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An interlaboratory study of perfluorinated alkyl compound levels in human plasma

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Abstract

We conducted an interlaboratory study which differed from the typical study of this type because of its emphasis on comparing intralaboratory variability in results. We sent specimens to six laboratories experienced in the analysis of perfluorinated alkyl compounds in blood matrices and that use stringent procedures to control and assure accuracy and precision. Each received an identical set of 60 plasma specimens that were analyzed in 6 completely independent batches. Split specimens were included so that within- and between-batch coefficients of variation could be calculated. All laboratories used liquid chromatography-tandem mass spectrometry (LC-MS/MS). The concentrations of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexanesulfonate (PFHxS) measured in the specimens in general showed a high level of agreement, although in some cases the agreement was only moderate. The average within- and between-batch coefficient of variation for PFOS was 9.1% and 9.3%; for PFOA was 14.5% and 14.5%; and for PFHxS was 14.5% and 17.0%. The recent availability of labeled internal standards, among other advances, has facilitated

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improvement in the accuracy and precision of the assays. Considering the degree of between-subject variation in levels among people in background exposed populations, the results indicate that biomarker-based epidemiologic studies of associations with health could have reasonable precision.

Keywords

Perfluorinated alkyl compounds; interlaboratory study; persistent organic pollutants; interdisciplinary studies; research design; epidemiologic methods

The identification of several perfluorinated alkyl compounds at low concentrations in wildlife (Giesy and Kannan, 2001) and human sera from the general population (Hansen et al., 2001) has resulted in a growing interest in biomonitoring (Kannan et al., 2004; Houde et al., 2006; Butenhoff et al., 2006; Calafat et al., 2006; Calafat et al., 2007) and assessing the possibility that such exposure could affect human health. Epidemiologic studies among populations with such exposure are needed to confirm or assess the safety of current exposures. At the low concentrations in blood matrices from the general population, accurate and precise measurements of these compounds pose analytical challenges. Marked imprecision in measurements, as suggested by a recent interlaboratory study (van Leeuwen et al., 2006), could hobble epidemiologic studies in which concentrations of perfluorinated alkyl compounds in blood matrices are examined in relation to aspects of health.

Perfluorinated alkyl compounds have been used to manufacture many industrial and consumer products such as repellent coatings for paper, fabric, and carpets, in heat resistant or impermeable materials, and insecticide formulations (Kissa, 2001; Lewandowski et al., 2006; Dinglasan-Panlilo and Mabury, 2006). Perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS), and perfluorohexanesulfonate (PFHxS) are three representative perfluorinated alkyl compounds that have been recently shown to have relatively long elimination half-lives in humans (Olsen et al., 2005). Resistance to degradation and potential for accumulation have led to assessments of hazard (OECD, 2006). Health risks have been evaluated based on serum levels (Butenthoff et al., 2004; 3M Company, 2003; Swedish Kemikalieinspektionen, 2004). Many of the animal toxicology studies have also measured serum levels, and have shown LOAELs at serum concentrations hundreds to thousands of times higher than the median general population levels; however, for several outcomes no effect levels were not established (Luebker et al., 2005).

The analytical methodology underlying these biomonitoring data has continued to evolve, and the number of laboratories engaged in analyzing samples has continued to increase. Martin et al. (2004) and de Voogt et al. (2006) have reviewed the challenges in conducting these analyses. These challenges include sample preparation methods (e.g., avoiding coextractants), separation of isomers, lack of pure standards or stable isotope labeled standards for quantitation, matrix effects and tandem mass spectrometry detection, and contamination of solvents, labware, and matrix. These factors underscore the need for inter-laboratory studies aimed at developing a better understanding of the potential uncertainty in analyses based on precision and accuracy of measurements.

One prior interlaboratory study has been reported (van Leeuwen et al. 2006). In that study, among 18 participating laboratories, the interlaboratory coefficients of variations for analysis of plasma unknowns were: PFOS, 32%; PFOA, 51%; and PFHxS, 64%. In that study, the procedures used by the laboratories to control and assure accuracy and precision of the assays were not clear, nor were the details of the methods.

The present study was designed to investigate intra- and interlaboratory variability. In an interlaboratory study, or “round robin”, a sample of material is divided into identical aliquots and analyzed at different laboratories. The design of such studies depends on whether they address technical issues of primary interest to analytical chemists, or more general aspects of the assay relevant to scientists who must choose a laboratory collaborator or interpret results. While this type of exercise is frequently done as part of routine quality control or study planning, the results can be of wider interest when a particular assay is new or especially challenging.

