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Analysis of PFOA in dosed CD1 mice: Part 1. Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups

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Abstract

The number of studies involving the analysis of perfluorooctanoic acid (PFOA) has increased recently because PFOA is routinely detected in human blood samples from around the world. Recent studies with mice have shown that dosing pregnant dams with PFOA during gestation gives rise to a dose-dependent mortality in the litters, a reduction in neonatal bodyweight for the surviving pups, and subsequent deficits in mammary gland development when compared to control animals. The actual body burdens of PFOA in dams and pups associated with these endpoints have not been determined, in part due to a lack of robust analytical methods for these matrices. The goal of the current study was to develop reliable methods with acceptable performance characteristics for the analysis of PFOA in several matrices relevant to pregnant mouse studies. Dam and pup serum, amniotic fluid, urine, milk, mammary tissue, and whole mouse pups were isolated for method development and analysis. The resulting method provided excellent accuracy (92.1–111%) and reproducibility (relative standard deviation 4.3–21%) making them very useful for future studies. These methods were then applied to dosed animal fluids and tissues in order to conduct a thorough evaluation of the pharmacokinetics *in utero*. Resulting tissue specific measurements of PFOA in serum, amniotic fluid, urine, milk, mammary tissue, and whole pup homogenate will be used to more completely describe the dose–response relationships for the most sensitive health outcomes and inform pharmacokinetic models that are being developed and evaluated.

Keywords

PFOA; Reproductive; Serum; Amniotic fluid; Urine; Milk; Mammary tissue; Whole pups

1. Introduction

Perfluorooctanoic acid (PFOA) has been used in a wide range of industrial products and processes. During the production of fluoropolymers, the PFOA ammonium salt is used as a solubilizer to aid in the polymerization process of polytetrafluoroethylene (PTFE) and

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Conflict of interest statement

None.

polyvinylidene fluoride (PVDF) [1]. PTFE has been used in a wide variety of products such as stovetop cookware, sealant tape, and dental floss [2]. Sinclair et al. have recently shown off-gassing of PFOA from the surface of nonstick cookware, demonstrating that residual PFOA is present in some kitchenware [3]. Human exposure to PFOA may occur through contact with consumer goods [3], and many studies investigating PFOA in the serum of the general public now confirm the widespread distribution of PFOA in the United States and other countries [4–8].

PFOA serum concentrations range between 4.8 and 5.5 ng/mL in the United States general population [4] and are higher than 300 ng/mL in some occupationally exposed workers [7] and among some individuals living in exceptionally contaminated communities [5]. A draft risk assessment for PFOA was conducted by the U.S. Environmental Protection Agency. A peer review by the Science Advisory Board (SAB) indicated that PFOA may adversely affect human health [9]. PFOA has been characterized as a peroxisome proliferator-activated receptor alpha (PPAR- α) agonist in rats, with exposure to leading to hepatotoxicity in rodents [10]. Although PFOA is not expected to cause hepatotoxicity in humans, studies have suggested that PFOA may impact reproductive tissues and be relevant during fetal and neonatal development [11]. One study focusing on the impact of PFOA in mouse reproductive tissues demonstrated altered mammary gland development in female pups, but the disposition of PFOA was not determined due to the lack of analytical methods available at the time of the study [11]. Another study reported decreased body weight gain in mouse neonates following *in utero* exposure [12]. In this study, lactational exposure was linked to reduction in weight gain; however, due to lack of sufficient analytical procedures, the amount of PFOA transferred in the milk was not measured.

Little is known about the potential impact of PFOA on mouse reproductive tissues, but all studies intending to evaluate the consequences of PFOA exposure would benefit greatly from the use of well characterized analytical methods to determine the distribution of PFOA in different tissues and body fluids. While some methods for the measurement of PFOA in mouse blood serum have been published, most lack any information regarding analytical validation (accuracy, precision, etc.). Moreover, methods designed to evaluate other important tissues and fluids have not yet been established.

The objective of the present work was to develop and evaluate liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods for the isolation and measurement of PFOA in mouse serum, amniotic fluid, urine, milk, mammary tissue, and whole pups. To our knowledge, there are no methods for the determination of PFOA in mouse amniotic fluid, urine, milk, mammary tissue, or whole pups in the current literature. The methods described here use a reduced sample volume in order to simplify preparation procedures and maximize throughput, but overall sensitivity is maintained to accurately determine tissue specific concentrations and to distinguish between the various dose groups and controls. This paper is the first to describe the analytical methods for these matrices and to characterize their performance in detail. Data resulting from the use of these new methods are given in a companion paper which explores the tissue distribution of PFOA in gestationally exposed dams and pups [13].

