ALKYLATED PERFLUORINATED SULFONAMIDES, METABOLISM AND THE RELATIONSHIP TO PRECURSOR AND PERFLUOROALKYL SULFONATE RESIDUES IN ARCTIC MAMMALIAN MARINE WILDLIFE

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Introduction

Poly- and per-fluoroalkyl substances (PFASs) are a class of chemicals that are used in many industrial and commercial applications, primarily for their stain repellency properties[1]. PFASs include perfluorinated sulfonates (PFSAs), which are globally distributed contaminants[2,3]. The PFSA perfluorooctane sulfonate (PFOS) has and continues to receive considerable attention due to its persistence, bioaccumulation and high concentrations in free-ranging wildlife worldwide. Over most of the last decade, PFOS is consistently reported to be among the most concentrated contaminant known in Arctic wildlife, particularly in the liver of polar bears (*Ursus maritimus*)[4-7]. There are several pathways in the environment that can account for the presence of PFOS and other PFSAs of shorter or longer alkyl chain length, in Arctic biota. As reviewed recently by Wang et al.[8] and Liu and Avendaño[9], pathways and sources of PFSAs in the environment include the release and subsequent degradation of PFSA-precursors such as via biotransformation.

In the majority of Arctic wildlife, PFOS is the dominant PFAS, but in contrast, perfluorooctane sulfonamide (FOSA) and *N*-alkyl-perfluorooctane sulfonamide (e.g. *N*-EtFOSA) levels are measured at much lower or non-detectable concentrations. In the present study, we examined existing data for free-ranging ringed seals (*Pusa hispida*) and polar bears, which have been shown to have very high PFOS:FOSA concentration ratios in the liver[4,6,7]. We also examined beluga whales (*Delphinapterus leucas*), where animals from the eastern Canadian Arctic[10], have shown PFOS:FOSA ratios that are in contrast to most other arctic wildlife species[2]. The discrepancy in PFOS:FOSA concentration ratio trends between cetaceans and other mammals may be due to differences in in the ability to biotransform PFOS precursors. In the present study, we examined three top Arctic mammalian predators, polar bear, beluga whale, and ringed seal and their capacity to deplete *in vitro*, pure linear isomer precursors of PFOS (i.e. FOSA and/or *N*-EtFOSA). Using enzymatically viable tissues, a liver microsomal *in vitro* assay approach was used where microsomes were extracted from cryopreserved polar bear, beluga whale and ringed seal liver tissues as well as the laboratory rat (*Rattus rattus*), which served as a mammalian control model.

Materials and methods

Full details of sample collection, preparation of liver microsomes and analysis can be found elsewhere[11,12]. Briefly, fresh (< 60 min post mortem) liver specimens were collected from a stranded polar bear from Iceland (in 2008) and from subsistence hunted beluga whale (in 2003) and ringed seal (in 2001) from Canada. At the time of collection, the liver tissues were temporarily stored in a liquid nitrogen dry shipper and subsequently transferred to a -80 °C freezer at Environment Canada's Wildlife Specimen Bank. Liver microsomes were prepared from the polar bear, ringed seal and beluga whale liver samples in June 2009. These microsomal suspensions included the use of buffers with DTT (10 nM to preserve reductase and deiodinase enzyme activities), and subdivision into 1 mL aliquots of 10 mg microsomal protein/mL, and storage at -80 °C until further use. No substantial enzyme activity was lost over this microsomal storage period since there was no decrease in the cytochrome P450 (CYP)1A enzyme catalyzed 7-ethoxyresorufin-*O*-deethylase (EROD) activity at the time of microsome preparation[11], and after approximately 8 months and prior to using the same microsomes for the present *N*-EtFOSA/FOSA assays performed in 2010.