In some interlaboratory studies one aliquot is sent to each participating laboratory. This allows calculation of the “method variation”, which is also called the interlaboratory coefficient of variation. This value gives insight into assay performance, and provides a means to compare the results of the assay with minor variations. However, if multiple aliquots are sent to each laboratory, this can allow, in addition, a standardized characterization of the within- and between-batch coefficients of variation at each laboratory, which are often more crucial to epidemiologic validity than is calibration (Hankinson et al., 1994). By batch, we mean an independent unit of analysis, including a full standard curve, blanks, control samples, and a group of unknowns all run in the same contiguous time frame. Given the unusual degree of difficulty in accurately quantitating perfluorinated alkyl compounds, the authors conducted an interlaboratory study in which the participating laboratories were sent multiple aliquots of multiple specimens comprising both unknowns and quality control samples at a prepared concentration. We included six completely independent laboratories that employ stringent procedures to control and assure the accuracy and precision of their measurements. The laboratories were selected because either they had extensive experience with the assay or their work on other low-level contaminants was well-regarded by us or others, and they were able to commit to having the assays completed according to a specified schedule. Other laboratories that were not included also met these criteria. With careful selection of the 6 laboratories, this number was sufficient for us to achieve an important goal—objective selection of a strong scientific partner. Sending multiple split samples to each laboratory increased the cost per laboratory relative to a traditional interlaboratory study, and these assays are relatively expensive. With just 6 laboratories we were limited in some ways, e.g., our ability to identify specific reasons for interlaboratory variability in results.

Thus, the present study was done to gain an improved understanding of the range in quality of laboratory assays of perfluorinated alkyl compounds in plasma that were available in 2006—therefore allowing comparison to the true variation in levels among subjects and an assessment of whether biomarker-based epidemiologic studies of associations with health could have reasonable precision.

Experimental Design

Laboratory Methods

Six laboratory groups participated, designated 1-6. The laboratories were in Canada, Pennsylvania, Minnesota, and North Carolina. There were two labs each from MN and NC, but they had nothing in common other than the same corporate affiliation in MN; no laboratory at NIEHS was involved. The analytical methods were selected independently at each laboratory. The laboratories were asked to measure PFOS, PFOA, and PFHxS. All used liquid chromatography followed by tandem mass spectrometry using electrospray ionization (LC/MS/MS). The distinguishing features of the laboratories' procedures used are shown in Table 1. The extraction and sample preparation procedures varied more than the methods for separation and detection of analytes. Labeled internal standards were used by all except laboratory 1. Adjustments for blanks or recovery was not done by any of the laboratories.

Specimens for Analysis

Plasma of three types was sent to each laboratory (Table 2). The first type was from pregnant women from North Carolina; their blood was collected in 9 ml EDTA containing plastic tubes (polyethylene terephthalate) and refrigerated at 4 °C. Within 24 hours the specimens were centrifuged and all the subject's plasma was placed in a 50 ml polypropylene container, homogenized for 5 minutes using a tube rocker, and 1 ml aliquots were put into 1.2 ml plastic vials. These were stored at -80 °C until shipping to the laboratories, about 6 weeks later. The 11 pregnant subjects were aged 22-33 years (mean 27), were 16-30 weeks pregnant (mean 22), and were from the Research Triangle Park, North Carolina area. Their blood was drawn in 2005. The second type of plasma was taken from a large pool derived from donations by 40 female and male adult employees of the Norwegian Institute of Public Health in Oslo, Norway, in 2003; this blood was collected in EDTA-containing glass tubes. The tubes were immediately centrifuged and the subjects' plasma was combined (total of 1.7 L) in a 3 L glass container and stored overnight at 4 °C. The next day the material was homogenized and 30 ml aliquots were put into 50 ml polypropylene tubes. These were frozen at -80 °C. In 2005 one aliquot was shipped frozen to the coordinating laboratory in North Carolina, and the specimens were handled as described above.

