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ScienceDirect
Environmental Research 103 (2007) 176–184
Environmental Research
www.elsevier.com/locate/envres

Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals

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Received 27 January 2006; received in revised form 16 June 2006; accepted 21 June 2006

Abstract

Interest in human exposure to perfluorinated acids, including perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), perfluorooctanesulfonate (PFOS), and perfluorooctanoate (PFOA) has led to their measurement in whole blood, plasma and serum. Comparison of measurements in these different blood-based matrices, however, has not been rigorously investigated to allow for across-matrix comparisons. This research evaluated concentrations of PFBS, PFHS, PFOS, and PFOA in whole blood collected in heparin (lithium) and ethylenediamine tetraacetic acid (EDTA), plasma samples collected in heparin and EDTA, and serum (from whole blood allowed to clot). Blood samples were collected from 18 voluntary participants employed at 3M Company. Solid phase extraction methods were used for all analytical sample preparations, and analyses were completed using high-pressure liquid chromatography/tandem mass spectrometry methods. Serum concentrations ranged from: limit of quantitation (LOQ, 5 ng/mL) to 25 ng/mL for PFBS; LOQ (5 ng/mL) to 75 ng/mL for PFHS; LOQ (5 ng/mL) to 880 ng/mL for PFOS; and LOQ (5 or 10 ng/mL) to 7320 ng/mL for PFOA. Values less than the LOQ were not included in the statistical analyses of the mean of the ratios of individual values for the matrices. PFBS was not quantifiable in most samples. Serum to plasma ratios for PFHS, PFOS, and PFOA were 1:1 and this ratio was independent of the level of concentrations measured. Serum or plasma to whole blood ratios, regardless of the anticoagulant used, approximated 2:1. The difference between plasma and serum and whole blood corresponded to volume displacement by red blood cells, suggesting that the fluorochemicals are not found intracellularly or attached to the red blood cells.

Keywords: Biomonitoring; Perfluorobutanesulfonate; Perfluorohexanesulfonate; Perfluorooctanesulfonate; Perfluorooctanoate

1. Introduction

Recent interest in human exposure to perfluorinated alkyl acids, including perfluorohexanesulfonate (PFHS), perfluorooctanesulfonate (PFOS), and perfluorooctanoate (PFOA), has led to reports of measurements of these substances in whole blood, plasma, or serum in samples from the general population (Hansen et al., 2001; Olsen et al., 2003a, 2004a, b, 2005; Harada et al., 2004a, b; Inoue et al., 2004a, b; Kärrman et al., 2005; Kannan et al., 2004; Kuklenyik et al., 2004; Calafat et al., 2006) and measured in 3M Company fluorochemical production workers (Olsen et al., 2003b). Perfluorobutanesulfonate (PFBS) is a fluorochemical surfactant and final degradation product of perfluorobutanesulfonyl fluoride and materials manufactured with it that are being introduced as replacements for the six and eight-carbon analogs. Some 3M fluorochemical production workers have been occupationally exposed to perfluorobutanesulfonyl fluoride and other materials that may degrade to PFBS. All four of the perfluorinated acids mentioned above are highly bound to serum and/or plasma proteins (Luebker et al., 2002; Kerstner-Wood et al., 2004).

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0013-9351/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2006.06.008

PFOS has been measured primarily in human blood serum (Olsen et al., 2003a, 2004a, b, 2005; Guruge et al., 2004; Harada et al., 2004a, b, 2005; Kannan et al., 2004; Kubwabo et al., 2004; Kuklenyik et al., 2004; Calafat et al., 2006); however, data are also available on PFOS in human whole blood (Kannan et al., 2004; Kärrman et al., 2004; Lindström et al., 2004), plasma (Kannan et al., 2004; Olsen et al., 2005a), liver (Olsen et al., 2003c), cord blood (Inoue et al., 2004a; Tittlemier et al., 2004), breast milk (Kuklenyik et al., 2004) and seminal plasma (Guruge et al., 2005). There are several published methods for analysis of these fluorochemicals in the various blood-based matrices (Hansen et al., 2001; Inoue et al., 2004; Flaherty et al., 2005; Kärrman et al., 2005; Kuklenyik et al., 2004). However, comparison of measurements of these compounds in different blood-based matrices has never been rigorously evaluated in spite of the burgeoning literature of human fluorochemical biomonitoring data. In addition, the potential effect of different anticoagulants used during collection of blood samples has not been investigated.

The purpose of this study was to compare the measurement of PFBS, PFHS, PFOS, and PFOA in whole blood, plasma, and serum samples collected from the same individuals. As part of this comparison, the possible effect of different anticoagulants for whole blood and plasma was also investigated.

