PERFLUORINATED ACID ISOMER PROFILING IN OCEAN WATER BY LARGE VOLUME INJECTION-HPLC-MS/MS

Benskin, Jonathan P[1]; Yamashita, Nobuyoshi[2]; Taniyasu, Sachi[2]; Lam, Paul KS[3]; Tomy, Gregg T[4]; Muir, Derek CG[5]; Scott, Brian F[5]; Spencer, Christine[5]; Rosenberg, Bruno[5]; Martin, Jonathan W[1]

[1]Department of Laboratory Medicine and Pathology, University of Alberta, Canada
[2]National Institute of Advanced Industrial Science and Technology (AIST), Japan
[3]Department of Biology and Chemistry, City University of Hong Kong, China
[4]Department of Fisheries and Oceans, Arctic Aquatic Research Division, Winnipeg, MB, Canada
[5]Aquatic Ecosystem Protection Research Branch, Environment Canada, Burlington, ON, Canada

Introduction

Perfluorinated acids (PFAs) are now widespread contaminants of the global environment, and have been detected in humans [1] and remote arctic wildlife [3, 4]. Their toxicity [5], persistence, bioaccumulation potential [6], and long range transport potential [7] can be considerable, depending on chain length. Among the prominent environmental PFAs, each may exist as several structural isomers resulting from the electrochemical fluorination (ECF) manufacturing process. While ECF-derived fluorochemicals were largely phased out in 2002 [8], the production of others was continued by a telomerization process that yields exclusively the linear isomer [9]. Among the most important questions today, which will have many implications for future regulation of fluorochemicals, is to what extent the environmental burden of PFAs is a consequence of historic or current-use fluorochemicals.

It was previously hypothesized that PFA isomer patterns in biota could be correlated to exposure from either historical releases of ECF fluorochemicals (mixture of isomers, pre-2002 phase-out) or on-going releases of telomer-derived product (strictly linear, historic and current use) based on the isomer profile in samples [10, 11]. However, recent data suggests that branched PFA isomer profiles are preferentially excreted from animals [12, 13], and thus it is not clear whether the absence of branched perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) in polar bears [11] and humans [10, 14] reflects exposure to a telomer source, or whether differential excretion of branched isomers can result in tissue isomer patterns which *appear* to be telomer-based. While biological samples may therefore be of limited use for source tracking, isomer patterns in abiotic samples are likely to be a better indication of PFA manufacturing source. Environmental models [15, 16, 17] predict that historic releases of ECF PFAs, and their subsequent slow transport in oceans, are the dominant source of global PFA transport, however little empirical evidence exists. To validate these model predictions, we examined the PFA isomer patterns in various ocean waters. The greatest practical challenge however, is the very small concentrations in ocean water, thus we first developed a large-volume injection-HPLC-MS/MS method for ultra-trace isomer profiling.

Materials and Methods

HPLC-grade methanol was purchased from Fisher Scientific. HPLC grade formic acid (50%), potassium perfluorohexane sulfonate (PFHxS), potassium perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotetradecanoic acid (PFTA) were purchased from Sigma-Aldrich (Oakville, ON, Canada). ECF PFOS and PFOA (Lot # TCR 279) standards were provided by the 3M Co. (St. Paul, MN). Linear PFOS and internal standards ^{13}C-PFOS, ^{13}C-PFOA, ^{13}C-PFNA, ^{13}C-PFDA, and ^{13}C-PFDoA were obtained from Wellington Labs (Guelph, ON, Canada). Isolated and characterized standards of *iso*-, 5*m*-, 4*m*-, 3*m*-, 1*m*-, 4,4m_2-, and *tb*-PFOS, as well as *iso*-, 5*m*-, 4*m*-, 3*m*-, 4,4m_2-, and *tb*-PFOA were also obtained from Wellington Labs. A standard of 5,3m_2- and 5,4m_2-PFOA was also obtained as a mixture (3:5 ratio based on NMR).

