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Effect of temperature and duration of storage on the stability of polyfluoroalkyl chemicals in human serum

Kayoko Kato*, Lee-Yang Wong, Brian J. Basden, and Antonia M. Calafat

Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Abstract

We assessed the potential impact of temperature on the long-term stability of several polyfluoroalkyl chemicals in serum. We evaluated the concentrations of perfluorooctane sulfonate, perfluorohexane sulfonate, perfluorooctanoate and perfluorononanoate in 16 human serum samples stored at room temperature, 5 °C, –20 °C and –70 °C at several time points during an eight month period. Concentrations of the target analytes remained unchanged under all studied conditions, even when serum was kept at room temperature for 10 days.

Keywords

Polyfluoroalkyl chemicals; Stability; Storage temperature; Biomonitoring

1. Introduction

Polyfluoroalkyl chemicals (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are man-made chemicals that have been used in a wide range of products since 1950 (Key et al., 1997; Lindstrom et al., 2011). The remarkable strength of the fluorine-carbon covalent bond makes PFCs thermally and chemically stable. These unique properties make some PFCs highly resistant to both chemical and biological degradation under normal environmental conditions (Lau et al., 2007; Awad et al., 2011). However, unlike other persistent organic pollutants (e.g., organochlorine compounds), which are lipophilic (CDC, 2009), the unique surfactant nature of PFCs results in strong PFCs-protein interactions, and PFCs concentrations are greatest in body compartments high in protein content, such as the liver, kidney, and blood (Bischel et al., 2010). The global occurrence of certain PFCs (Suja et al., 2009; Ahrens, 2011), persistence in the environment and bioaccumulation in biota have raised concerns about exposures to PFCs (Fromme et al., 2009; White et al., 2011).

*Corresponding author. Address: Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Mailstop F53, Atlanta, GA 30341, USA. Tel.: +1 770 488 7295; fax: +1 770 488 4371. kkato1@cdc.gov (K. Kato).

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Biomonitoring or the measurement of trace levels in humans and wild animals of PFCs has gained importance for the exposure assessment of these compounds (Houde et al., 2006; Kato et al., 2011; Lindstrom et al., 2011; Reiner et al., 2011). The use of bio-monitoring in environmental epidemiology involves collection, shipping, preservation and storage of biological specimens. Any one of these processes may influence the integrity of the specimens and compromise their future analyses. Therefore, we assessed the potential impact of temperature on the long-term (i.e., months) stability of several PFCs in human sera. Specifically, we investigated the stability of four PFCs in human serum for a period up to 240 days at controlled temperatures and up to 10 days at room temperature.

2. Materials and methods

2.1. Human serum specimens

We purchased 16 serum samples, collected between 1998 and 2003, from 5 male and 11 female donors, from Tennessee Blood Services (Memphis, TN). We had no access to other demographic data, including information regarding the donors' potential exposure to the target PFCs or information on the collection and storage methods until the samples reached our laboratory. Upon receipt, we stored the frozen samples at -70°C until use.

For the present study, we thawed the samples, split each one into four equal aliquots (5 mL each), and dispensed them into 5-mL polypropylene tubes. We stored the aliquots at four different temperatures: room temperature (aliquot I), 5°C (aliquot II), -20°C (aliquot III), and -70°C (aliquot IV). We chose to evaluate storage at room temperature to reflect the worst-case scenario (e.g., shipping delays) that may occur during the collection, shipping and handling of the serum specimens before their long-term storage at controlled temperatures.

2.2. Analytical method

By using a modification of our analytical method (Kuklenyik et al., 2005), we measured the following four PFCs: perfluorohexane sulfonate (PFHxS), PFOS, PFOA, and perfluorononanoate (PFNA). We used the following isotope-labeled internal standards for quantification: $^{18}\text{O}_2$ -PFOS (for PFOS and PFHxS), $^{13}\text{C}_2$ -PFOA, and $^{13}\text{C}_5$ -PFNA. To compensate for the lack of stable isotope-labeled internal standard for PFHxS and to account for potential matrix effects, we spiked the calibration standards into calf serum (Gibco, Grand Island, NY). Briefly, we added 275 μL of 0.1 M formic acid and 25 μL of internal standard solution to 100 μL of serum in a 1.5 mL polypropylene autosampler vial, and the spiked serum was vortex-mixed. The sera vials were placed on a Symbiosis on-line SPE system (Spark Holland, Plainsboro, NJ) for the pre-concentration 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 \times 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 TurboIonspray-tandem mass spectrometry on an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). The limits of detection (LOD) were 0.1 ng/mL for PFHxS, PFOA and PFNA, and 0.2 ng/mL for PFOS. Low-concentration quality control materials (QCs) and high-concentration QCs, prepared

from a calf serum pool, were analyzed with the study samples and with reagent and serum blanks to ensure the accuracy and reliability of the data (Kuklenyik et al., 2005).

Statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc., Cary, NC). The serum concentrations followed log-normal distributions. Therefore, all data were log₁₀-transformed before statistical analysis. We used log linear regression, incorporating the repeated-measures correlation, to test for significant differences in the mean of the log concentrations of the target analytes between storage temperatures, and to test for significant trends over time at each temperature. The initial model included day, temperature, and the interaction term between day and temperature. For each analyte, we determined statistically significant time trend changes by repeated-measures ANOVA with the spatial structure correlation using the SAS PROC MIXED procedure. Significance was set at $p < 0.05$.

3. Results and discussion

We used the concentrations from day 0 (i.e., the day of serum aliquoting) of aliquot I (room temperature) as the baseline value for all temperatures tested (Table 1). We analyzed aliquot I on days 0, 1, 2, 3, 4, 8, and 10. Because storage of serum samples to be used for biomonitoring purposes for longer than 10 days at room temperature would be not advisable, we selected 10 days as the upper time limit for this temperature. We analyzed aliquot II (5 °C) at 11 time points during a 240 day storage period (1, 3, 4, 10, 21, 30, 50, 75, 90, 120 and 240 days). We analyzed aliquot III (-20 °C) and aliquot IV (-70 °C) at 7 time points (4, 10, 30, 60, 90, 120 and 240 days).

We found quantifiable concentrations ($>3 * \text{LOD}$) of the four PFCs in all of the samples at all of the time points examined. Table 1 lists the concentrations of PFHxS, PFOS, PFOA and PFNA in the 16 serum samples analyzed on day 0. Table 1 also lists the mean and median concentrations for each analyte on day 0. In Table 2, we present the standard deviations of the serum concentrations of each analyte for the 16 commercial samples at each temperature. The standard deviations for the conditions evaluated ranged from 8.7% to 12.3% (PFHxS), 8.3% to 9.0% (PFOS), 7.6% to 12.1% (PFOA), and 6.6% to 8.7% (PFNA) (Table 2). These standard deviations are within the precision of the analytical method (Kuklenyik et al., 2005).

The repeated measurement regression showed that there was no significant interaction between day and temperature. Therefore, the final model included day and storage temperature only. The differences in the mean of the log concentrations between storage temperatures were not statistically significant for any of the analytes (p -values were 0.9600 for PFHxS, 0.9620 for PFOS, 0.6895 for PFOA and 0.8739 for PFNA). Similarly, the mean of the log concentration did not show a statistically significant time trend for any of the target analytes (p -values were 0.5445 for PFHxS, 0.5141 for PFOS, 0.9477 for PFOA and 0.2319 for PFNA). Figs. 1 and 2 show the individual PFNA concentrations with time at 5 °C and PFOA concentrations with time at -20 °C.

To our knowledge, this is the first study to report on the stability of PFCs in serum. However, our study has several limitations. First, although the concentrations of the four PFCs examined were quantifiable in all samples, the sample size was relatively small.

Second, we used archived sera. We had no information on the methods used for the collection and storage of these samples, and we cannot rule out that some degradation might have occurred before we procured the samples. Because of these potential limitations, the findings above need to be replicated and confirmed in future studies with larger sample size and preferably using newly-collected samples. Nonetheless, taken together, our data suggest that the serum concentrations of all target analytes—PFOS, PFOA, PFNA and PFHxS—were stable for at least 240 days at 5 °C, -20 °C and -70 °C, and even when the sera was kept at room temperature for 10 days.

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Highlights

- To assess the potential impact of temperature on the long-term stability of polyfluoroalkyl chemicals in serum.
- Levels of polyfluoroalkyl chemicals in human serum remained unchanged even when serum was kept at room temperature for 10 days.