The third type of specimen was spiked bovine plasma (Valley Medical Products, Winchester, VA) quality-control samples (n = 3). Plasma samples spiked with PFOA and PFOS were prepared to allow comparison of laboratory results for samples with a known concentration of analytes. No PFHxS was available to us when we were preparing the spiked samples. PFOS and PFOA were procured from Waco Chemical (Dalton, GA) and Sigma Aldrich Chemical (St. Louis, MO), respectively. Certificates of analysis provided by the suppliers indicated a purity of 98.9 % by chromatography and 101.6 % by titration with sodium hydroxide for the PFOA and 97.9 % and 98.5 % for two lots of PFOS that were combined for use in this study. Identity characterization using Fourier transform infrared (FTIR) showed the PFOA to be the free acid, however this analysis could not distinguish between the free acid and salt form of PFOS. Stock formulations of PFOS and PFOA were prepared at 1000 ug/mL in acetonitrile and were combined and diluted in acetonitrile in a manner to generate intermediate stock solutions of three specific ratios of PFOS to PFOA. A 1 mL aliquot of these intermediate stocks diluted to 100 mL in MilliQ water to form working stocks, and a 0.5 mL aliquot of a given working solution was shaken by hand with 50 mL of bovine plasma for approximately 30 seconds to prepare the plasma samples. The resulting mixtures were refrigerated (2-8 °C) when not in use and were placed in polycarbonate bottles for shipping on dry ice to the coordinating laboratory for handling as described above. The gravimetric concentration of PFOS and PFOA in the 3 spiked preparations (Table 2) were selected so that we would have data for low, medium, and high levels within the range expected for background-exposed populations. The spiked preparations were analyzed for concentration and homogeneity by taking four replicate 1 mL samples from the top, middle, 1/3 bottom, and bottom of each flask after mixing. To the samples (100 µL), 500 µL of a working internal standard (50 ng/mL perfluoroundecanoic acid in acetonitrile) was added, then 500 µL of 1.0 % formic acid in acetonitrile was added (protein precipitation sample preparation), samples were then vortexed and centrifuged, and 20 µL was injected onto the system. Chromatographic separations were performed with a Waters XTerra-MS (Milford, MA) C-18 column and mobile phase composition of 80:20 (v/v), 0.1% formic acid in Acetonitrile:2mM ammonium acetate, column temperature of 40° C, and flow rate of 0.5 mLs/min. A Sciex LC/MS/MS API 3000 system (Toronto, CA) was operated in negative electrospray mode with an Agilent Series model 1100 HPLC (Palo Alto, CA). Multiple reaction monitoring (MRM) transitions of m/z 499 to 80 and 413 to 169 were monitored for PFOS and PFOA, respectively. Precision was within 20% for the spiked samples and the QC samples that were run with them. Spiked plasma at the final concentrations were confirmed within 15% of

the gravimetric concentration and were homogeneous. The spiking and confirmation were done at the Battelle Memorial Institute, Columbus, OH.

Thus, we compiled 15 different source samples. Each was divided into 24 1-ml aliquots and placed in polypropylene tubes. The laboratories received 4 aliquots of each source sample, with a total to 60 specimens sent to each laboratory. Within these sets each specimen was labeled only with a number from 1 to 60; these were analyzed in that order in batches of 10 unknowns. Within each batch there were 2 replicates, and the second pair of replicates from a given source was in another batch. The order of samples within batch, and the assignment of pairs to batch, was done at random. The specimens were shipped from North Carolina on dry ice within the span of one week.

To compare the average concentrations across laboratories we used mixed effects analysis of variance (ANOVA), where sample was a random effect and laboratory was a fixed effect. Where labs differed, Tukey's multiple comparisons procedure at $\alpha = 0.05$ was used to determine which labs differed significantly from each other. Standard deviations needed to calculate the interlaboratory coefficient of variation (CV), the average within-batch CV across laboratories, the average between-batch CV across laboratories, and the laboratory-specific within- and between-batch CVs were obtained from fixed effects ANOVAs. Spiked plasmas were fortified with PFOS and PFOA but not with PFHxS. As a result PFOS and PFOA were above each lab's LOQ; however this was not the case for PFHxS. Thus, the results for PFHxS, for the spiked samples, were not included in the data analysis. Statistical analyses were conducted using the SAS 9.1 (SAS Institute, Cary, NC) software package.

Results

The concentrations of PFOS, PFOA, and PFHxS measured in the specimens in general showed a high level of agreement across laboratories, although in some cases the agreement was only moderate (Figure 1). The agreement among individual measurements within and across laboratories tended to be better at lower levels of analyte. For the spiked samples, each laboratory's observed mean value of PFOS and PFOA concentration was within 50% or less of the gravimetric value; for the 6 spikes the mean absolute percent difference (observed – gravimetric) across laboratories ranged from 12 to 26%.

