RETROSPECTIVE ANALYSIS OF PERFLUORINATED ALKYL ACIDS IN NORTHERN FUR SEALS FROM ALASKA

Reiner JL[1]*, Pugh RS[2], Moors, AJ[2], Becker PR[2]

[1]Analytical Chemistry Division, National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD, USA [2]Analytical Chemistry Division, National Institute of Standards and Technology, Hollings Marine Laboratory, 331 Fort Johnson Rd., Charleston, SC, USA

Introduction

The Alaska Marine Mammal Tissue Archival Project (AMMTAP) was established in 1987 through an agreement between the National Oceanic and Atmospheric Administration (NOAA), the National Institute of Standards and Technology (NIST), and the Minerals Management Service (MMS) to help determine contaminant levels in marine mammal samples that were taken primarily during native subsistence hunts in Alaska[1]. Tissues were collected and banked as a part of the National Marine Mammal Tissue Bank (NMMTB) for long-term archival using NIST standardized protocols. Archived samples from the NMMTB were provided for this retrospective analysis of emerging contaminants.

Perfluorinated alky acids (PFAAs), mostly perfluorinated carboxylic (PFCA) and sulfonic (PFSA) acids, have been identified in wildlife worldwide[2-7]; with some of the highest levels of PFAAs being measured in marine mammals from the Arctic. It is not exactly clear how PFAAs are transported to arctic wildlife, but it is thought to happen via two major routes: atmospheric transport of volatile precursors and oceanic transport from their release in lower latitudes [8-10]. A previous study has indicated atmospheric transport having a greater influence on PFAA contamination in the Arctic by examining the spatial trends of PFAAs in ringed seal (*Phoca hispida*) populations from East and West Greenland[3]. Another study noted that circulation patterns in the Arctic Ocean may also have an effect on spatial differences of PFAAs in the arctic region[11]. Due to the large distribution of some arctic species it is important to understand if there are similarities or differences in the concentrations and patterns among species from different locations. While there have been numerous studies focusing on PFAAs in wildlife from the Canadian and European Arctic, few studies have looked at the wildlife in the Alaskan Arctic[2, 12]. Recognizing possible spatial patterns will help highlight sources of PFAAs to the Arctic and help examine atmospheric and oceanic transport.

In this study, 12 PFAAs, consisting of PFCA and PFSA acids, as well as the perfluorooctane sulfonate (PFOS) precursor perfluorooctane sulfonamide (PFOSA), were measured in northern fur seal (*Callorhinus ursinus*) livers collected in Alaska between 1987 and 2007 to understand transport of PFAAs to the Arctic and the temporal trends. This work was made possible because of the collection and banking efforts through AMMTAP and emphasizes the importance of environmental specimen banks as a sample resource for assessing chemical trends.

Materials and Methods

Northern fur seal livers (n=49) were selected for PFAA analysis from the NMMTB. Northern fur seal liver samples came from male animals from three rookeries on St. Paul Island, AK. Samples, calibrants, quality control materials, and blanks were extracted using a method similar to the potassium hydroxide (KOH) in methanol method as described previously[13] and cleaned-up using a solid phase extraction (SPE) step also described previously[14]. NIST Standard Reference Material (SRM) 1947 Lake Michigan Fish Tissue was analyzed with each set of samples for quality control.

Samples were injected onto a liquid chromatograph (Agilent 1100 HPLC, Palo Alto, CA) interfaced to a negative electrospray ionization tandem mass spectrometer (LC-MS/MS) (API 4000, Applied Biosystems-MDS Sciex, Foster City, CA) using the NIST method described in Keller et al. [14]. The MS/MS method included the

optimization parameters for each analyte; and two to three of the most abundant transitions for each PFC were monitored.

All statistical analyses were performed using JMP 9.0.0 (SAS Institute, Cary, NC). Statistical tests were performed for individual PFCAs, individual PFSAs, total perfluorocarboxylate (ΣPFCA), and total PFAA (ΣPFAA) concentrations, which were the compounds detected in >70% of the samples. Compound concentrations less than the reporting limit (RL) were set equal to half the RL prior to running the statistical tests.

