

**National Health and Nutrition  
Examination Survey 2001–2002**

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**Documentation, Codebook,  
and Frequencies**

**Polyfluoroalkyl Chemicals (NHANES  
Surplus Serum – Pooled Samples)**

**Laboratory  
Surplus Sera**

**Survey Years:  
2001 to 2002**

**SAS Export File:  
PFC\_Pool.XPT**



October 2008

# NHANES 2001–2002 Data Documentation

## Laboratory Assessment: Polyfluoroalkyl Chemicals (NHANES Surplus Serum – Pooled Samples)

First Published: October 2008

Last Revised: N/A

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### Component Description

The primary objective of this study was to characterize serum concentrations of selected polyfluoroalkyl chemicals (PFCs) in pooled serum samples collected from children 3-11 years old participants in NHANES 2001-2002.

PFCs have been used extensively since the 1950s in commercial applications, including surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams. Some of these PFCs, including perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), persist in humans and the environment and have been detected worldwide in wildlife. Because of the known animal toxicity of several PFCs; their ubiquitous presence; and their persistence in humans, wildlife, and the environment, PFCs research is of interest. Biomonitoring data for these PFCs in the general population are needed to assess human exposures to these compounds.

By using a modification of an existing analytical method (Kuklennyik et al. 2005), the following 11 PFCs were measured: perfluorooctane sulfonamide (PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorobutane sulfonic acid (PFBuS), perfluorohexane sulfonic acid (PFHxS), PFOS, perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), and perfluorododecanoic acid (PFDoA).

### Eligible Sample

Study participants aged 3-11 years from of NHANES 2001 - 2002 with stored serum available to prepare pooled samples.

### Description of Laboratory Methodology

Serum samples from NHANES 2001-2002 were stored frozen before analysis. The eleven PFCs were measured by using on-line solid-phase extraction (SPE) coupled to isotope dilution-high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The following isotope-labeled internal standards were used for quantification: 18O2-PFOS (PFOS, PFBuS and PFHxS), 13C2-PFOA

(PFOA, PFHpA), <sup>13</sup>C<sub>5</sub>-PFNA (PFNA), <sup>13</sup>C<sub>2</sub>-PFDeA (PFDeA and PFDoA), <sup>18</sup>O<sub>2</sub>-PFOSA (PFOSA), D<sub>3</sub>-Me-PFOSA-AcOH (Me-PFOSA-AcOH), and D<sub>5</sub>-Et-PFOSA-AcOH (Et-PFOSA-AcOH). Briefly, 0.25 mL of 0.1 M formic acid and 0.025 mL of internal standard solution were added to 0.1 mL of serum, and the spiked serum was vortex-mixed and sonicated. The samples were placed on a commercial Symbiosis on-line SPE system (Spark Holland, Plainsboro, NJ) for the preconcentration of the analytes on a Polaris C18 cartridge (7 µm, 10 × 1 mm; Spark Holland). The analytes were transferred onto a Betasil C8 HPLC column (3 × 50 mm, 5 µm; ThermoHypersil Keystone, Bellefonte, PA), separated by HPLC (mobile phase A: 20 mM ammonium acetate in water, pH = 4; mobile phase B: methanol), and detected by negative-ion Turbolonspray-MS/MS on an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). The limits of detection ranged from 0.1 ng/mL to 0.4 ng/mL.

### **Laboratory Quality Control and Monitoring**

CDC's laboratory is CLIA '88 certified and practices all quality control (QC) and assurance procedures dictated by this certification. QC procedures included the daily analysis of characterized serum pools and the periodic analysis of proficiency testing materials. Low-concentration (QCL; ~3 ng/mL to ~9 ng/mL, depending upon the analyte) and high-concentration (QCH; ~10 ng/mL to ~30 ng/mL, depending upon the analyte) QC materials were prepared from a base calf serum pool, dispensed in 3-mL aliquots and stored at -20 °C. QC materials were characterized through repeated measurements spanning at least 3 weeks, to define the mean concentrations and the 95% and 99% control limits of PFCs. Calibration standards, 2 QCH, 2 QCL, reagent and serum blanks were analyzed with the samples. The concentrations of the two QCH and the two QCL were averaged to obtain one measurement of QCH and of QCL per batch; these concentrations were evaluated using standard statistical probability rules.

### **Data Processing and Editing**

Specimens were processed, stored, and shipped to DLS, NCEH, CDC (Atlanta, Georgia). The analytical approach used, including data processing, was a modification of a method described in detail in a peer-reviewed publication (Kuklennyik et al. 2005). Reported results met the Division of Laboratory Sciences' quality control and quality assurance performance criteria for accuracy and precision (similar to specifications outlined by Westgard (1981)).