On a specimen-by-specimen basis, the laboratories were fairly consistent in their differences from the overall mean concentration (data not shown). Therefore, to summarize laboratories' results across specimens we looked at the overall mean concentration (Figure 2). For all three analytes there were statistically significant differences among the results from the 6 laboratories. Laboratories 2 and 5 tended to obtain higher values for PFOS, and laboratory 1 tended to get lower values for PFOA and PFHxS. When we repeated this type of analysis on the spiked samples, and compared the observed values to the gravimetric mean, the results for PFOS were similar to those shown in Figure 2. For PFOA, the results were also similar though the tendency for laboratories 2 and 3 to give higher values was more pronounced (not shown).

The between-batch coefficients of variation for PFOS were less than 10% for all laboratories except 4 and 5 (Table 3). The between-batch variation in measurements of PFOS for laboratory 3 was remarkably low. The within-batch coefficients of variation for PFOS were below 10% for all laboratories except 4. The interlaboratory coefficient of variation for PFOS was 23.7%

The between-batch coefficients of variation for PFOA were, in general, higher than for PFOS, and the range in values across laboratories was greater. The within-batch coefficients of variation for PFOA, were, in general, also greater than for PFOS, and laboratory 6's value was much higher than the rest. The interlaboratory coefficient of variation for PFOA was 20.3%

The between-batch coefficients of variation for PFHxS showed a wider range across laboratories than for the other two compounds, though for most laboratories the coefficients were less than 15%. The within-batch coefficients of variation for PFHxS were less than 10% except in laboratory 5. The interlaboratory coefficient of variation for PFHxS was much larger than for the other two compounds, primarily due to the results from laboratory 5. Because the measured values for PFHxS were near the LOQ of each laboratory (Table 1), the greater coefficients of variation compared with the other analytes was expected.

The calculations used to obtain the results shown in Table 3 were repeated after using a logarithmic transformation of the data; the results were not materially different than those shown. We also evaluated the findings after excluding the data from the spiked samples; again the general patterns present were essentially the same as those shown in the Table 3.

Discussion

The acceptable accuracy and precision of the results obtained from the six laboratories was reassuring, and suggests that sensitive epidemiologic studies are possible despite the challenges of quantifying low levels of perfluorinated alkyl compounds in human plasma. The success in the participating laboratories was likely attributable, at least in part, to the stringent procedures used to control and assure the accuracy and precision of measurements.

The interlaboratory coefficients of variation among the six laboratories in our study (PFOS, 23.7%; PFOA 20.3%; and PFHxS, 33.1%) were relatively low compared with those for plasma among 18 laboratories recently reported by van Leeuwen and colleagues (2006) (PFOS, 32%; PFOA, 51%; and PFHxS, 64%). Van Leeuwen et al. found that the coefficients of variation for matrices such as fish liver, fish tissue, and water were even higher. The source of variability among the laboratories' results in that study was not attributable to any specific cause, and they concluded that analytical approaches to perfluorinated alkyl compounds could not be considered routine at this time. But improvements might result with increasing use of stable-isotope labeled internal standards, as used by most of the laboratories in the present study. In addition, standards that are characterized with respect to the percentage of isomers present would allow for greater accuracy when using LC-MS/MS, due to potential differences in response factors for the different isomers. As consensus develops within the analytical community on well characterized external standards, further improvements are expected.

The specific aspects of the techniques in each laboratory that accounted for minor differences in accuracy and precision were beyond the power of this study to identify. Three distinctly different methods of sample preparation were used, yet all the data were acceptably close given the concentration range of the samples. This suggests different techniques can be used as long as appropriate quality control and quality assurance procedures are followed. For example, while 5 of the 6 laboratories utilized matrix matched calibration for LC/MS/MS quantitation (U.S. Food and Drug Administration, 2001), the laboratory that did not use them (lab 1) still obtained acceptable results. Five of the 6 laboratories used labeled internal standards, reflecting what has been considered a major improvement in just the past few years (Martin et al. 2004). We note, however, that both laboratories 2 and 5 were higher than others for PFOS and these were the only two that used 18O PFOS as their internal standard.

We had plasma from only 11 subjects, thus comparison of the perfluorinated alkyl compound levels in our study with results from others is precarious. Nonetheless, the levels for the 11 were similar to those in the National Health and Nutrition Examination Survey 2003-2004 data (Calafat et al., 2007). Calafat et al. (2007) noted that the 2003-2004 values were lower than in the NHANES 1999-2000 data. Olsen et al. (2003; 2007) also have presented U.S. data showing a decrease in PFOS over approximately the same period.