Results and Discussion:

PFAAs were found in the northern fur seal liver samples (Table 1). Consistently measured PFAAs were the odd chain PFCAs, perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnA), and perfluorotridecanoic acid (PFTriA), PFSAs including perfluorohexane sulfonate (PFHxS) and PFOS, and PFOSA. PFUnA was found to be at the highest concentration measured in all three rookeries. PFOS concentrations measured in this study are lower than PFOS concentrations measured in fur seals from the west coast of the United States[15]and ringed seals sampled from the Canadian Arctic[5].

Table 1. Ranges of PFAA concentrations (ng/g, as received) in northern fur seal liver samples

	Polavina Rookery (n=23)	**Reefpoint Rookery (n=16)**	**Zapadni Rookery (n=9)**
PFNA	<RL - 19.8	0.545 - 13.1	1.17 - 8.55
PFDA	<RL - 1.70	<RL	<RL - 1.42
PFUnA	1.50 - 122	1.90 - 138	1.69 - 12.2
PFDoA	<RL - 2.21	<RL	<RL - 2.78
PFTriA	<RL - 13.8	<RL - 25.9	0.823 - 4.69
PFHxS	<RL - 1.63	<RL - 0.460	0.111 - 0.358
PFOS	0.927 - 15.8	1.07 - 17.5	3.09 - 13.6
PFOSA	<RL - 3.99	<RL - 1.39	0.574 - 3.19

<RL = below the reporting limit

Concentration differences related to year were assessed for these samples. Similar to the other Alaskan marine mammal samples[16], concentrations of PFNA, PFUnA, and PFTriA showed a significant increase from 1987 to 2007 ($p < 0.05$, Figure 1). There was no significant increase in concentrations of PFOS seen in the northern fur seal samples. Rookery differences were seen for PFUnA concentrations. Polavina and Reefpoint rookeries had significantly higher concentrations ($p < 0.05$) compared to concentrations in the northern fur seals from the Zapadni rookery (Figure 2).

