[b]
PCB 187	96±17[b]	**517±28**[b]	**15.5±0.5**[b]	**411±38**[b]
PCB 189		**432±39**[b]		**402±31**[b]
PCB 194	50±16[b]	**484±6**[b]	**11.9±0.3**[b]	**387±20**[b]
PCB 195	11.9±1.6[c]	**459±19**[b]		**385±25**[b]
PCB 196				**397±2**[b]
PCB 196/203		**870±31**[b]	10.9±0.7[c]	
PCB 199		**469±17**[b]	11.2±0.8[c]	**362±15**[b]
PCB 201				**397±7**[b]
PCB 203				**398±49**[b]
PCB 206		**463±12**[b]	6.98±0.37[c]	**366±8**[b]
PCB 209		**441±26**[b]	3.33±0.59[c]	**338±16**[b]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [33] and later revised by Schulte and Malisch [34] to conform with IUPAC rules. For the specific congeners mentioned in this table, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch. When two or more congeners are known to coelute under the GC analysis conditions used, the PCB congener listed first is the major component and the additional congeners may be present as minor components

[b] Values are weighted means of the mass fractions determined by two to five analytical methods [29]. The uncertainty listed with each value is an expanded uncertainty about the mean [29, 35], with coverage factor, $k = 2$ (except $k = 4$ for PCB 180/193 in SRM 1957), calculated by combining a pooled within method variance with a between method variance [29, 36] following the ISO Guide [37, 38]

[c] 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

Table 2

Certified, Reference, and *Information* mass fractions (ng/kg milk or reconstituted serum) of selected pesticides in SRM 1953 organic contaminants in non-fortified human milk, SRM 1954 organic contaminants in fortified human milk, SRM 1957 organic contaminants in non-fortified human serum, and SRM 1958 organic contaminants in fortified human serum

Pesticide	SRM 1953	SRM 1954	SRM 1957	SRM 1958
2,3,4,5-Tetrabromophenol			*120*	*150*
2,3,4,6-Tetrachlorophenol			*99*	*98*
Pentachlorophenol			2710±880[a]	2780±550[a]
Pentabromophenol				445±93[a]
Pentachloronitrobenzene				*480*
Pentachlorobenzene		362±24[b]		
Hexachlorobenzene	**261±30**[a]	**671±48**[a]	**29.7±3.5**[a]	**442±46**[a]
Octachlorostyrene		374±28[b]		**359±19**[a]
α-HCH				260±44[a]
β-HCH	**610±4**[a]	**820±6**[a]	31.3±6.0[a]	278±47[a]
γ-HCH		**587±54**[a]		315±43[a]
Oxychlordane	**612±68**[a]	**1060±150**[a]		226±43[a]
cis-Chlordane		**368±9**[a]		**412±25**[a]
trans-Chlordane		**378±7**[a]		**412±3**[a]
cis-Nonachlor	**126±6**[a]	**499±5**[a]		**425±23**[a]
trans-Nonachlor	**1240±70**[a]	**1690±110**[a]	**58.3±1.9**[a]	**469±6**[a]
Mirex	68±25[a]	**512±7**[a]		**384±59**[a]
2,4′-DDE		403±6[a]		**450±20**[a]
4,4′-DDE	**7430±420**[a]	**8150±200**[a]	**921±76**[a]	**1250±130**[a]
2,4′-DDD		**421±7**[a]		**347±46**[a]
4,4′-DDD		**422±8**[a]		**416±17**[a]
2,4′-DDT	18.2±1.2[b]	**443±9**[a]		**313±43**[a]
4,4′-DDT	**229±16**[a]	**691±37**[a]		**293±12**[a]
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (Parlar 26)				*340*
2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (Parlar 50)				*230*
2,2,5,5,8,9,9,10,10-nonachlorobornane (Parlar 62)				*320*

[a]Values are weighted means of the mass fractions determined by two to five analytical methods [29]. The uncertainty listed with each value is an expanded uncertainty about the mean [29, 35], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between method variance [29, 36] following the ISO Guide [37, 38]

[b]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

Table 3

Certified and Reference mass fractions (ng/kg milk or reconstituted serum) of selected PBDE congeners and PBB 153 in SRM 1953 organic contaminants in non-fortified human milk, SRM 1954 organic contaminants in fortified human milk, SRM 1957 organic contaminants in non-fortified human serum, and SRM 1958 organic contaminants in fortified human serum

