Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013–2014 National Health and Nutrition Examination Survey

Antonia M. Calafat, Kayoko Kato, Kendra Hubbard, Tao Jia, Julianne Cook Botelho, Lee-Yang Wong

Supporting Information

**Quantification of C4-C11 PFAS and three fluorinated alternatives in urine**

The analytical method was described before (Kato et al., 2018). For full method-specific details, please consult the publication. Some general aspects of the method are described below.

Urine preparation

Urine is dispensed (50 µL) into a polypropylene snap-cap autosampler vial, spiked with the internal standard containing a mixture of the isotopically labeled analytes (13C3-PFBS, 18O2-PFHxS, 13C4-PFOS, 13C2-PFBA, 13C5-PFPeA, 13C2-PFHxA, 13C5-PFHpA, 13C4-PFOA, 13C5-PFNA, 13C2-PFDA, 13C2-PFUnDA, 13C3-HFPO-DA, D3-MeFOSAA, D5-EtFOSAA, and 18O2-FOSA), β-glucuronidase in ammonium acetate buffer, and incubated at 37 °C for 240 min for the enzymatic hydrolysis of urinary conjugates, followed by the addition of formic acid.

Instrumental analysis

For preconcentration of the target analytes, the pretreated urine sample is loaded onto a SPE Oasis WAX 30 mm, 10mm×1mm (Waters) cartridge on a Symbiosys PICO (Spark Holland, VE Emmen, The Netherlands) coupled with a Sciex Triple Quad 5500 or Qtrap 6500 mass spectrometer (Sciex, Framingham, MA, USA). Elution with NH4OH in MeOH transfers the analytes onto a Chromolith® HighResolution RP-18e guard column (4.6 × 5 mm) followed by a Chromolith® High-Resolution RP-18e column (4.6 × 25 mm) (Merck KGaA, Germany). The analytes are chromatographically resolved using a gradient of ammonium acetate (pH 4.0) in acetonitrile (mobile phase A) and acetonitrile (mobile phase B) at 1000 µL/minute, and detected using negative ion TurboIonSpray ionization. Data acquisition uses Analyst 1.6.2 software, and data analysis MultiQuan 3.0.2 software.

Quality control and daily operation

Analytes are quantified using calibration curves of the peak area ratio of each analyte to its corresponding isotopically labeled internal standard (or 13C3-PFBS for PFPrS, 13C4-PFOS for PFHpS, 13C2-PFHxA for DONA, 13C2-PFUnDA for 9Cl-PF3ONS) versus standard concentration.

Daily calibration curves include nine standard solutions for each analyte, spiked into 0.1M formic acid, that encompass the entire linear range of the method (0.07-20 ng/mL). Two low concentration quality control materials (QCs), two high concentration QCs, and three reagent blanks (made using 0.1M formic acid) are analyzed concurrently with 20 samples and the calibration standards. For each analytical run, concentrations of reagent blanks are examined (of note, PFAS were not detected in any of the reagent blanks analyzed). Also, the concentrations of the QCs, averaged to obtain one measurement of high- and one of low-concentration QC for each run, are evaluated by use of standard statistical probability rules (Caudill et al., 2008).

Kato K, Kalathil AA, Patel AM, Ye X, Calafat AM (2018) Per- and polyfluoroalkyl substances and fluorinated alternatives in urine and serum by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Chemosphere 209:338-345.

Caudill SP, Schleicher RL, Pirkle JL (2008) Multi‐rule quality control for the age‐related eye disease study. Stat. Med. 27, 4094e4106.

Table S1. Estimated proportion of the U.S. general population with detectable urinary concentrations of PFAS (NHANES 2013–2014)

|  | **Estimated percent of the U.S. general population with detectable PFAS** | | |
| --- | --- | --- | --- |
|  |  | **Paired urine-serum** | |
| **No of detected PFAS** | **People ≥6 years** | **Adults 12+** | **Children 6-11** |
| 3 | 0.6 | 0.5 | 6.1 |
| 2 | 4.7 | 5.0 | 15.8 |
| 1 | 27.2 | 27.0 | 42.6 |
| 0 | 67.5 | 67.5 | 35.5 |
|  |  |  |  |

Figure S1. Estimated proportion of the U.S. general population 6 years of age and older with detectable urinary concentrations of 17 PFAS in urine (2013­­–2014 NHANES)