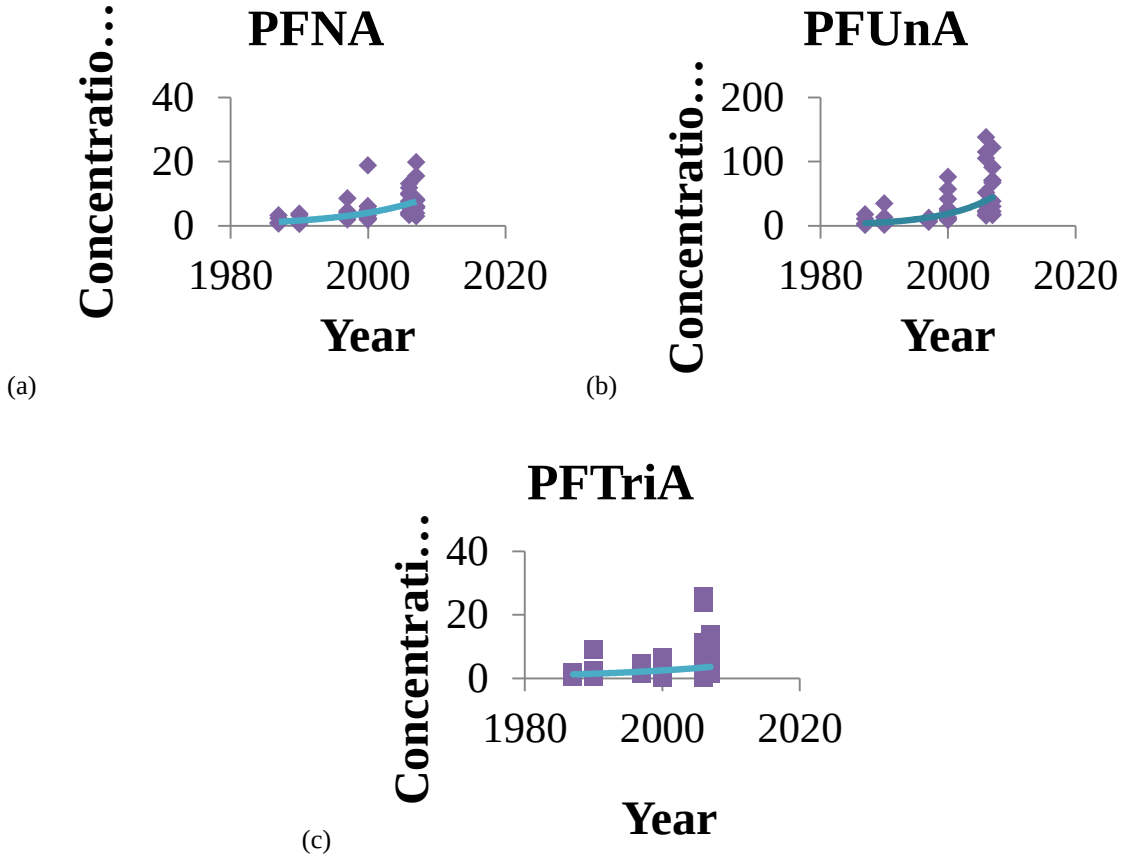

Figure 1. Temporal trend of a) PFNA (R^2 = 0.57) b) PFUnA (R^2 = 0.56) and c) PFTriA (R^2 = 15) concentrations (ng/g) in northern fur seal livers from Alaska.

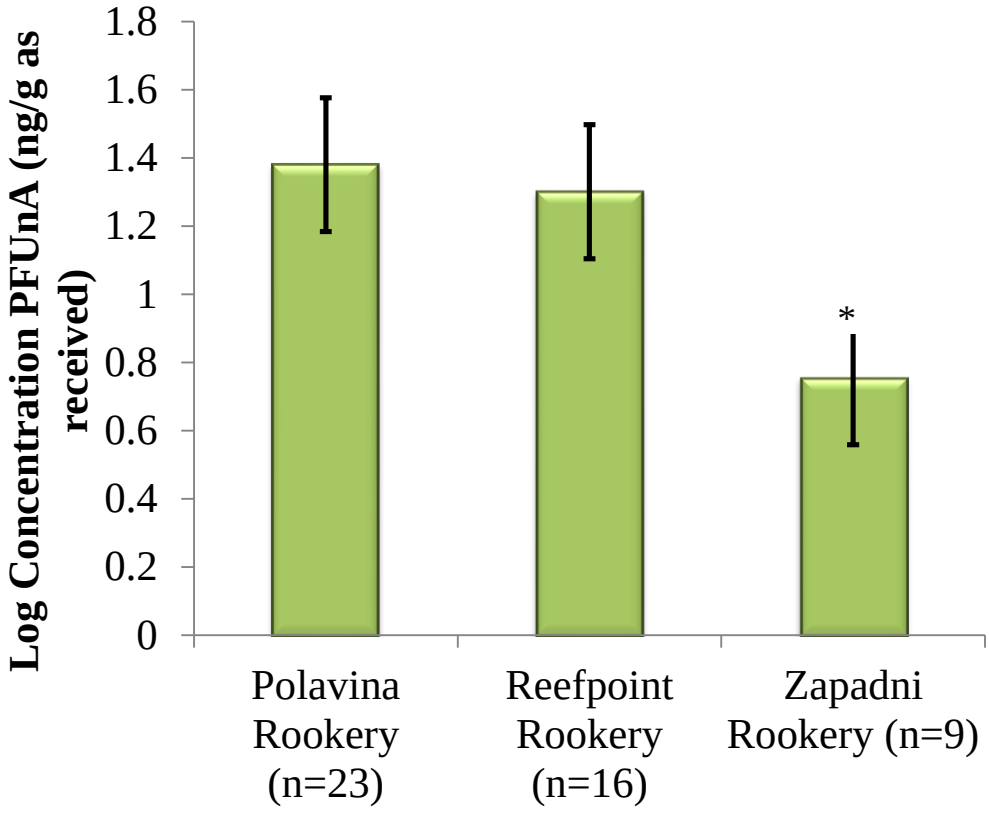

Figure 2. Rookery concentrations of PFUnA in northern fur seal livers. Asterisk (*) indicates a significantly ($p < 0.05$) lower concentration of PFUnA. Error bars represent the standard deviation.

