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Comparative hepatic *in vitro* depletion and metabolite formation of major perfluorooctane sulfonate precursors in arctic polar bear, beluga whale, and ringed seal

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HIGHLIGHTS

- A hepatic microsomal *in vitro* assay examined *N*-EtFOSA to FOSA metabolism.
- *N*-EtFOSA depletion and FOSA formation was 95% for polar bear after 90 min.
- *N*-EtFOSA depletion and FOSA formation was 65% for ringed seal after 90 min.
- There was negligible *N*-EtFOSA or FOSA formation for beluga whale after 90 min.
- Biotransformation of accumulated *N*-EtFOSA may contribute to FOSA in Arctic biota.

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ABSTRACT

Perfluorooctane sulfonate (PFOS) has been reported to be among the most concentrated persistent organic pollutants in Arctic marine wildlife. The present study examined the *in vitro* depletion of major PFOS precursors, *N*-ethyl-perfluorooctane sulfonamide (*N*-EtFOSA) and perfluorooctane sulfonamide (FOSA), as well as metabolite formation using an assay based on enzymatically viable liver microsomes for three top Arctic marine mammalian predators, polar bear (*Ursus maritimus*), beluga whale (*Delphinapterus leucas*), and ringed seal (*Pusa hispida*), and in laboratory rat (*Rattus rattus*) serving as a general mammalian model and positive control. Rat assays showed that *N*-EtFOSA (38 nM or 150 ng mL^{-1}) to FOSA metabolism was >90% complete after 10 min, and at a rate of 23 pmol min^{-1} mg^{-1} protein. Examining all species in a full 90 min incubation assay, there was >95% *N*-EtFOSA depletion for the rat active control and polar bear microsomes, ~65% for ringed seals, and negligible depletion of *N*-EtFOSA for beluga whale. Concomitantly, the corresponding *in vitro* formation of FOSA from *N*-EtFOSA was also quantitatively rat ≈ polar bear > ringed seal >>> beluga whale. A lack of enzymatic ability and/or a rate too slow to be detected likely explains the lack of *N*-EtFOSA to FOSA transformation for beluga whale. In the same assays, the depletion of the FOSA metabolite was insignificant ($p > 0.01$) and with no concomitant formation of PFOS metabolite. This suggests that, in part, a source of FOSA is the biotransformation of accumulated *N*-EtFOSA in free-ranging Arctic ringed seal and polar bear.

1. 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 (Lindstrom et al., 2011). Commercial applications include carpets, textiles, paper and food packaging, and aqueous film forming foam for

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fire-fighting. PFASs include perfluorinated sulfonates (PFSAs), which are globally distributed contaminants (Houde et al., 2011). 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 (Houde et al., 2006, 2011). 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*) from East Greenland (Smithwick et al., 2005; Dietz et al., 2008; Butt et al., 2010; Letcher et al., 2010; Greaves et al., 2012; Rigét et al., 2013). For example, PFOS in polar bears collected in 2006 from East Greenland had mean liver and fat concentrations of 3271 ± 290 and 15.4 ± 1.9 ng g^{-1} wet weight (ww), respectively (Greaves et al., 2012), as compared to mean concentrations of 9690 ± 3726 and 1085 ± 510 ng g^{-1} ww for ΣPCB and Σchlordane pesticides, respectively, in the fat of the same bears (Dietz et al., 2013).

There are multiple pathways in the environment that can account for the presence of PFOS and other PFSAs of shorter or longer alkyl chain length. One pathway has been shown to be due to direct PFSA release (Paul et al., 2009). As reviewed recently by Wang et al. (2013) and Liu and Avendaño (2013), PFSAs in the environment can also be sourced from the release and subsequent degradation of PFSA-precursors, which includes pathways such as atmospheric oxidation (D'eon et al., 2006; Martin et al., 2006) and biotransformation (Xu et al., 2004).