PFAs in Figure 1 were quantified by relating the response of the native analyte to the corresponding ^{13}C-labelled internal standard. In cases where a labeled internal standard was not available, we used the internal standard with the closest chain length to the given PFA, for example PFTA with ^{13}C-PFDoA. Each sample was spiked with 5uL of a 2.5 ng/mL ^{13}C internal standard mixture (12.5 pg of each labeled ^{13}C-labelled PFA). The small quantity

of internal standard was well above detection limits, yet small enough that any native PFA contamination (assumed to be <10% of ^{13}C concentration) would not be observable.

Ocean water sample collection and solid phase extraction methods are described elsewhere [18, 19, 20]. Spike/recovery experiments demonstrated that isomer patterns were conserved throughout the extraction procedure. Sample extracts (5mL MeOH) were evaporated in a warm water bath (40^0C) under ultra high purity nitrogen to 100uL at which time the flow of nitrogen was stopped and samples were allowed to come to dryness by air. All samples were taken up in 300uL of 60% 5mM ammonium formate (pH 4) / 40% MeOH, (the starting HPLC mobile phase composition) and transferred to 300uL polypropylene micro vials. Using multiple injection stacking (2 x 100uL, 1x 75uL) into an expanded 400uL injector seat capillary, we were able to perform semi-quantitative injections (275 uL out of 300uL, 92% of sample).

Isomer separations were conducted on a perfluoroctyl stationary phase (3µ 100A 15cm x 2.1 mm, ES Industries, West Berlin, NJ) by modifying a previously developed method [14]. A 15 cm C18 column was inserted upstream of the injector to separate "system" PFAs from analyte PFAs. Starting conditions were 175uL/min, 60% A (water adjusted to pH 4.0 with ammonium formate) : 40% B (100% methanol). Initial conditions were held for 0.3 min at which time the flow was increased to 190uL/min, ramped to 64% B by 1.9 min, increased to 66% B by 5.9 min, 70% B by 7.9 min at which point the flow was increased to 200uL/min and the gradient was increased to 78% B by 40 min, 88% B by 42 min, 100% B by 60 min which was held for 10min prior to returning to initial conditions by 71 min and allowing 25 min for equilibration. Mass spectral data were collected using a hybrid triple-quadrupole linear ion trap mass spectrometer (4000QTRAP, MDS Sciex, Concord, ON, Canada) equipped with an electrospray interface operating in negative mode. Chromatograms were recorded by multiple reaction monitoring (MRM).

Results and Discussion

A large-volume, HPLC-MS/MS method was developed for ultra-trace PFA isomer profiling of abiotic samples. Limits of detection for most PFAs in ocean water were 5-10pg/L, based on response of the linear isomer. Concentrations of linear PFAs (Figure 1) were similar to those observed in previous studies using total PFA analysis [18, 19, 20]. Relative quantities of *n*-PFAs off the coast of Hobart and Auckland decreased in the order PFOA > FOSA > PFOS ≈ PFHxS > PFDA > PFNA > PFUnA. PFTA and PFDoA were below our limit of quantification in all samples. These ratios were similar to what was observed in Tokyo Bay, yet remarkably different than Shanghai, whereby a decrease in FOSA concentrations accompanied decreases in both PFOS and PFHxS levels.

PFOA isomer patterns observed in ocean water (Figure 2) and river water (Figure 3) around Tokyo Bay were unlike anything observed to date in biota, with the ratio of linear : branched PFOA closely matching what was observed in a technical 3M ECF PFOA standard (Figure 4 bottom panel). *iso*-PFNA and branched PFHxS isomers (Figures 2 and 3) were also observed at relative concentrations above what was previously observed in human serum [14]. Interestingly, PFOS isomer profiles from around Tokyo Bay showed remarkable similarities to a 3M ECF PFOS standard (~68% *n*-PFOS, Figure 4 middle panel) compared to that of the Fluka standard (78% *n*-PFOS, Figure 4 top panel). In contrast, the isomer profile of PFOS in human blood from Edmonton, Canada was nearly identical to that of the Fluka standard [14]. While ECF-based manufacturing of perfluorochemicals is known to have taken place in Japan, [21] and may still be occurring, it is not clear whether isomer patterns in and around the sampling location are indicative of recent emissions or, alternatively, legacy ECF manufacturing which is hypothesized as the dominant global source of PFOA. Procurement of ECF-based PFOA and PFOS standards from individual manufacturers may allow isomer patterns in the environment to be more accurately correlated to their manufacturing source. Future work will include isomer profiling of North Atlantic and Arctic oceans in an effort to elucidate contributions from direct releases of PFAs to Arctic wildlife concentrations.

