ABNORMALLY HIGH SERUM LEVELS OF PERFLUOROHEXANE SULFONATE(PFHxS) IN A CANADIAN FAMILY - A CASE STUDY.

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Introduction.
Perfluorochemicals (PFCs) have been manufactured on an industrial scale since the 1950s, and have been extensively used in multiple consumer products such as stain-resistant carpets and fabrics[1]. Since the first discovery of PFCs in the environment in 2001[2], multiple cross sectional studies[3-8] have documented ng/mL concentrations of these chemicals in human blood from North America, Europe, Australia and Asia. The most prevalent PFCs detected in humans are perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) and perfluorooctanoate (PFOA, $C_7F_{15}COO^-$). It was therefore surprising when a licensed physician contacted us in 2008 about 2 individuals, related to each other, whose serum samples had very high levels of perfluorohexane sulfonate (PFHxS, $C_6F_{13}SO_3^-$), with concentrations 3-4 times higher than PFOS or PFOA. Here we describe a study designed to thoroughly assess the possible sources and pathways of exposure to this family through biomonitoring and home sampling.

Materials and Methods.
Sampling and Data Gathering: Ethics approval was obtained from the Health Research Ethics Board of the University of Alberta prior to sample collection. Blood, urine and stools samples were collected from all 7 family members: father, aged 52, mother, aged 48, four sons aged 14, 16, 20 and 22, and a 17 year old daughter. Samples of carpet (10 x 2 cm each, n=10) were collected from different parts of the house, as well as a piece of uninstalled carpet that was purchased with the rest of the carpets 20 years ago. A sample of vacuum dust from the central main canister was also taken. Active air samples were collected on OSHA (Occupational Safety and Health Administration) versatile sampler (OVS) tubes using a calibrated pump (Airchek Sampler-Model 224-PCXR8). A questionnaire based interview was administered to the family members to determine other potential exposure sources such as household characteristics, dietary habits, and possible occupational exposures.

Extraction: PFCs were extracted from 0.5 ml serum samples using a protein precipitation step followed by ultracentrifugation[9], while urine samples (10 ml) were extracted using a solid phase extraction method[10] employing Oasis HLB (60 mg) cartridges. The method of Yoo et al[11] for sludge was adapted for analyzing the feces. Samples (~2g) were extracted using MeOH, vortexed for 5 minutes, sonicated for 1 hour, and then centrifuged at 4000 g for 10 minutes. Moisture content was calculated by drying a portion of the feces in an oven overnight and comparing the wet and dry weights.

Vacuum dust samples were sieved using an AS200 Analytical Retsch sieve shaker and a 150 μm mesh size stainless sieve (Retsch GmbH, Rheinische, Germany). The sieved dust was collected in a MeOH rinsed stainless steel pan. Approximately 0.5 g of sieved dust was weighed into a 15 ml polypropylene tube to which 5 ml MeOH was added followed by vortexing, sonication and centrifugation. A 2 ml aliquot was transferred to a clean 15 ml centrifuge tube, reduced to 300 μl under nitrogen, centrifuged for 10 min at 4000g. The supernatant was then transferred to a 0.5 ml autosampler vial for analysis.
PFCs were extracted from ~1 g carpet samples using the method of L'Empereur et al[12]. For air samples, the OVS tubes were separated into the filter (airborne dust), and the sorbent material (gas-phase analytes). PFCs on the sorbent material were extracted by passing 3 ml of MeOH through it, and the dust was extracted by washing the filter with 3 x 1 ml of MeOH and processing the same way as the vacuum dust.