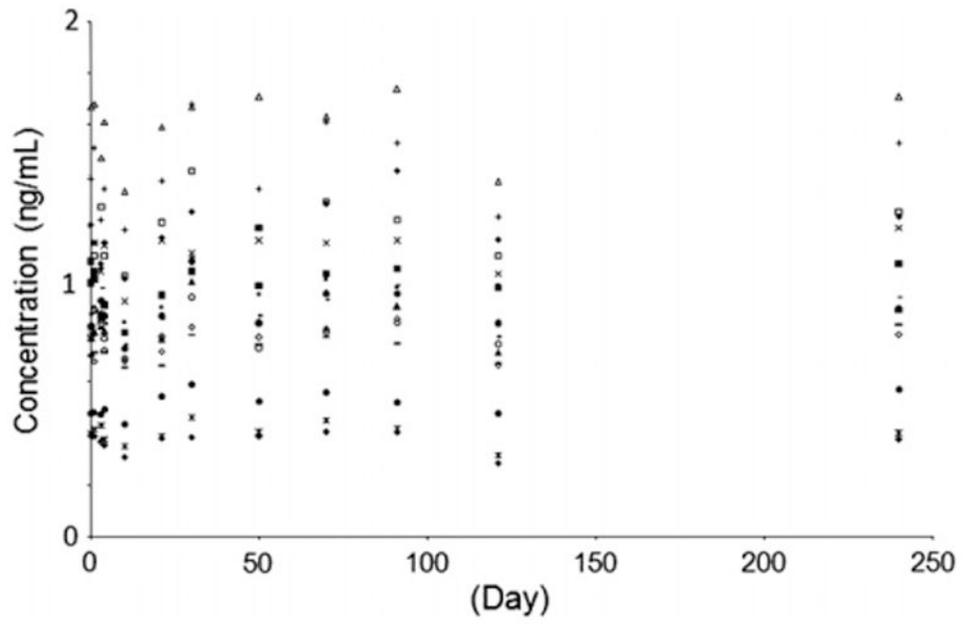


Fig. 1. PFNA serum concentrations over 8 months of storage at 5 °C (the different symbols represent each of the 16 commercial sera examined).

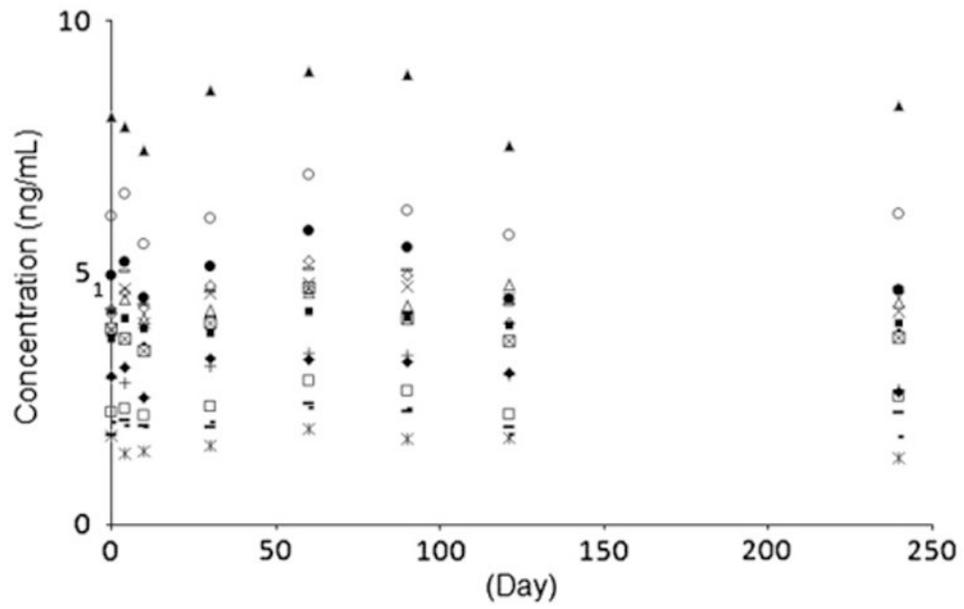


Fig. 2. PFOA serum concentrations over 8 months of storage at -20°C (the different symbols represent each of the 16 commercial sera examined).

Table 1

Day 0 serum concentrations (in ng/mL) of 4 PFCs in 16 individual samples, and their mean and median concentrations.

	PFHxS	PFOS	PFOA	PFNA
1	1.50	36.3	2.94	1.21
2	2.52	16.1	4.21	0.98
3	2.68	35.8	8.09	1.67
4	2.30	29.3	3.90	1.07
5	0.83	8.82	1.76	0.39
6	0.95	13.1	4.94	0.48
7	0.67	17.5	2.90	1.39
8	1.01	23.1	2.03	0.81
9	1.13	26.6	4.27	0.70
10	1.89	25.6	4.19	0.70
11	0.95	52.6	2.24	1.07
12	1.33	22.3	4.31	0.77
13	0.94	20.8	3.85	0.86
14	2.44	14.2	3.70	0.40
15	3.00	46.4	6.11	0.78
16	1.25	8.26	1.78	0.82
Mean	1.59	24.8	3.83	0.88
Median	1.29	22.9	3.98	0.81

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Table 2

Standard deviations (%) of the concentrations of each analyte at four different storage temperatures.

	Room temperature ^a	5 °C ^b	-20 °C ^c	-70 °C ^c
PFHxS	8.7	9.9	10.7	12.3
PFOS	8.2	9.0	8.3	8.3
PFOA	7.6	9.2	11.8	12.1
PFNA	6.6	7.4	8.7	8.6

^aThis aliquot was analyzed on days 0, 1, 2, 3, 4, 8, and 10.

^bThis aliquot was analyzed on days 1, 3, 4, 10, 21, 30, 50, 75, 90, 120 and 240.

^cThis aliquot was analyzed on days 4, 10, 30, 60, 90, 120 and 240.

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