In the majority of Arctic wildlife, PFOS is the dominant PFAS, but in contrast, perfluorooctane sulfonamide (FOSA) levels are measured at much lower concentrations. Liver tissues of free-ranging ringed seals and polar bears have been shown to have very high PFOS to FOSA (PFOA:FOSA) concentration ratios (Dietz et al., 2008; Butt et al., 2010; Greaves et al., 2012, 2013; Rigét et al., 2013). This information corresponds well with the primary observation and review of the literature by Galatius et al. (2013) where it was concluded that Carnivora species including Pinnipedia have a much higher capacity of transforming FOSA to PFOS than cetacean species. For beluga whales (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*) from the eastern Canadian Arctic, Tomy et al. (2004a) was the first to report on *N*-ethyl-perfluorooctane sulfonamide (*N*-EtFOSA) in any Arctic biota, and suggested that *N*-EtFOSA and other FOSA-type precursors are likely present but are being biotransformed to FOSA. In contrast to most other arctic wildlife species (Houde et al. 2006), it has been reported that beluga whale have liver concentrations of PFOS that are lower than FOSA concentrations, and in the range of *N*-EtFOSA (Tomy et al. 2004a). The discrepancy in PFOS:FOSA concentration ratio trends between cetaceans and other mammals (notably ringed seals (*Pusa hispida*) and polar bears) may also be due to differences in diet. Alternatively, these trends may represent differences in the ability to biotransform PFOS precursors. To our knowledge, there have been no reports examining the biotransformation of PFOS precursors to PFOS in Arctic wildlife using *in vitro* or *in vivo* study approaches.

In the present study, we tested the hypothesis that three top Arctic mammalian predators, polar bear, beluga whale, and ringed seal can differentially 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.

2. Materials and methods

2.1. Standards and chemicals

Dithiothreitol (DTT; Cleland's reagent) and NADPH regenerating systems were purchased from Sigma–Aldrich and BD Gentest, respectively. Standard solutions of perfluoro-1-octanesulfonamide (FOSA), *N*-ethyl-perfluoro-1-octanesulfonamide (*N*-EtFOSA), $^{13}C_8$-FOSA and d_3-*N*-EtFOSA were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

HPLC grade methanol and diethyl ether was purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada) and VWR International (Mississauga, ON, Canada), respectively. Ultrapure water was obtained from a Milli-Q system. Ammonium acetate was obtained from Sigma–Aldrich (Oakville, ON, Canada).

A suspension of rat liver microsomes (protein content 20 mg mL^{-1}) from pooled adult male Wister–Han rats (BD Gentest, Woburn, MA, USA), NADPH regeneration system solutions (A) and (B) were obtained from BD Biosciences. Buffer containing 80 mM NaH_2PO_4, 6.0 mM $MgCl_2$, and 1.0 mM disodium ethylenediaminetetraacetate [Na_2EDTA] (pH = 8.0) was prepared in our lab (Organic Contaminants Research Lab (OCRL), NWRC, Ottawa, Canada).

2.2. Sample collection, microsome preparation and analysis

Full details of sample collection, preparation of liver microsomes and analysis can be found in McKinney et al. (2011). 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 (Table 1). At the time of collection, the liver tissues were temporarily stored in a liquid nitrogen dry shipper and

Table 1
Collection location and date and biological data for the liver samples of polar bears, ringed seal and beluga from the Arctic, and liver microsomal protein content and ethoxyresorufin-*O*-deethylase (EROD) activity.[a]

Sample ID	Species	Collection region	Collection date (YYYY/MM)	Sex	Age class	Microsomal yield[c] (mg protein/g tissue)	EROD[c] (pmol mg^{-1} protein min^{-1})
PB1	Polar bear	Iceland[b]	2008/06	Female	Adult	15 (2)	2167 (99)
BW	Beluga whale	Western Hudson Bay, Canada	2003/08	Female	Adult	9.0 (0.3)	309 (6)
RS1	Ringed seal	Cumberland Sound, Canada	2001/07	Female	Adult	18 (1)	397 (15)
RS2	Ringed seal	Cumberland Sound, Canada	2001/07	Female	Adult	19 (0.4)	199 (10)
RAT	Wistar–Han rat	N/A	N/A	Male	Adult	20	120

[a] From McKinney et al. (2011).
[b] PB1 was stranded in Iceland as the sea ice retreated during summer. It is thus likely to be an individual from the East Greenland subpopulation.
[c] Mean (±SD) of interday duplicate assays (n = 3 replicates/assays), except RS2 microsomal yield is mean (±SD) of single day replicates, and RAT EROD data is from microsome provider (BD Gentest).