2. Materials and methods

2.1. Chemicals

PFOA (98%) was purchased from Fluka Chemicals (Steinheim, Switzerland) and 1,2-¹³C₂-perfluorooctanoic acid (¹³C₂-PFOA) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA).

2.2. Animals and blank matrices

Timed pregnant female CD-1 mice were purchased from Charles River Laboratories (Raleigh, NC). Sperm-positive females were designated gestation day (GD) 0 and delivered on the same day to the U.S. EPA's Association for Assessment and Accreditation of Laboratory Animal Care facility. Animals were weighed upon arrival and randomly distributed among PFOA treatment groups. Animals were housed individually in polypropylene cages with Alpha-dri (Shepherd Specialty Papers, Kalamazoo, MI) bedding and provided food (LabDiet 5001, PMI Nutrition International LLC, Brentwood, MO) and tap water *ad libitum*. Animal facilities were maintained at 20–24 °C and 40–60% relative humidity with a 14:10-h light-dark cycle. Mice received either water vehicle or a single dose (0.1, 1.0 or 5.0 mg/kg) of PFOA (in water) by oral gavage on gestation day 17 [13]. Animal tissues and fluids were collected as detailed in Fenton et al. [13].

To obtain blank control material for calibrations, matrix blanks, and spiked quality control (QC) samples, hemolyzed Swiss–Webster mouse serum was purchased from Pel-Freeze Biologicals (Rogers, AR). CD-1 mouse milk was purchased from Bioreclamation (Hicksville, NY). Amniotic fluid, urine, mammary tissue, and whole pup matrix blanks were obtained from vehicle dosed CD-1 mice during the study.

2.3. Preparation of standard solutions and quality control (QC) material

Six spiking solutions were prepared by serial dilutions of a 10,000 ng/μL PFOA standard in methanol to obtain final concentrations encompassing the entire linear range for all of the matrices to be analyzed. The spiking solutions were added to blank matrices and the calibration curve concentration ranges chosen for a given analysis depending on the matrix to be analyzed and the dose that each group of mice received. Since PFOA concentrations covered a wide range in each matrix, multiple calibration curves were made to cover the appropriate ranges when necessary. For example, serum samples were analyzed with four separate calibration curves covering a range of 5–25,000 ng/mL (5–100 ng/mL for control animals; 25–1500 ng/mL for the 0.1 mg/kg dose group; 100–5000 ng/mL for the 1 mg/kg dose group; 500–25,000 ng/mL for the 5 mg/kg dose group) (Table 1). QC materials were prepared from serum, amniotic fluid, urine, milk, mammary tissue, and whole pups, by enrichment with PFOA to obtain QC pools at multiple levels (Table 1). Because limited volumes of control amniotic fluid were available, only two different amniotic fluid pools were prepared. Both pools were enriched with PFOA to obtain low and high QC pools. Because serum concentrations were expected to cover a wider range, eight different QC pools were prepared from hemolyzed Swiss–Webster mouse serum. All remaining QC pools (urine, milk, mammary tissue and whole pup) had 3–4 QC pools prepared (Table 1). All QC pools were prepared in advance and stored at –20 °C until used.

2.4. Chromatographic and mass spectrometer conditions

The analysis of PFOA was performed using a Waters Acquity™ Ultra Performance Liquid Chromatograph interfaced with a Waters Quattro Premier XE triple quadrupole mass spectrometer (UPLC-MS/MS) (Waters, Milford, MA). Either 40 μL (for serum and amniotic fluid) or 20 μL (for all other matrices) aliquots of the sample were injected onto an Acquity UPLC® BEH C18 column (2.1 mm i.d. × 50 mm, 1.7 μm; Waters, Milford, MA). For serum and amniotic fluid, the mobile phase was held at 70% acetonitrile: 30% 2 mM ammonium acetate in water at a constant flow of 0.3 mL/min for 1.5 min. For urine, milk, mammary tissue, and whole pups, a gradient mobile phase, consisting of 2 mM ammonium acetate aqueous solution with 5% methanol (solvent A) and 2 mM ammonium acetate in methanol (solvent B), starting with 50% solvent A at a constant flow rate of 0.5 mL/min was used. The gradient was increased to 90% solvent B at 1.75 min and 100% solvent B at 1.8 min and held for 0.5 min. At 2.3 min the gradient was returned to the original conditions and held