As we have reported elsewhere[12,13], *N*-EtFOSA and FOSA metabolism *in vitro* was investigated using a model rat liver microsomal assay (as a positive assay control), as well as using liver microsomes from East Greenland

polar bear (n=1), and Canadian Arctic beluga whale (n=2) and ringed seal (n=2). *N*-EtFOSA, FOSA and PFOS in the incubation solutions were analyzed by liquid chromatography-tandem quadrupole-mass spectrometry (LC-ESI(-)-MS/MS).

Results and discussion

In our preliminary time-course study using rat microsomes, we found a rapid depletion of *N*-EtFOSA at a calculated rate of 23 pmol/min/mg protein (Figure 1). In fact, after only 10 min., *N*-EtFOSA metabolism and FOSA formation was > 90% complete. As also shown in Figure 1, even after 90 min. there was no significant ($p > 0.01$) depletion of FOSA in the rat assay and thus no PFOS could be detected. The FOSA formed from *N*-EtFOSA is therefore a "stable" metabolite as it did not undergo any further transformation. Thus, in the microsomal suspensions, the necessary enzymes to catalyze FOSA to PFOS were either absent or the rate of transformation was too slow to detect any change within the 90 min incubation period. This is consistent with Benskin *et al*.[14] who reported no FOSA depletion or PFOS formation in assays using human microsomes and recombinant human CYPs 2C9 and 2C19. Xu *et al*.[15] also observed no *in vitro* formation of PFOS from FOSA using a rat liver microsomal assay, but did report PFOS formation *in vitro* after incubation with rat liver slices with FOSA.

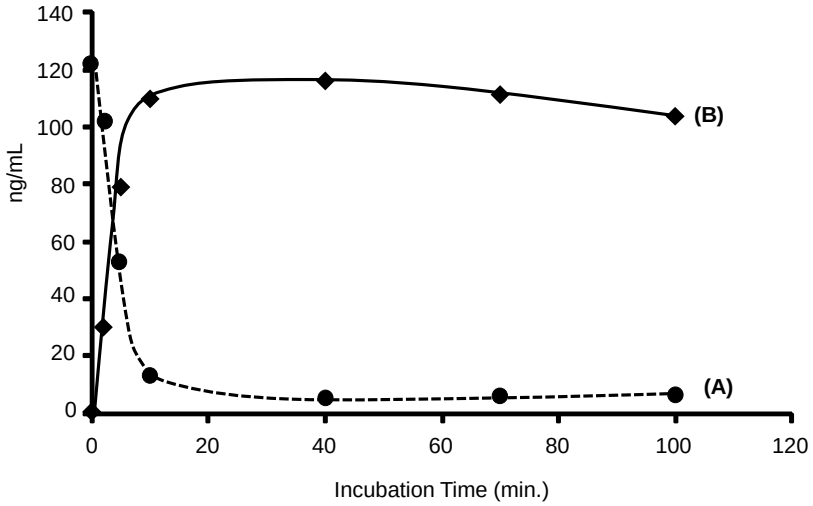

Figure 1. Time course incubation of (A) *N*-EtFOSA depleted and (B) FOSA formed concentrations (based on n = 3 replicate assays for each time point) in an *in vitro* liver assay using adult male Wistar-Han rat microsomes. The initial *N*-EtFOSA concentration was 300 nM.