Quantification: A previously published LC-MS/MS method[13] was used for total and isomer specific analysis of non-volatile PFCs in all samples except for air. Precursor and product ions were monitored using a triple-quadrupole mass spectrometer (5000Q, MDS Sciex, Concord, ON, Canada) with electrospray ionization operating in negative mode. For each analyte, at least 2 transitions were monitored and specifically for PFHxS, the 399/119 m/z transition was used to rule out possible interferences[14]. PFOS and PFOA isomer profiles were compared against standards provided by 3M. Semi volatile PFCs in air samples were analysed using gas chromatography-positive chemical ionization mass spectrometry (GC-PCIMS) with a 30 m DB-5 column, as adapted from Martin et al[15].

Results and Discussion.

Information gathered through personal interviews revealed that there was no occupational exposure in this family of high socio-economic status, and that their dietary habits did not show any departure from the conventional Canadian diet. However, the family's household carpets had been regularly cleaned and treated for stain and soil repellence every 1-2 years since 1989. Furthermore, the house was heated by radiant heaters in the floor, underlying the carpet. Serum analysis revealed disproportionately high concentrations of PFHxS, coupled with PFOS and PFOA concentrations that were also higher than the average Canadian levels. Serum concentrations for PFCs in the family ranged from 27.5 – 423, 15.2 – 108, and 2.4 – 9.2 ng/mL for PFHxS, PFOS, and PFOA, respectively, with the levels of all three chemicals being highest in the younger members of the family (Figure 1).

Samples of dust and carpet collected from the family's home showed high levels of all PFCs monitored, in particular of PFHxS in the main family room(FR) and dining room(DR) (Figure 2). This is consistent with receipts from the carpet cleaning company showing that for the past 20 years the whole house was cleaned, but it was specifically in these 2 areas of the house where the stain repellents were regularly applied. Interestingly, the PFHxS/PFOS ratio in the blood of most of the family members was the same as that in the family and dining room carpet and dust. This may be a reflection of the PFHxS/PFOS ratio of ≈ 3 reported to be found in a particular formulation of ScotchGard™ Carpet Protector[16]. According to a report from the epidemiology/medical department of the 3M company, PFHxS was a major constituent that was intentionally added to an after market carpet protector, but discontinued in 1999[17]. Although production of this latter product ceased in 1999, we have no information about its continued use in North America or production in developing countries.

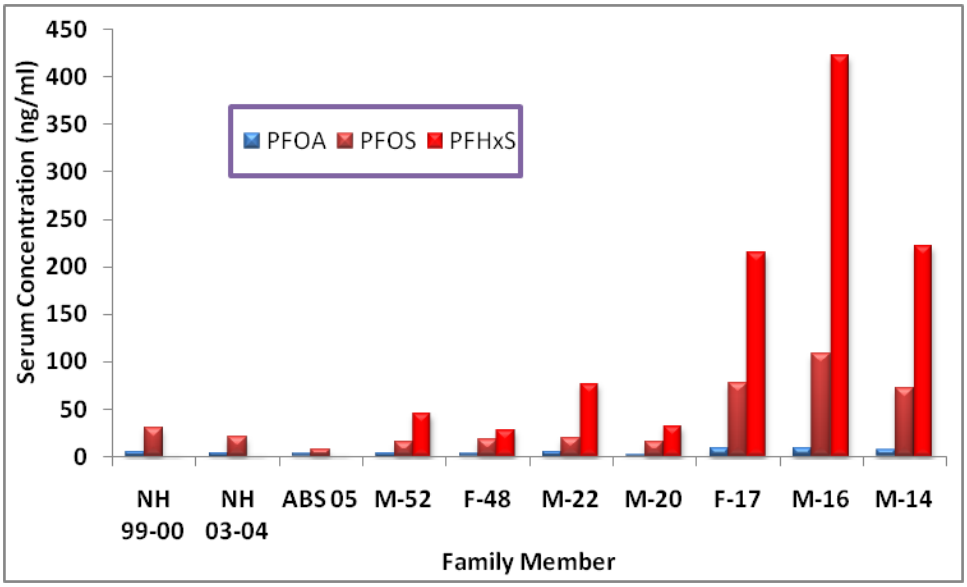

Figure 1. Serum concentrations of PFCs in family members (gender-age) compared to U.S. NHANES 1999-2000 (NH 99-00), and 2003-2004 (NH 03-04) studies, and an Alberta Biomonitoring Study (ABS 05).