The between-batch coefficients of variation at the levels of perfluorinated alkyl compounds studied were, in general, low compared to those assessed blindly for other persistent organic pollutants, present at about the same levels in serum. For example, the between-batch coefficient of variation for 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) was 19% at 29 ng/mL (Longnecker et al., 2001), and for polychlorinated biphenyls (PCBs) was 19% at 3.5 ng/mL (Daniels et al., 2003). An important issue in epidemiologic studies of the health effects, if any, of exposure at ambient levels is the relative size of between-subject variation in exposure compared with the between-batch coefficient of variation in the measurement. We had too few subjects in our study to derive reliable estimates of between-subject variation in levels of perfluorinated alkyl compounds, but based on the data presented by Olsen et al. (2003), we estimate that the between-subject coefficient of variation for PFOS is at least 33%, for PFOA is at least 34%, and for PFHxS, is at least 43% (assuming that the interquartile distance divided by 2 is roughly equal to the standard deviation). These values, when compared with the corresponding average between-batch variation in measurement across labs in the present study, are relatively large. Thus, the true variation between subjects is enough larger than the error of measurement that present analytical methods could work reasonably well to rank subjects according to level of exposure, especially when the relatively long half life of these compounds is considered (Olsen et al., 2007).

In conclusion, we found that for the laboratories included in our study, the assays were relatively precise. This, considered with the amount of between-subject variation in plasma levels of the main perfluorinated alkyl compounds among subjects with ambient exposure, means epidemiologic estimates of health effects, if any, would undergo little attenuation due to measurement error (Willett, 1990). Thus, epidemiologic studies conducted in conjunction with laboratories such as those characterized here could yield precise effect estimates, assuming that other errors were minimized. While the techniques employed among the laboratories in this study were more than adequate to support epidemiologic research, as consensus develops within the analytical community on well characterized external standards, additional improvements in precision and accuracy will result.

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Figure 1.

Concentration of perfluorinated alkyl compound measured by the six laboratories, according to specimen. Each laboratory conducted 4 determinations per specimen. Gravimetric values of the samples spiked with PFOS and PFOA are shown by horizontal lines. (No specimens were spiked with PFHxS.)

Figure 2.

Overall mean concentration in specimens measured by the six laboratories. Laboratories adjacent to the same vertical line have similar means at $\alpha = 0.05$. For example, for PFOS, laboratories 1, 3, and 4 are not statistically different, whereas laboratories 2, 5, and 6 are each statistically different from laboratories 1, 3, and 4; laboratories 2 and 5 are neither statistically similar to each other nor to any of the other laboratories.

Table 1
Laboratory-specific analytical procedures for measurement of perfluorinated alkyl compounds in plasma

	1	2	3	4	5	6
Sample Handling						
Aliquot volume (μL)	500	100	100	50	200	1,000
SPE column type	Waters Oasis WAX	Waters Oasis HLB	None	None	Waters Oasis HLB	None
Elution solvent(s)	20:80:2 Acetonitrile:methanol: ammonium hydroxide	Acetonitrile	n.a. ^b	n.a.	Methanol	n.a.
Other sample preparation			Protein precipitation	Protein precipitation		Liquid-liquid extraction (ion pairing)
LC/MS-MS						
Injection volume (μL)	5	10	5	5	10	20
Mobile phase	Ammonium acetate: methanol 95:5 to acetone:methanol 80:20 gradient	PFOA: ammonium acetate: acetonitrile 57:43 PFOS & PFHS ammonium acetate: acetonitrile 47:53 isocratic	Ammonium acetate to methanol gradient	Ammonium acetate:methanol 80:20 to 20:80 gradient	Ammonium acetate: acetonitrile 40:60 isocratic	Methanol: ammonium acetate 40:60 to 100% methanol gradient
Stationary phase	C18 2.1×50 mm 3 μm	C18 2.1×100mm 5 μm, base deactivated ACE column	C8 20×40mm 3μm	C18 2×100mm 5 μm	C8 3.0×50mm 5 μm	C18 2.1×50mm 5 μm
Column temperature	Ambient	34 °C	35 °C	30 °C	Ambient	Ambient