Acknowledgements

Funding for materials, supplies, and instrument time was provided through an NSERC Discovery Grant (JWM), an Alberta Ingenuity New Faculty Grant (JWM), and an Alberta Ingenuity Student Scholarship (JPB). Alberta

Health and Wellness is thanked for support of laboratory activities. 3M is thanked for providing ECF PFOS and PFOA standards. Wellington Labs is thanked for providing pure isomer standards.

References

[1] Calafat A.M., Needham L.L., Kuklenyik Z., Reidy J.A., Tully J.S., Aguilar-Villalobos M., Naeher L.P. *Chemosphere* 2006; 63:490-496.
[2] Karrman A., Mueller J.F., van Bavel B., Harden F., Toms L.M., Lindstrom G. *Environ Sci Technol* 2006; 40:3742-3748.
[3] Martin J.W., Smithwick M.M., Braune B.M., Hoekstra P.F., Muir D.C., Mabury S.A. *Environ Sci Technol* 2004; 38:373-380.
[4] Butt C.M., Muir D.C., Stirling I., Kwan M., Mabury S.A. *Environ Sci Technol* 2007; 41:42-49.
[5] Lau C., Anitole K., Hodes C., Lai D., Pfahles-Hutchens A., Seed J. *Toxicol Sci* 2007; 90:510-518.
[6] Houde M., Martin J.W., Letcher R.J., Solomon K.R., Muir D.C. *Environ Sci Technol* 2006; 40:3463-3473.
[7] Wallington T.J., Hurley M.D., Xia J., Wuebbles D.J., Sillman S., Ito A., Penner J.E., Ellis D.A., Martin J.W., Mabury S.A., Nielsen O.J., Sulbaek Andersen M.P. *Environ Sci Technol* 2006; 40:924-930.
[8] Olsen G.W., Mair D.C., Reagen W.K., Ellefson M.E., Ehresman D.J., Butenhoff J.L., Zobel L.R. *Chemosphere* 2007; 68:105-111.
[9] Dupont. 2005. *US EPA Public Docket*, AR226-1914.
[10] De Silva A.O., Mabury S.A. *Environ Sci Technol* 2006; 40:2903-2909.
[11] De Silva A.O., Mabury S.A. *Environ Sci Technol* 2004; 38:6538-6545.
[12] Benskin J.P., De Silva A.O., Martin L., Arsenault G., McCrindle R., Ridell N., Mabury S.A., Martin J.W. 2007. Platform at 2007 SETAC NA Conference, Milwaukee Wis.
[12] De Silva A.O., Benskin J.P., Martin L., Arsenault G., McCrindle R., Ridell N., Martin J.W., Mabury S.A. 2007. Platform at 2007 SETAC NA Conference, Milwaukee Wis.
[14] Benskin J.P., Bataineh M., Martin J.W. *Anal Chem* 2007; 79:6455-6464.
[15] Armitage J., Cousins I.T., Buck R.C., Prevedouros K., Russell M.H., MacLeod M., Korzeniowski S.H. *Environ Sci Technol.* 2006; 40: 6969-6975.
[16] Wania F. A global mass balance analysis of the source of perfluorocarboxylic acids in the Arctic ocean. *Environ. Sci. Technol.* 2007; 41: 4529–4535.
[17] Schenker U., Scheringer M., MacLeod M., Martin, J. W., Cousins I. T., Hungerbühler K. *Environ Sci Technol* 2008; ASAP Article.
[18] Yamashita N., Kannan K., Taniyasu S., Horii Y., Okazawa T., Petrick G., Gamo T., *Environ Sci Technol* 2004; 38: 5522-5528,
[19] Yamashita N., Kannan K., Taniyasu S., Horii Y., Petrick G., Gamo T., *Mar Pollut Bull* 2005; 51: 658-668, England
[20] Yamashita N, Taniyasu,S.; Petrick,G.; Wei,S.; Gamo,T.; Lam,P.K.; Kannan,K. 2008. *Chemosphere*, 70: 1247-1255.
[21] OECD 2002. Document ENV/JM/RD(2002)17.