until 3.0 min. Both chromatographic separations (isocratic/gradient) were found to be acceptable for the purposes of this work. Electrospray negative ionization was used in the mass spectrometer source. The capillary voltage, for PFOA and $^{13}\text{C}_2$ -PFOA, was set at negative 0.4 kV. Cone gas and desolvation gas flows were 2 and 1200 L/h, respectively. The source temperature was 150 °C and the desolvation temperature was 350 °C. Transitions for all ions were observed using multiple reaction monitoring (MRM) and analyte-specific mass spectrometer parameters were optimized for each compound. For quantitation purposes, the mass transition of 412.60 $\rightarrow$ 368.70 was used for PFOA. The second mass transition, 412.60 $\rightarrow$ 168.65, was monitored for confirmation of PFOA. The transition used for the quantification of $^{13}\text{C}_2$ -PFOA was 414.75 $\rightarrow$ 369.70.

2.5. Preparation and extraction of samples

2.5.1. Serum—The serum method was adapted from a previous method used to determine the concentration of PFOA in mouse serum, described elsewhere [14,15]. Briefly, 25–50 μL of mouse serum was transferred into 15 mL polypropylene tubes. An appropriate amount of internal standard ($^{13}\text{C}_2$ -PFOA) was added to approximate the midpoint of the calibration curve for the anticipated sample range. For example, for serum samples expected to be in the 100–1500 ng/mL range approximately 20 ng of $^{13}\text{C}_2$ -PFOA was added to 25 μL of serum, approximating the midpoint of the calibration curve (800 ng/mL). Formic acid (0.1 M) was added to the serum to denature proteins at a volume of at least twice the serum volume. The sample was vortex mixed and cold (–20 °C) acetonitrile was added to precipitate proteins in a volume of at least 10 times the formic acid volume used above. The sample was then vortex mixed and centrifuged at $2000 \times g$ for 3 min. An aliquot of the acetonitrile supernatant was placed in a LC vial with 2 mM ammonium acetate buffer (pH 6.5) (1:1), and the PFOA concentrations were determined using UPLC–MS/MS analysis.

The volume of formic acid and acetonitrile in the protein precipitation step may be altered to increase the dilution factor for samples that are expected to be high in PFOA concentration. In some instances, it is necessary to dramatically dilute dosed animal serum samples in order to prevent carry-over contamination of analytical equipment with PFOA. The analyst should determine the range of acceptable on-column mass of analyte (limit of detection to maximum allowable) to maintain adequate sensitivity while not overwhelming the instrumentation with carry-over contamination. It should be noted that the formic acid/acetonitrile ratio should not exceed 1/9. A greater proportion of formic acid leads to poor protein precipitation and matrix-rich extracts which could lead to an under or overestimation of the concentration of PFOA during UPLC–MS/MS.

2.5.2. Amniotic fluid—Amniotic fluid methods were adapted from the above serum method. Twenty-five microliters of amniotic fluid were transferred into 15 mL polypropylene Falcon tubes (Becton Dickinson, Franklin Lakes, NJ). One hundred microliters of methanol containing 0.1 ng $^{13}\text{C}_2$ -PFOA/ μL and 0.5 mL formic acid (0.1 M) were added to the amniotic fluid sample. The sample was vortex mixed and 5 mL of acetonitrile was added to precipitate proteins. The sample was vortex mixed again and then centrifuged at $2000 \times g$ for 3 min. A 200- μL aliquot of the sample was placed in a LC autosampler vial with 200 μL of 2 mM ammonium acetate buffer (pH 6.5). PFOA concentrations were determined by UPLC–MS/MS analysis.