PBDE congener[a]	SRM 1953	SRM 1954	SRM 1957	SRM 1958
PBDE 17	16.8±0.1[b]	**401 ±50**[b]	4.2±1.5[b]	**458±32**[b]
PBDE 28	137±18[b]	**576±7**[b]	20.0±2.4[b]	**462 ±19**[b]
PBDE 47	**2240±30**[b]	**2590±80**[b]	**268±14**[b]	**651 ±29**[b]
PBDE 66	16.2±3.4[b]	**414±10**[b]	6.70±0.13[b]	**440±41**[b]
PBDE 85	49.2±8.1[b]	**475 ±22**[b]	8.2±1.9[b]	**475 ±39**[b]
PBDE 99	**341±8**[b]	**738±47**[b]	**76.0±3.8**[b]	**492±15**[b]
PBDE 100	**822±15**[b]	**1280±40**[b]	**49.7±2.7**[b]	**475 ±27**[b]
PBDE 153	**971 ±23**[b]	**1440±100**[b]	**61.0±3.2**[b]	**455±54**[b]
PBDE 154	**42.7±2.3**[b]	**470 ±13**[b]	7.0±1.0[c]	**441 ±39**[b]
PBDE 183		**507±43**[b]		**453±42**[b]
PBDE 203		494±44[c]		
PBDE 206		820±280[b]		**426±4**[b]
PBDE 209		**423±24**[b]		**417±5**[b]
PBB 153	**36.5±0.6**[b]	**476±9**[b]	**15.5±0.1**[b]	**421±13**[b]

[a] PBDE congeners and PBB 153 are numbered according to IUPAC rules

[b] Values are weighted means of the mass fractions determined by two to five analytical methods [29]. The uncertainty listed with each value is an expanded uncertainty about the mean [29, 35], with coverage factor, $k = 2$ (except $k = 4$ for PBDE 206 in SRM 1958), calculated by combining a pooled within method variance with a between method variance [29, 36] following the ISO Guide [37, 38]

[c] 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

Table 4

Reference mass fractions (pg/kg milk or reconstituted serum) of selected PCDD and PCDF congeners in SRM 1953 organic contaminants in non-fortified human milk, SRM 1954 organic contaminants in fortified human milk, SRM 1957 organic contaminants in non-fortified human serum, and SRM 1958 organic contaminants in fortified human serum

Dioxin or furan congener[a]	SRM 1953	SRM 1954	SRM 1957	SRM 1958
2,3,7,8-TCDD	55.2±4.6[a]	162±32[a]		94.2±7.8[a]
1,2,3,7,8-PCDD	123±11[a]	240±27[a]	17.3±2.9[a]	106 ± 12[a]
1,2,3,4,7,8-HxCDD	77.7±9.0[a]	180±21[a]	11.8±1.7[a]	95.3±9.9[a]
1,2,3,6,7,8-HxCDD	501±91[a]	890±240[a]	82±18[a]	340±60[a]
1,2,3,7,8,9-HxCDD	100±11[a]	206±33[a]	18.7±0.3[a]	99.6±7.2[a]
1,2,3,4,6,7,8-HpCDD	480±160[a]	1080±410[a]	104±36[a]	565±90[a]
OCDD	2240±360[a]	4890±1430[a]	716±81[a]	2570±280[a]
2,3,7,8-TCDF	16.4±0.6[a]	125±13[a]		104±3[a]
1,2,3,7,8-PCDF		132±29[a]		98±15[a]
2,3,4,7,8-PCDF	127±11[a]	344±33[a]	16.4±2.5[a]	199±30[a]
1,2,3,4,7,8-HxCDF	72±10[a]	171±22[a]	17.0±3.1[a]	95.9±9.3[a]
1,2,3,6,7,8- HxCDF	66.0±1.3[a]	183±20[a]	14.1±2.5[a]	102±11[a]
1,2,3,7,8,9- HxCDF	31.6±0.8[a]	126±9[a]		94±11[a]
2,3,4,6,7,8- HxCDF		1080±160[a]		900±110[a]
1,2,3,4,6,7,8-HpCDF	100±12[a]	405±66[a]	39.8±6.2[a]	289±9[a]
1,2,3,4,7,8,9-HpCDF				84±12[a]
OCDF	11.6±5.0[b]	94.3±12.7[b]		83.3±1.7[a]

[a]Values are weighted means of the mass fractions determined by two to three analytical methods [29]. The uncertainty listed with each value is an expanded uncertainty about the mean [29, 35], with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between method variance [29, 36] following the ISO Guide [37, 38]

[b]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