The source of PFOS in Arctic wildlife is not fully understood. In particular, the degradation of PFOS precursors (or "PreFOS") is largely unknown, which refers to the complexity of fluorinated precursors that can degrade and give rise to PFOS in exposed biota[8,9]. Regardless, the key intermediate in the abiotic or biotic formation of PFOS appears to be FOSA[2,3]. We found that after 90 min., the comparative extent of *in vitro* depletion of *N*-EtFOSA was > 95% for rat and polar bears microsomes, an average of 65% for ringed seals, and with no significant ($p>0.05$) depletion of *N*-EtFOSA for beluga whale (Figure 2a). Concomitantly, the corresponding FOSA formation *in vitro* was also quantitatively in the order of rat ≈ polar bear > ringed seal >>> beluga whale, and followed the same change trend of *N*-EtFOSA depletion (Figure 2b). Similar to the rat assay, after 90 min., for polar bear, ringed seal and beluga whale, there was no significant ($p > 0.01$) decrease in depletion of the FOSA metabolite as well as no concomitant PFOS formation. Therefore, a mass balance was achieved in the assays with polar bear and ringed seal microsomes, since the depletion of *N*-EtFOSA was inversely proportional to the formation of the only measureable metabolite, FOSA. Our *N*-EtFOSA *in vitro* depletion results were consistent with that of Benskin *et al*.[14], who reported similar isomer-specific *in vitro* biotransformation rates of *N*-EtFOSA in assays based on human microsomes and recombinant human CYPs 2C9 and 2C19.

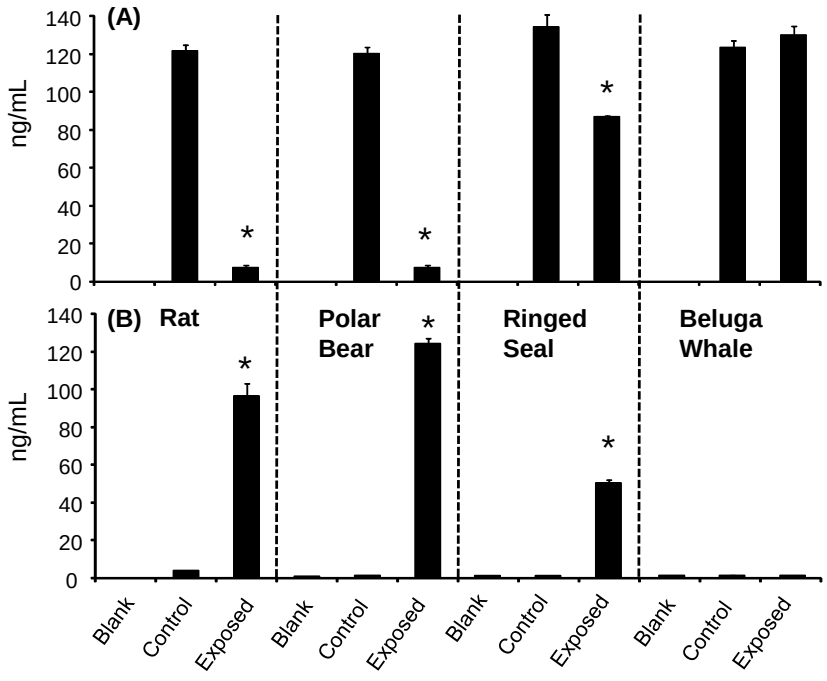

Figure 2. The mean concentration (±SD) of (A) *N*-EtFOSA depleted and (B) FOSA formed in an *in vitro* liver assay using adult male Wistar-Han rat (RAT), polar bear (PB), ringed seal (RS) and beluga whale (BW) microsomes. There were n = 1 blank, n=4 inactive control replicate and n=4 active replicate *in vitro* assays The asterisk indicates a significantly ($p < 0.01$) different mean concentration between the inactive and corresponding active assays. The initial *N*-EtFOSA concentration in the *in vitro* assays was 38 nM.

Biomonitoring in humans, wildlife and fish continues to show tissue concentrations of FOSA, which may be biotransformed to PFOS[2,8,10,15,16]. The present study on "PreFOS" *in vitro* metabolism, as shown by *N*-EtFOSA to FOSA transformation, was consistent with the relative residue concentrations of *N*-EtFOSA, FOSA and PFOS that have been reported in the liver of free-ranging wildlife in the order of polar bear ≥ ringed seal >>> beluga whale. These PFOS:FOSA concentration ratio trends correspond well with results and reviewed literature values conducted by Galatius *et al.*[16] where it was concluded that a general pattern could be observed with Carnivora species including Pinnipedia having a much higher capacity of transforming FOSA to PFOS than cetacean species including beluga whales.