2.5.3. Urine and milk—Twenty microliters of urine or milk was mixed with 1 mL of 0.1 M formic acid containing 10 ng of $^{13}\text{C}_2$ -PFOA in a 1.7 mL polypropylene micro centrifuge tube. After vigorous vortexing, each sample was loaded onto an Oasis® Weak Anion-Exchange (WAX, 3 cm^3 , 60 mg, 60 μm) solid phase extraction (SPE) cartridge (Waters, Milford, MA) that had been preconditioned with 4 mL of methanol, followed by 4 mL of

deionized (DI) water. The empty microcentrifuge tube was rinsed with 0.5 mL of 0.1 M formic acid and the rinse loaded onto the same WAX cartridge. The cartridge was washed with 4 mL of 25 mM sodium acetate buffer (pH 4) followed by 4 mL of methanol, and then dried under vacuum. PFOA was eluted from the cartridge with 1 mL of methanol containing a 1% (v/v) ammonium hydroxide solution. The eluate was reduced in volume down to approximately 200 μ L in a Zymak TurboVap LV sample concentrator (Caliper Life Sciences, Mountain View, CA). The concentrated eluate was centrifuged at $16,800 \times g$ for 5 min to settle any floating debris, and 100 μ L of this supernatant was mixed with 100 μ L of 2 mM ammonium acetate buffer (pH 6.5) and vortex mixed and analyzed by UPLC–MS/MS.

2.5.4. Mammary tissue and whole pups—The analytical procedure for the analysis of mammary tissue and whole pups was a modification of a method described previously [16]. Briefly, mammary tissue or whole pups were weighed and DI water was added to the sample at a ratio of 3 mL water per gram of mouse tissue. The sample was homogenized using a 10/35 PT Polytron homogenizer (Brinkmann Instruments, Westbury, NY). A portion (0.5 mL) of the homogenate was transferred into a clean pre-weighed 15 mL polypropylene Falcon tube and the net weight of the homogenate aliquot was determined by reweighing the tubes. Two milliliters of 0.01 M NaOH in methanol was added and the polypropylene tubes were sonicated for 30 min at room temperature followed by centrifugation at $2000 \times g$ for 5 min. Three hundred microliters of the digested extract supernatant were transferred into a clean 15 mL polypropylene tube, diluted 1:10 with 2.7 mL of DI water containing 10 ng of $^{13}\text{C}_2$ -PFOA, and vortex mixed.

SPE cleanup was then performed on the diluted digest extract using WAX (3 cm³, 60 mg, 60 μ m) cartridges. Cartridges were conditioned using 3 mL of methanol followed by 3 mL of DI water. The entire 3 mL aliquot of diluted digest extract was loaded onto a WAX cartridge and the sample was washed using 3 mL of 25 mM sodium acetate buffer (pH 4.0) followed by 3 mL of methanol. The sample was eluted using 3 mL of 0.1% ammonium hydroxide (NH₄OH) in methanol. A 200- μ L aliquot of the eluate was transferred to a LC vial containing 200 μ L of 2 mM ammonium acetate aqueous solution and vortex mixed. The samples were then analyzed using UPLC–MS/MS.

2.6. Quality control

Calibration curves were generated by plotting the ratio of PFOA peak area to $^{13}\text{C}_2$ -PFOA peak area versus concentration and were fitted to a linear regression equation with $1/x$ weighting. Depending on the matrix and PFOA concentration, batch specific calibration curves were prepared to obtain linear curves in the range from 1 to 25,000 ng/g or ng/mL. For example, for serum, 4 different curves were prepared to cover the range of expected concentrations (Table 1). A minimum of six standards were used to generate each calibration curve and the coefficients of determination (r^2) values were 0.99 or greater for each analysis. The limit of quantitation (LOQ) for the assays was defined as the lowest point on the standard curve that had back prediction of within $\pm 30\%$ of the theoretical value. Matrix matched standard curves and QA/QC pools were used to minimize potential errors associated with matrix enhancement or suppression of analyte signal due to co-eluting matrix interferences. QC pools were prepared at a minimum of 2 levels (low and high) expected to represent the range of the unknown samples for each matrix (Table 1).

Method accuracy and precision were determined by analyzing the QC samples repeatedly, using the complete analytical method (i.e., extraction, SPE cleanup, UPLC–MS/MS). Accuracy was calculated as the percentage of the concentration found compared with the theoretical concentration. The precision of the methods were determined by calculating the

average relative standard deviation (R.S.D.) of the replicate analysis of the QC materials (Table 1).