This presentation will highlight the importance of specimen banking for the purpose of monitoring emerging contaminants in samples, helping to understand the implications of the release of PFAAs from lower latitudes, their transport to the Arctic, and temporal trends.

Acknowledgements:
All specimens used in this study were collected and banked through the Alaska Marine Mammal Tissue Archival Project with funding support from the Minerals Management Service, U.S. Geological Survey Biological Resources Division (USGS BRD), National Marine Fisheries Service (NMFS) Office of Protected Resources (Teri Rowles), and the National Institute of Standards and Technology (NIST). The following individuals and organizations are acknowledged for their aid in the northern fur seal specimen collections and banking: from the Aleut Community of St. Paul Island - Phillip A. Zavadil, Aquilina D. Lestenkof, Pamela Lestenkof, Pat Kozloff, and Darleen Melovidov, and from the NMFS Alaska Regional Office – Mike Williams and Steve Zimmerman. A special acknowledgment goes to Terry Spraker, DVM, Colorado State University, who provided special aid at St. Paul in obtaining all of the specimens used in this study. Also Barbara Porter, Lauren Rust, Danielle Peterson, and Jody Rhoderick, NIST Marine Environmental Specimen Bank. Funding support for the analysis was provided by NIST.

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National

Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

References:

1. Becker PR, Wise SA, Koster BJ, Zeisler R. (1991) NISTIR 4529. USDOC, National Institute of Standards and Technology, Gaithersburg, MD
2. Kannan K, Yun SH, Evans TJ. (2005) *Environ Sci Technol* 39:9057-9063
3. Bossi R, Riget FF, Dietz R. (2005) *Environ Sci Technol* 39:7416-7422
4. Butt CM, Mabury SA, Muir, Braune BM. (2007) *Environ Sci Technol* 41:3521-3528
5. Butt CM, Muir DC, Stirling I, Kwan M, Mabury SA. (2007) *Environ Sci Technol* 41:42-49
6. Houde M, De Silva AO, Muir DCG, Letcher RJ. (2011) *Environ Sci Technol* 45:7962-7973
7. Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R, Gobas FAPC. (2009) *Environ Sci Technol* 43:4037-4043
8. Smithwick M, Norstrom RJ, Mabury SA, Solomon K, Evans TJ, Stirling I, Taylor MK, Muir DC. (2006) *Environ Sci Technol* 40:1139-1143
9. Ellis DA, Martin JW, DeSilva AO, Mabury SA, Hurley MD, SulbaekAndersen MP, Wallington TJ. (2004) *Environ Sci Technol* 38:3316-3321
10. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. (2006) *Environ Sci Technol* 40:32-44
11. Armitage JM, MacLeod M, Cousins IT. (2009) *Environ Sci Technol* 43:1134-1140
12 Hart K, Gill VA, Kannan K. (2009) *Arch Environ Contam Tox* 56:607-614
13 Taniyasu S, Kannan K, So MK, Gulkowska A, Sinclair E, Okazawa T, Yamashita N. (2005) *J Chrom A* 1093:89-97
14. Keller JM, Calafat AM, Kato K, Ellefson ME, Reagen WK, Strynar M, O'Connell S, Butt CM, Mabury SA, Small J, Muir DCG, Leigh SD, Schantz MM. (2010) *Anal Bioanal Chem* 397:439-451
15. Kannan K, Koistinen J, Beckman K, Evans T, Gorzelany JF, Hansen KJ, Jones PD, Helle E, Nyman M, Giesy JP. (2001) *Environ Sci Technol* 35: 1593-1598
16. Reiner JL, O'Connell SG, Moors AJ, Kucklick JR, Becker PR, Keller JM. (2011) *Environ Sci Technol* 45: 8129-8136