SRMS FOR MONITORING HUMAN EXPOSURE TO ORGANIC CONTAMINANTS

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Introduction

The National Institute of Standards and Technology (NIST) is collaborating with the Centers for Disease Control and Prevention (CDC) to develop several new Standard Reference Materials (SRMs) to meet the expanding needs for measurement of organic contaminants in human body fluids (serum, milk, and urine) to support human exposure monitoring programs. In 1985 NIST issued the first serum-based Standard Reference Material (SRM) 1589 Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum for the determination of organochlorine contaminants in human serum. This material was prepared by fortifying human serum with Aroclor 1260 prior to freeze-drying. SRM 1589a PCBs, Pesticides, and Dioxin/Furans in Human Serum was issued in 2000 and contained natural levels of these organochlorine constituents. Because the concentrations of PCBs, pesticides, and dioxins/furans in the human population have decreased by approximately 50% since SRM 1589a was prepared in 1996 and the concentrations of emerging contaminants such as brominated flame retardants have increased significantly in the same period, a more contemporary serum SRM is needed to represent these changing concentrations of contaminants in human serum. For the development of both the serum and milk SRMs, two SRMs were prepared from one pool of serum (200 L) or milk (100 L) collected from donors across the United States (US). The pool was divided and one portion of the materials represents a natural level (nonfortified) and the other portion is a fortified material. The solution used to fortify both the serum and milk contained 172 selected chlorinated dioxins and furans, brominated dioxins and furans, pesticides, polychlorinated biphenyls, brominated flame retardants, polychlorinated naphthalenes, phenols, and toxaphenes, and it was added to provide concentrations in the serum or milk approximately 5 to 10 times higher than median concentrations found in the US population. The two serum SRMs were freeze-dried, and the two milk SRMs were frozen. SRM 1953 is Organic Contaminants in Nonfortified Human Milk and SRM 1954 is Organic Contaminants in Fortified Human Milk. SRM 1957 is Organic Contaminants in Nonfortified Human Serum and SRM 1958 is Organic Contaminants in Fortified Human Serum. NIST and CDC are both providing measurements of target contaminants in all four SRMs using gas chromatography/mass spectrometry (GC/MS), GC with high-resolution MS, liquid chromatography/MS (LC/MS), and LC with tandem MS (LC/MS/MS). For most of the MS-based measurement techniques, isotope dilution approaches are being used.

Urine is the third matrix of interest and three urine SRMs are planned; two materials will be natural level (nonfortified) urine, one urine pool collected from smokers (Candidate SRM 3672) and the second pool collected from non-smokers (Candidate SRM 3673). The third material (Candidate SRM 3674) will be prepared from the same urine pool as the natural-level urine from non-smokers but will be fortified with the compounds of interest at appropriate levels, including 25 hydroxylated polycyclic aromatic hydrocarbons (PAHs) 13 methyl naphthols, 13 amino PAHs, and 2 nitrated hydroxy PAHs.

Materials and methods

The 200 L of serum (acquired as plasma and then converted to serum) was acquired from eight states within the US: Arkansas, Arizona, Florida, Illinois, Maine, New Mexico, North Carolina, and Tennessee. The 100 L of milk was acquired from six mothers' milk banks located within the following US states: California, Delaware, Florida, Iowa, North Carolina, and Texas. (This milk was considered "research milk" that could not be fed to infants for various reasons.) The serum and milk were thoroughly mixed prior to dividing into two approximately equal portions (each 100 L ± 10% for the serum and each 50 L ± 10% for the milk). One of the portions for each matrix was stored frozen while the other portion was aliquoted into vials with approximately 10 mL per vial for the serum and 5 mL per vial for the milk. The vials of serum were then freeze-dried (candidate SRM 1957) while the vials of milk were frozen (candidate SRM 1953). Following thawing of the remaining portion of each matrix, a spiking solution in methanol containing compound classes listed in Table 1

was added. The fortified samples were stirred for 4h and then dispensed and stored in the same manner as the nonfortified materials. Candidate SRM 1954 is the fortified human milk while candidate SRM 1958 is the fortified human serum.