Each sample batch of tissue or fluids contained a maximum of 60 unknowns (10% of the unknown samples run in replicate) along with at least 10% QC samples. Each sample batch also included a minimum of six calibration standards, a matrix blank, and a methanol blank. All calibration curve standards were prepared in blank matrix matching the unknown samples. Blanks, calibration curve standards, and QCs were all subjected to the same preparation procedure as unknown samples, as detailed in Sections 2.5.1–2.5.4. QC samples were intermixed with unknown samples. Acceptance criteria specified that if a single QC sample exceeded $\pm 30\%$ of the theoretical value, or there was an aggregate R.S.D. of $\pm 30\%$, the entire batch was to be rejected and new aliquots of the samples would be put through the sample preparation procedure again and rerun.

3. Results

3.1. Evaluation of method performance

The LOQ was determined to be 5.0 ng/mL for serum and 1.0 ng/mL for amniotic fluid, urine, and milk. The LOQ for mammary tissue and whole pups was determined to be 1.0 ng/g. The chromatograms of PFOA extracted from spiked serum, amniotic fluid, urine, milk, mammary tissue, and whole pups at the LOQ (Fig. 1) always had a signal to noise ratio (S/N) of at least 30 (Fig. 1). Average accuracy and precision of QC samples were found to be acceptable for each of the various matrices with accuracies ranging between 92.1 and 111% and the average R.S.D. values (precision) ranging from 4.3 to 21% (Table 1). Replicate analysis of unknown samples showed good agreement, with variation between samples being consistently less than 20%. QC samples never exceeded $\pm 30\%$ of the theoretical value during the analysis period, or an R.S.D. of $\pm 30\%$ with the highest R.S.D. of 21.0% found in mammary tissue (Table 1).

3.2. Application of the assays

The methods described above were applied in a study involving a single dose of PFOA by oral gavage given to pregnant to CD-1 mice at gestation day 17 [13]. Resulting data are provided in the companion paper [13].

4. Discussion

This is the first study to describe analytical techniques that have been developed for the analysis of PFOA in mouse amniotic fluid, urine, milk, mammary tissue, and whole pups. Moreover, there are few studies that measure PFOA in mouse serum [11,12,14,17], but these studies show very little, if any, method performance parameters in the manuscripts. Given the challenges associated with measuring PFOA in complex biological matrices [18], it is necessary to develop methods that are well characterized for the specific matrices in order to obtain reliable results. The methods described here help to reduce uncertainties associated with the analytical determination. Because the method performance characteristics have been thoroughly evaluated, researchers can have confidence in the data obtained and can use the data to assess even subtle differences in tissue concentrations that may arise in reproductive and other investigations.

The methods presented here were for the purpose of measuring PFOA in different reproductive mouse matrices. These studies provide accurate and precise information on internal dose, excretion, and other pharmacokinetic endpoints following a 1-day gestational exposure to PFOA. Because sample quantity available from animals can sometimes be a limiting factor for establishing analytical methods, it is important to develop methods that

use a minimal amount of sample. The methods described here are sensitive and have reliable performance characteristics for the analysis of PFOA in dosed mice. The methods presented offer a wide linear range that allows quantification from 1 to 25,000 ng/g or ng/mL. The average accuracies, for all methods, were between 92.1 and 111%, showing good precision (4.3–21%) R.S.D. for the different matrices. We established methods that were sensitive enough to successfully meet the demands of a reproductive study (high sample throughput, adequate sensitivity [low LOQ], and small sample volumes). The methods presented here can be used for future mouse reproductive studies that would measure PFOA in serum, amniotic fluid, urine, milk, mammary tissue, and whole pups and should help to address areas of uncertainty dealing with the potential reproductive health effects associated with PFOA exposure through different exposure routes (*in utero*, lactational).

It is possible that applications of these developed methods to other similar perfluorinated compounds and/or other biological species would lead to comparable results. However, this would have to be confirmed and evaluated in a similar exercise before application to samples. This work should be useful as a point of departure in the development of novel methods for other perfluorinated compounds not yet investigated.