Although the number of individual animals was limited, in the present study the liver microsomal assays showed that *N*-EtFOSA to FOSA biotransformation was in the order of rat ≈ polar bear > ringed seal >>> beluga whale, while no *in vitro* PFOS formation occurred from any FOSA metabolite formed. This suggests that the occurrence of FOSA in free-ranging Arctic ringed seal and polar bear tissues is partly due to biotransformation of accumulated *N*-EtFOSA. In contrast, this *N*-EtFOSA to FOSA metabolism appears unlikely in beluga whales due to much lower enzyme-mediated dealkylation activity and capacity in the liver. There was no evidence for *in vitro* biotransformation of FOSA to PFOS, which may be due to lack of enzymatic ability and/or that the transformation rate was too slow to measure in the *in vitro* incubation time of 90 min. However, this does not preclude that in free-ranging polar bears and ringed seals, PFOS residue levels may be due in part to metabolism of accumulated FOSA. Overall, our study adds further evidence to the growing importance of "PreFOS" degradation to more terminal PFSAs in biota.

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References:

1. Lindstrom AB, Strynar MJ, Libelo EL. (2011); *Environ Sci Technol*. 45: 7954-61.
2. Butt CM, Berger U, Bossi R, Tomy GT. (2010); *Sci Total Environ*. 408 : 2936−65.
3. Houde M, De Silva AO, Muir DCG, Letcher RJ. (2011); *Environ Sci Technol.* 45: 7962-73.
4. Dietz R, Bossi R, Rigét FF, Sonne C, Born EW. (2008); *Environ Sci Technol*. 42: 2701−7.
5. Letcher RJ, Bustnes J-O, Dietz R, Jenssen BM, Jorgensen EH, Sonne C, Verreault J, Vijayan MM, Gabrielsen GW. (2010); *Sci Total Environ*. 408: 2995−3043.
6. Greaves AK, Letcher RJ, Sonne C, Dietz R, Born EW. (2012); *Environ Sci Technol*. 46: 11575-83.
7. Rigét FF, Bossi R, Sonne C, Vorkamp K, Dietz R. (2013); *Chemosphere*. 93: 1607–14.
8. Wang Z, Cousins IT, Scheringer M, Hungerbühler K. (2013); *Environ Int.* 60: 242-8.
9. Liu J, Avendaño SM. (2013); *Environ Int.* 61: 98-114.
10. Tomy GT, Budakowski W, Hallorson T, Helm PA, Stern GA, Friesen K, Pepper K, Tittlemier SA, Fisk, AT. (2004); *Environ Sci Technol.* 38: 6475–81.
11. McKinney MA, Dietz R, Sonne C, De Guise S, Skirnisson K, Karlsson K, Steingrímsson E, Letcher RJ. (2011); *Environ Toxicol Chem*. 30: 1506-14.
12. Letcher RJ, Chu SG, McKinney MA, Tomy GT, Sonne C, Dietz R. (2014); *Chemosphere*. In press.
13. Chu SG, Letcher RJ. (2014); *Environ Sci Technol*. In press.
14. Benskin JP, Holt A, Martin JW. (2009); *Environ Sci Technol.* 43: 8566-72.
15. Xu L, Krenitsky DM, Seacat AM, Butenhoff JL, Anders MW. (2004); *Chem Res Toxicol.* 17: 767–75.
16. Tomy GT, Tittlemier SA, Palace VP, Budakowski WR, Braekevelt E, Brinkworth L, Friesen K. (2004); *Environ Sci Technol*. 38: 758–62.
17. Galatius A, Bossi R, Sonne C, Rigét FF, Kinze CC, Lockyer C, Teilmann J, Dietz R. (2013); *Environ Sci Pollut Res*. 20: 8013–20.