The certification of the serum and milk SRMs is a joint effort between NIST and CDC with CDC also coordinating a small interlaboratory study using the four materials. The methods are summarized in Figure 1. Extractions of the materials were done using liquid-liquid extraction, open-focused (OF) microwave extraction, or solid phase extraction (SPE). The clean-up steps involved sulfuric acid or size exclusion chromatography (SEC) to remove the lipid interferences followed by silica or alumina to remove some of the more polar interferences. The final analysis step is GC with either low-resolution MS or high-resolution MS (HRMS), LC/MS, or LC/MS/MS.

Results and discussion

The results from the different methods of analysis shown in Figure 1 are summarized in Figure 2 for selected analytes in the two serum and two milk SRMs. Method 1 was used to test duplicate samples from 10 bottles selected using a stratified random sampling to assess the homogeneity of each of the materials. No significant differences were noted in the variability of the concentrations within bottles compared to those between bottles indicating that the materials are homogeneous. Methods 2 and 3 were used for analysis of single samples from six bottles, method 4 (CDC) was used to test single samples from nine bottles, and each of the laboratories participating in the interlaboratory study analyzed single samples from four bottles. The bottles were all chosen using a stratified random sampling. The data from the interlaboratory study were combined into one data set (Method 5).

As shown in Figure 2, the agreement among the concentrations determined by the different methods is good, especially considering the low concentrations in the nonfortified serum (approximately 5 pg/g reconstituted serum to 1000 pg/g reconstituted serum) and in the nonfortified milk (approximately 10 pg/g milk to 8000 pg/g milk). The data from the different methods will be combined to provide certified, reference, and information concentration values for the organic contaminants of interest in each of these SRMs.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Components included in the solution used to spike SRM 1954Organic Contaminants in Fortified Human Milk and SRM 1958 Organic Contaminants in Fortified Human Serum

Compound Class	**Number of Compounds**	**Concentration Range in Serum or Milk**
Planar Organochlorine Compounds (chlorinated dioxins/furans and coplanar PCBs)	22	0.10 to 2.40 pg/mL
Organochlorine Pesticides	22	500 pg/mL
Chlorobenzenes and octachlorostyrene	8	500 pg/mL
Polychlorinated Biphenyls (PCBs)	38	50 to 500 pg/mL
Brominated Flame Retardants including HBCD, BTBPE	25	500 pg/mL
Polychlorinated Naphthalenes (PCNs)	9	1 pg/mL
Halogenated Phenolic Compounds	12	500 pg/mL
Hydroxylated PCBs	5	500 pg/mL
Brominated Dioxins and Furans	17	0.05 pg/mL
Chloro-bromo Dioxins and Furans	8	0.05 pg/mL
Toxaphene Congeners	6	500 pg/mL

Figure 1. Analytical Scheme for the Certification of Human Milk and Human Serum SRMs