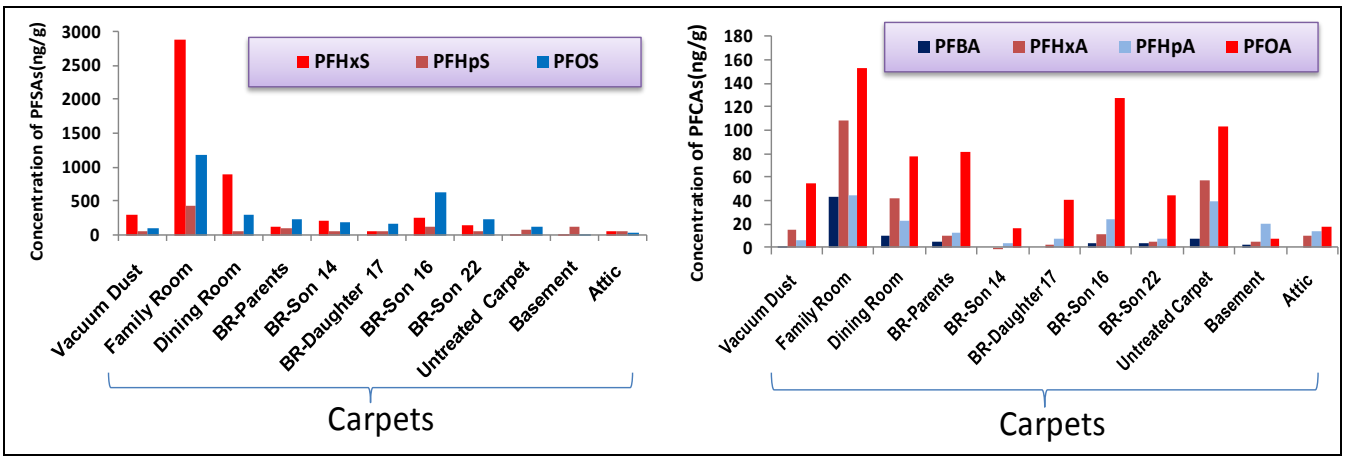

Figure 2. Perfluorinated carboxylate (PFCA) and perfluorinated sulfonate (PFSA) concentrations in carpet and dust samples. PFHpS- perfluoroheptane sulfonate ($C_7F_{15}SO_3^-$), PFBA- perfluorobutanoic acid (C_3F_7COOH), PFHxA-perfluorohexanoic acid ($C_5F_{11}COOH$), PFHpA-perfluoroheptanoic acid ($C_6F_{13}COOH$), BR- Bedroom.

This pilot study shows a unique exposure scenario whereby multiple factors have contributed to the elevated serum levels of PFCs in the family; firstly, the repeated application of Scotchgard™ to the household carpet over a 20 year period, secondly, the house is equipped with an infloor heating system and thirdly, given the weather conditions in that part of the country the house is completely closed for nearly 7 months of the year, hence poorly ventilated. The comparatively higher levels of PFCs, especially PFHxS, in the younger members of the family compared to the adults is not unprecedented [4, 6, 18] and is probably due to greater contact of the children with the treated carpets. The presence of PFHxS in urine but undetected in feces is suggestive that urine is the main mode of excretion. Furthermore, a paired analysis of PFHxS isomer profiles in serum and urine indicated that there is a preferential excretion of branched isomers compared to linear.

Overall the family appears healthy, but the long-term health risks associated with this exposure cannot currently be addressed, as there is little toxicological or epidemiological data available on PFHxS, except that among the currently studied PFCs, this latter compound is known to have the longest elimination half-life in humans[19].

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