Whole blood is one of the most difficult matrix choices from an analytical standpoint and is not typically used for clinical chemistry measurements. Whole blood is not stable on long-term storage unless frozen, but the freezing process results in the lysis of its cellular components. Refrigerating whole blood cells allows continuous glucose metabolism and other clinically significant changes within the sample, which results in a constantly changing matrix (Sacks, 1994). Plasma contains fibrinogen, which will result in coagulation unless treated with an anticoagulant, usually heparin or ethylenediamine tetraacetic acid (EDTA). EDTA is a commonly used anticoagulant for hematology studies; however, EDTA plasma is not the usual specimen of choice for most clinical chemistry determinations due to potential interferences with common clinical chemistry analytes and enzyme measurements (Young and Bermes, 1994). Heparinized plasma can be used for both clinical chemistry and analytical determinations; however, the change in the chemical composition of the sample upon long-term storage and fibrin clot formation are common problems associated with this matrix (Young and Bermes, 1994). For these reasons, serum, obtained from clotted blood, is the most common matrix employed for clinical chemistry and trace analyses.

This study investigated potential differences between measurements of PFBS, PFHS, PFOS, and PFOA in blood samples collected in commercially available tubes (Becton, Dickenson and Co., Franklin Lakes, NJ) containing either no anticoagulant (serum), or containing either EDTA or heparin (whole blood and plasma). Samples were obtained from 3M Company employees that represented different potential exposure levels. These voluntary participants included fluorochemical production workers, laboratory researchers, and administrative employees.

2. Materials and methods

2.1. Sample collection and storage

Blood samples for the fluorochemical analyses were drawn sequentially from a single venipuncture site from 18 voluntary participant employees of the 3M Company. These participants included six fluorochemical production workers, six fluorochemical laboratory researchers, and six administrative employees. All participants signed an informed consent form. Whole blood and plasma samples were collected in heparin (lithium) and in EDTA. Whole blood samples were also collected without anticoagulant for serum. Serum was obtained by allowing whole blood to clot at room temperature. All whole blood samples were refrigerated until analyzed; serum and plasma samples were frozen at −80 °C until analyzed.

2.2. Serum, plasma, and washed cells sample preparation

Cellular components and plasma were isolated from whole blood (EDTA and heparin) by centrifugation at $1500 \times g$ for 20 min. The cellular components were washed four times with commercially prepared 0.9% NaCl (physiological saline, Abbott Laboratories, Abbott Park, IL) to remove residual proteins. Each wash consisted of an addition of 4.0 mL of physiological saline with gentle rotation on a mixer for 20 min followed by centrifugation at $1500 \times g$. Cells were resuspended in physiological saline to a volume equivalent to the original sample volume of whole blood.

2.3. Analysis

All extractions were performed using solid phase extraction (SPE) techniques (Kuklenyik et al., 2004). All extractions were based on 100 μL of sample matrix and utilized Waters (Milford, MA) Oasis® hydrophilic–lipophilic balance (HLB) 3.0 mL cartridges. Initially, 10 μL of a 1 ng/μL (10 ng total) of PFOS labeled with two ^{18}O in the sulfonate group (internal standard, >99% purity, synthesized by Research Triangle Institute, Research Triangle Park, NC) was added to sample tubes for determination of PFBS, PFHS, and PFOS. PFBS, PFHS, and PFOS were quantitated based on matrix-matched, extracted standard curves using PFOS internal standard. PFOA, with carboxyl and alpha carbons labeled with ^{13}C stable isotope (greater than 97%, provided by DuPont, Wilmington, DE), was used as an internal standard. PFOA quantitation was done based on matrix-matched, extracted standard curves using PFOA internal standard. Internal-standard-only spiked blanks were shown to contain PFOA at less than the limit of quantitation (LOQ). Appropriate amounts of analyte were then spiked into the tubes labeled for the standard curve. Commercially purchased human whole blood (Lampire Biological, Pipersville, PA) was used for the whole blood standard curve matrix. Newborn calf serum (Invitrogen Corp., Carlsbad, CA) was used for the serum standard curve matrix. Previous evaluation of serum and plasma samples in our laboratory has shown no significant differences when using an extracted serum spiked standard curve. Therefore, plasma samples were evaluated using calf serum extracted standard curves due to the difficulty of obtaining suitable blank human plasma matrix. Extracted solutions were analyzed by LC-MS-MS instrumentation. The LOQ was defined as the lowest concentration at which the analytical process could be reliably differentiated from background signal and was set at 10 times the signal-to-noise ratio. Signal-to-noise ratio was determined immediately prior to the peak-of-interest elution time equivalent to the peak width of the analyte of interest. The LOQ was 1.0 ng/mL. Standard curve matrices (blank matrices) were evaluated and had no measurable concentrations above the LOQ. Because