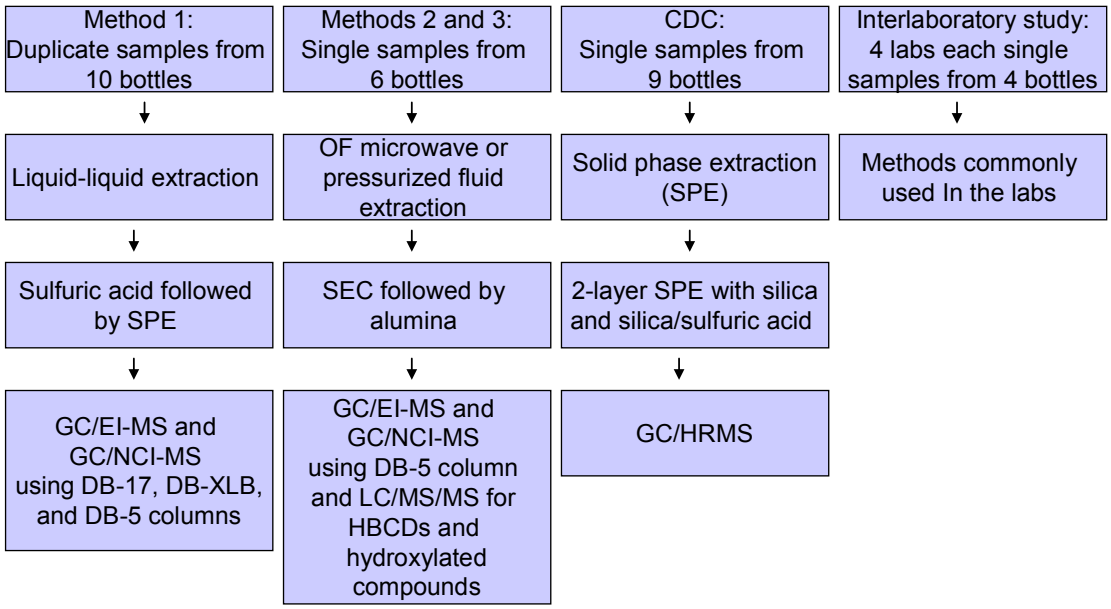
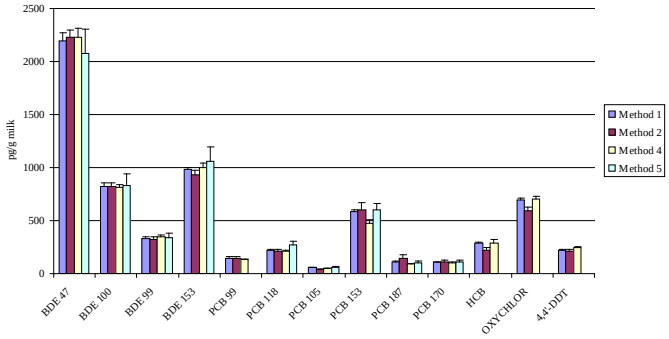
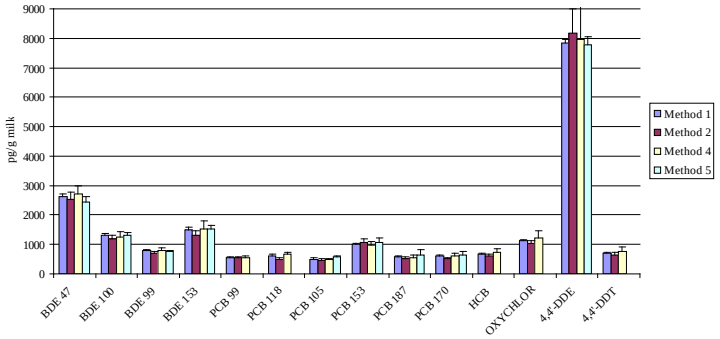


Figure 2. Comparison of data from different methods for selected analytes in the nonfortified and fortified human serum and milk SRMs. Concentrations shown are the means of the data for each method, and the error bars represent the standard deviation of the data for each method. See Figure 1 for the method descriptions (CDC is Method 4, and Interlaboratory study is Method 5).

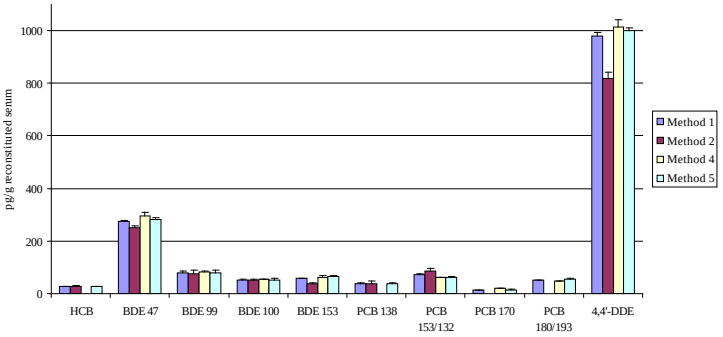
SRM 1953 nonfortified milk



SRM 1954 fortified milk



SRM 1957 nonfortified serum



SRM 1958 fortified serum