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References

1. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. Sources, fate and transport of perfluorocarboxylates. *Environ Sci Technol*. 2006; 40:32–44. [PubMed: 16433330]
2. Begley TH, White K, Honigfort P, Twaroski ML, Neches R, Walker RA. Perfluorochemicals: potential sources of and migration from food packaging. *Food Addit Contam*. 2005; 22:1023–1031. [PubMed: 16227186]
3. Sinclair E, Kim SK, Akinleye HB, Kannan K. Quantitation of gas-phase perfluoroalkyl surfactants and fluorotelomer alcohols released from nonstick cookware and microwave popcorn bags. *Environ Sci Technol*. 2007; 41:1180–1185. [PubMed: 17593716]
4. Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. Serum concentrations of 11 polyfluoroalkyl compounds in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES). *Environ Sci Technol*. 2007; 41:2237–2242. [PubMed: 17438769]
5. Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. *J Occup Environ Med*. 2006; 48:759–770. [PubMed: 16902368]
6. Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, et al. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol*. 2004; 38:4489–4495. [PubMed: 15461154]
7. Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, et al. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. *Environ Health Perspect*. 2003; 111:1892–1901. [PubMed: 14644663]
8. Olsen GW, Huang HY, Helzlsouer KJ, Hansen KJ, Butenhoff JL, Mandel JH. Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ Health Perspect*. 2005; 113:539–545. [PubMed: 15866760]

9. SAB Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts. 2006 May 30. http://www.epa.gov/sab/pdf/sab_06_006.pdf. More information of Draft PFOA Risk assessment can be found at <http://www.epa.gov/opptintr/pfoa/pubs/pfoarisk.htm>.
10. Intrasukri U, Rangwala SM, O'Brien M, Noonan DJ, Feller DR. Mechanisms of peroxisome proliferation by perfluorooctanoic acid and endogenous fatty acids. *Gen Pharmacol*. 1998; 31:187–197. [PubMed: 9688458]
11. White SS, Calafat AM, Kuklenyik Z, Villanueva L, Zehr RD, Helfant L, et al. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol Sci*. 2007; 96:133–144. [PubMed: 17132714]
12. Wolf CJ, Fenton SE, Schmid JE, Calafat AM, Kuklenyik Z, Bryant XA, et al. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol Sci*. 2007; 95:462–473. [PubMed: 17098816]
13. Fenton SE, Reiner JL, Nakayama SF, Delinsky AD, Stanko JP, Hines EP, et al. Analysis of PFOA in dosed CD-1 mice. Part 2: Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups. *Reprod Toxicol*. 2009; 27:365–372. [PubMed: 19429407]
14. DeWitt JC, Copeland CB, Strynar MJ, Luebke RW. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ Health Perspect*. 2008; 116:644–650. [PubMed: 18470313]
15. Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, et al. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol Sci*. 2006; 90:510–518. [PubMed: 16415327]
16. Taniyasu S, Kannan K, So MK, Gulkowska A, Sinclair E, Okazawa T, et al. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *J Chromatogr A*. 2005; 1093:89–97. [PubMed: 16233874]
17. Loveless SE, Finlay C, Everds NE, Frame SR, Gillies PJ, O'Connor JC, et al. Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology*. 2006; 220:203–217. [PubMed: 16448737]
18. van Leeuwen SP, Kärrman A, van Bavel B, de Boer J, Lindström G. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples. *Environ Sci Technol*. 2006; 40:7854–7860. [PubMed: 17256538]

Fig. 1.
Representative chromatograms for PFOA resulting from the analysis of: (a) methanol blank, (b) solvent standard, (c) serum, (d) amniotic fluid, (e) urine, (f) milk, (g) mammary tissue, and (h) whole pup.

Table 1

Summary of method performance parameters for all matrices and target concentrations.

Matrix	LOQ	Average Accuracy ^a (%)	Average R.S.D. ^a (%)	Number of calibration curves (range)	QC pool target values
Serum ^a	5.0	97.0	4.30	4(5–25,000)	25; 75; 500; 1,000; 5,000; 10,000; 25,000
Amniotic fluid ^b	1.0	106	9.81	2(10–10,000)	10; 1,000
Urine ^b	1.0	95.0	5.48	2(1–5,000)	5; 50; 100; 1,000
Milk ^b	1.0	92.1	11.0	3(1–2,500)	5; 50; 500; 5,000
Mammary Tissue ^c	1.0	111	21.3	4(1–2,500)	5; 50; 100
Whole pup ^c	1.0	101	19.6	3(1–1,000)	5; 100; 250

^a Average accuracy and %R.S.D. calculated from analysis of all QC samples for each individual matrix.

^b Concentrations of serum, amniotic fluid, urine, and milk are in ng/mL.

^c Concentrations of mammary tissue and whole pups are in ng/g.