	Laboratory					
	1	2	3	4	5	6
Masses monitored						
PFOs	499→99	499→80	499→80	499→99, 499→130, 499→80	499→80	499→99 499→169
PFOA	413→169	413→369	413→369	413→369 413→219 413→169	413→369	413→369 413→169
PFHxS	399→80	399→80	399→80	399→99 399→80	399→80	399→80 399→119
Internal standard	Perfluorododecanoic acid					
PFOs		Dual 180			Dual 180	13C4
PFOA		dual 13C	13C[1, 2]	13C[1, 2]	dual 13C	13C4
Calibration						
Design	Solvent internal standard curve	Matrix matched internal standard curve	Matrix matched external standard curve	Matrix matched external standard curve	Matrix matched internal standard curve	Matrix matched internal standard curve
Matrix/solvent	Solvent ^a	Newborn bovine serum	Rabbit serum	Rabbit serum	Newborn bovine serum	Bovine serum
Data handling/Method performance						
Calibration curve type	Weighted linear	Linear	Quadratic	Quadratic	Weighted linear	Weighted quadratic
Recovery (%)					Not assessed	
PFOs	86 (29.2 ng/mL)	94.0	91	102		87
PFOA	96 (23.1 ng/mL)	93.7	102	103		n.d. ^c
PFHxS	88 (28.1 ng/mL)	93.5	102	102		n.d.

	1	2	3	4	5	6
				Laboratory		
LLOQ (ng/mL)						
PFOs	0.1	1.0	0.5	2.5	1.09	0.3
PFOA	1.0	1.0	0.5	0.5	1.03	0.3
PFHxs	0.1	1.0	0.5	0.25	1.05	0.3
Quality control/quality assurance						
Solvent blanks	Before and after calibration curve, and after each solvent QC	No	No	Multiple ^d	1/batch	1 before the matrix blank, 1 after the highest calibration point
Matrix blanks	Yes	Yes	Yes	Multiple ^e	Yes	1 before the lowest calibration point
QC samples	Solvent QC of 10 ng/mL brackets each group of 10 samples. One spiked matrix control per batch	Yes	Yes	Multiple ^f	2 High and 2 Low/batch	In-house QC sample, 1 replica each 10 samples. This means: 1 before the first sample and 1 after the last one
Duplicates	Two samples per batch, selected from previous batch (10 total)	Yes	Yes	None	2/batch randomly chosen	None

^a A separate recovery experiment was done.

^b n.a., not applicable.

^c n.d., not done.

^d 3 acetonitrile solvent blanks (instrument blanks), 3 extracted H₂O blanks (method blanks), 3 extracted acetonitrile blanks (method blanks).

^e 3 extracted rabbit plasma blanks without surrogate, 3 extracted rabbit plasma blanks with 13C PFOA surrogate, 3 extracted human plasma blanks without surrogate, 3 extracted human plasma blanks with 13C PFOA surrogate.

^f 3 levels of extracted matrix spikes in rabbit plasma in triplicate, and 2 levels of extracted matrix spikes in human plasma in triplicate.

Table 2

Characteristics of the 3 types of specimens sent to each of the 6 laboratories^a

Type	Number of specimens	Source of plasma	Spiked level (ng/mL) ^b					
			#1	#2	#3	PFOA	PFOA	PFOA
U.S. pregnant women	11	human	N.A. ^c	N.A.	N.A.			
Norway pooled	1	human	N.A.	N.A.	N.A.			
Spiked	3	bovine	60	2.5	30	10	20	2.5

^a 4 aliquots of each specimen were sent to each laboratory (60 specimens per lab).

^b Gravimetric spiked concentration.

^c N.A., not applicable.

Table 3

Coefficients of variation (%) in measurements of perfluorinated alkyl compounds

Lab	Within-batch	Between-batch	Interlaboratory
PFOS			
1	9.7	8.4	
2	4.2	6.1	
3	5.7	3.5	
4	12.4	10.8	
5	4.5	13.8	
6	3.3	6.5	
All	9.1	9.3	23.7
PFOA			
1	6.4	8.9	
2	10.5	14.9	
3	7.4	7.6	
4	12.0	9.2	
5	9.4	13.9	
6	23.4	23.8	
All	14.5	14.5	20.3
PFHxS (excludes spiked samples)			
1	3.5	12.2	
2	6.7	9.1	
3	4.4	8.1	
4	2.5	3.4	
5	16.6	34.2	
6	6.7	10.9	
All	14.5	17.0	33.1