Figure 1. Concentrations of linear PFAs in coastal ocean water, n=1.

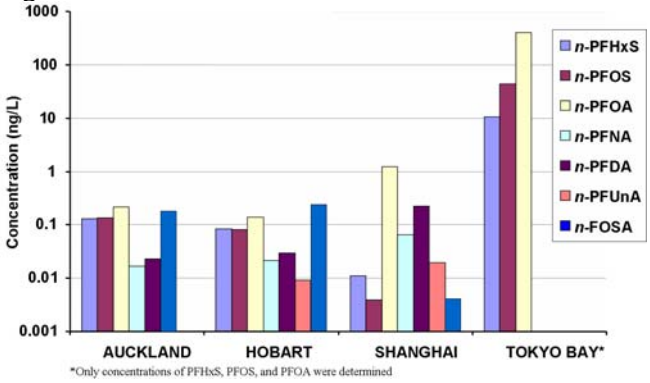

Figure 2. Tokyo Bay Ocean Water

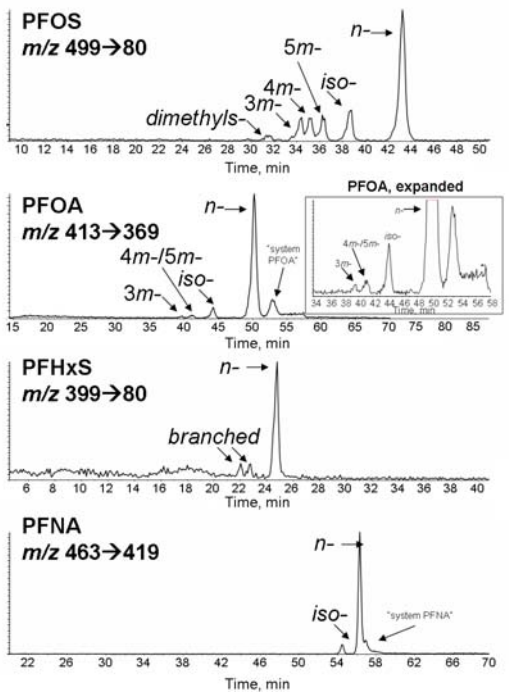

Figure 3. River Water near Tokyo Bay

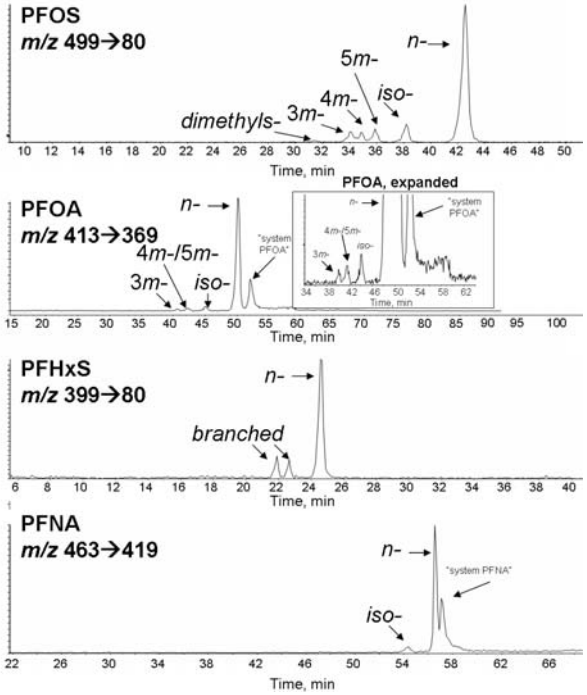

Figure 4. Isomer profiles in ECF Standards