## **Analytic Notes**

To prepare the pools, residual serum, collected from the 3–11 year old NHANES 2001–2002 participants and previously analyzed for cotinine, a marker of environmental tobacco smoke, was used. The 1049 individual samples available were categorized in 12 demographic groups, each representing a combination of race/ethnicity, sex, and age (3–5 years and 6–11 years). A total of 937 randomly selected individual samples were used to prepare 24 pools (two per demographic group). To ensure that no individual sample overly influenced the pooled results, all samples included in any one pool were of equal volume (i.e., 0.5 mL). Eleven of the individual serum samples from non-Hispanic black females 3–5 years of age contributed <0.5 mL. All pools included 21 (3–5 year old) or 57 (6–11 year old) individual samples randomly selected.

The limits of detection (LODs) were 0.1 ng/mL (PFOSA, PFBuS, PFHxS, PFOA, and PFNA), 0.2 ng/mL (PFOS, Me-PFOSA-AcOH, Et-PFOSA-AcOH, PFDeA, PFUA, and PFDoA), and 0.4 ng/mL (PFHpA). The detection limit divided by the square root of 2 is the value provided for results that are below the limit of detection.

## **Variables**

### **PFCANA**

Et-PFOS-A, 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid  
Me-PFOS-A, 2-(N-methyl-perfluorooctane sulfonamido) acetic acid  
PFBuS, perfluorobutane sulfonic acid  
PFDeA, perfluorodecanoic acid  
PFDoA, perfluorododecanoic acid  
PFHpA, perfluoroheptanoic acid  
PFHxS, perfluorohexane sulfonic acid  
PFNA, perfluorononanoic acid  
PFOA, perfluorooctanoic acid  
PFOS, perfluorooctane sulfonic acid  
PFOSA, perfluorooctane sulfonamide

### **PFCRACE**

1= non-Hispanic white  
2=non-Hispanic white  
3=Hispanic

### **PFCGENDER**

1=male  
2=female

**PFCAGE**

3= 2-5 years

6= 6-11 years

**PFCPOOL**

1=Pool#1

2=Pool#2

**PFCMNT**

0= at or above the detection limit

1=below the detection limit

Please refer to the Analytic Guidelines for further details on the use of sample weights and other analytic issues.

**References**

Kuklenyik Z, Needham LL, Calafat AM (2005) Measurement of 18 Perfluorinated Organic Acids and Amides in Human Serum Using On-Line Solid-Phase Extraction. *Anal Chem* 77:6085-6091.

Westgard JO, Barry PL, Hunt MR, Groth T. 1981. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 27: 493-501.

## Locator Fields

**Title:** Measurement of polyfluoroalkyl compounds (NHANES 2001 - 2002 surplus serum pools)

**Contact Number:** 1-770-488-7891

**Years of Content:** 2001–2002

**First Published:** October 2008

**Last Revised:** N/A

**Access Constraints:** None

**Use Constraints:** None

**Geographic Coverage:** National

**Subject:** Polyfluoroalkyl compounds

**Record Source:** NHANES 2001–2002

**Survey Methodology:** NHANES 2001–2002 is a stratified multistage probability sample of the civilian non-institutionalized population of the U.S.

**Medium:** NHANES Web site; SAS transport files

**National Health and Nutrition Examination Survey  
Codebook for Data Production (2001-2002)**

**Polyfluoroalkyl Chemicals (NHANES Surplus Serum - Pooled  
Samples) (PFC\_Pool)  
Person Level Data**

October 2008



<b>PFCANA</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Analyte Abbreviated Name			
<b>English Text:</b> Analyte Abbreviated Name				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
Et-PFOA-AcOH	Et-PFOA-AcOH	24	24	
Me-PFOA-AcOH	Me-PFOA-AcOH	24	48	
PFBuS	PFBuS	24	72	
PFDeA	PFDeA	24	96	
PFDoA	PFDoA	24	120	
PFHpA	PFHpA	24	144	
PFHxS	PFHxS	24	168	
PFNA	PFNA	24	192	
PFOA	PFOA	24	216	
PFOS	PFOS	24	240	
PFOSA	PFOSA	24	264	
< blank >	Missing	0	264	



<b>PFCRACE</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Race			
<b>English Text:</b> Race				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
1	non-Hispanic white	88	88	
2	non-Hispanic black	88	176	
3	Hispanic	88	264	
.	Missing	0	264	

<b>PFCGENDR</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Gender			
<b>English Text:</b> Gender				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
1	male	132	132	
2	female	132	264	
.	Missing	0	264	

<b>PFCAGE</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Age			
<b>English Text:</b> Age				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
3	3-5 years	132	132	
6	6-11 years	132	264	
.	Missing	0	264	

<b>PFCPOOL</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Pool Number			
<b>English Text:</b> Pool Number				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
1	Pool #1	132	132	
2	Pool #2	132	264	
.	Missing	0	264	

<b>PFCAMNT</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Amount (ng/ml)			
<b>English Text:</b> Amount (ng/ml)				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0.1 to 52.4	Range of Values	264	264	
.	Missing	0	264	

<b>PFCCMT</b>	<b>Target</b>			
	B(3 Yrs. to 11 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Comment Code			
<b>English Text:</b> Comment Code				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0	at or above the detection limit	195	195	
1	below the detection limit	69	264	
.	Missing	0	264	