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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).

subsequently transferred to a −80 °C freezer at Environment Canada's Wildlife Specimen Bank.

Microsomes were prepared from the polar bear, ringed seal and beluga whale liver samples in June 2009, which has been described in McKinney et al. (2011). 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^{-1}, and storage at −80 °C until further use. The present *N*-EtFOSA *in vitro* assays used these same liver microsomal suspensions. No substantial enzyme activity was lost over this microsomal storage period since there was no decrease in the CYP1A-catalyzed 7-ethoxyresorufin-*O*-deethylase (EROD) activity at the time of microsome preparation (McKinney et al., 2011) and after approximately 8 months and prior to using the same microsomes for the present *N*-EtFOSA assays performed in 2010.

The CYP1A-catalyzed EROD activity was used as an overall indicator of microsomal enzymatic capacity. The EROD and protein content were simultaneously determined using the fluorescence microplate assay of Kennedy and Jones (1994), but the reaction was terminated after 2 min to ensure resorufin concentrations fell within the calibration curve range. Calibration curve stock standard concentrations were determined spectrophotometrically immediately prior to the assay. Bovine serum albumin was used as the protein standard. Resorufin was the standard for EROD measurements.

2.3. In vitro metabolism assay

Details of the *in vitro* microsomal assay are described in full elsewhere (McKinney et al., 2011). Briefly, the incubation solution contained buffer (i.e. 80 mM NaH_2PO_4, 6.0 mM $MgCl_2$, 1.0 mM Na_2-EDTA, pH = 8.0), 3 μL of substrate solution (50 μg mL^{-1} in methanol) and 60 μL of NADPH regenerating system solutions (50 μL solution A and 10 μL of solution B). After pre-incubation in a water bath at 37 °C for 5 min, the reactions were initiated by addition of 1 mg of microsomal protein. The total final volume of incubation solution in each assay was 1 mL. The solution was incubated in a water bath (37 °C) with shaking (120 rpm). For negative control *in vitro* assays, liver microsomes that were used were heated to 100 °C for 5 min in a water bath to deactivate the enzymes. These deactivated microsomes were used in the negative control assays, and included *N*-EtFOSA substrate and NADPH, to assess for any potential non-enzymatic *N*-EtFOSA depletion, and/or FOSA formation and depletion, and/or PFOS formation. For buffer blank assays there was only buffer. The buffer blank assays did not contain microsomes or *N*-EtFOSA substrate, to assess for any *N*-EtFOSA or FOSA background contamination. In a time course experiment with just rat liver microsomes, at the 0, 2, 5, 10, 40, 70, and 100 min time points during the incubations (as described above), and 200 μL of each suspension was transferred into conical centrifuge tube. For all other *in vitro* wildlife assays, the maximum incubation time of 90 min was performed. The enzyme-catalyzed reactions were quenched by addition of 2 mL acetonitrile, and then 2 mL of the $^{13}C_8$-PFOSA and d_3-*N*-EtFOSA internal standards in acetonitrile were added (i.e., for each, 20 ng mL^{-1} X 2 mL = 40 ng). After 30 min of sonication and 10 min of centrifugation (5000 rpm), 1 mL of the supernatant phase was transferred to a LC vial for quantitative analysis by liquid chromatography–tandem quadrupole-mass spectrometry (LC–MS/MS). For each of the rat, beluga whale, polar bear and ringed seal, there were $n = 4$ replicate inactive control and $n = 4$ replicate enzyme-active *in vitro* assays. A ($n = 1$) buffer blank assay was included with each of the $n = 4$ replicate active and inactive enzyme assay blocks for each species.

2.4. PFOS, FOSA and N-EtFOSA extraction and quantitative determination

PFOS, FOSA and *N*-EtFOSA in the final incubation fractions were determined by LC–MS/MS. A volume of 50 μL of sample was injected into the LC system using an auto-injector. The LC separation was carried out using a Waters 2695 liquid chromatograph (Waters Limited, Milford, USA) and using a Luna C18(2) column (150 mm × 4.6 mm, 5 μm particle size) (Phenomenex Com., Torrance, CA, USA). LC mobile phases were water (A) and methanol (B) with both containing 2 mM of ammonium acetate. The mobile phase flow rate was 0.3 mL min^{-1}. The gradient for the LC system started at 5% B, increasing to 100% B in 10 min and was held for 30 min. Thereafter the mobile phase composition was returned to initial conditions and the column was allowed to equilibrate for 15 min between runs.

The MS/MS was a Waters Quattro Ultima triple quadrupole mass spectrometer (Waters Limited, Milford, USA). Nitrogen was used as nebulizing gas and dissolvent gas. Analyses were performed in negative ion ESI mode using multiple reaction monitoring (MRM) with argon as the collision gas. The capillary voltage was 1.0 kV. The source temperature and desolvation temperature were 120 and 350 °C, respectively. Cone and desolvation gas flow rates were 100 and 600 L h^{-1}, respectively. The compound-dependent MS/MS operating parameters are listed in Table 2.

Concentrations of metabolites in the extracts were measured by internal standard method ($^{13}C_8$-FOSA or d_3-*N*-EtFOSA as internal standards). The *N*-EtFOSA and FOSA calibration solutions were prepared with 5 levels of concentration of target compounds (from 0 to 40 ng mL^{-1}). All the calibration standards contained a fixed concentration of $^{13}C_8$-FOSA and d_3-*N*-EtFOSA internal standards (8 ng mL^{-1} each). The correlation coefficient (r^2) of the linear ESI(−) response was >0.99 for all target analytes.

The limit of detection (LOD) was defined as the concentration that would give a signal to noise ratio (peak to peak) of 3 in low level spiked samples. The method limit of quantification (LOQ) was defined as the concentration that would give a signal to noise ratio (peak to peak) of 10 in low level spiked samples. The LODs for FOSA and *N*-EtFOSA were 0.06 and 0.21 ng mL^{-1}, respectively. The LOQs for FOSA and *N*-EtFOSA were 0.19 and 0.7 ng mL^{-1}, respectively. The mean recovery efficiencies for $^{13}C_8$-FOSA and d_3-*N*-EtFOSA were 90 ± 9% and 90 ± 5%, respectively. For all the enzyme-deactivated, negative control assays, only *N*-EtFOSA was measureable, and with a small residual amount of FOSA, i.e. <3% of the *N*-EtFOSA concentration for the rat assays, and <1% for all

Table 2
The compound dependent operating parameters of MS/MS and retention times for FOSA and *N*-EtFOSA analysis.

Compound	MRM[a] transition	Cone voltage (V)	Collision energy (eV)	Dwell time (sec)	Retention time (min)
FOSA	498 > 78	60	70	0.20	18.2
$^{13}C_8$-FOSA	506 > 78	60	70	0.20	18.2
N-EtFOSA	526.2 > 169.1	60	25	0.20	19.4
d_3-*N*-EtFOSA	531.2 > 169.1	60	25	0.20	19.4

[a] MRM = multiple reaction monitoring.

of the polar bear, ringed seal and beluga whale assays. For buffer blank assays, *N*-EtFOSA and FOSA were below detection.

2.5. Statistical analysis

Statistical significance of the *N*-EtFOSA depletion and FOSA formation between replicates of the inactive (control) and the enzyme-active assays was tested using analysis of variance (ANOVA) (followed by post hoc Tukey's honestly significant difference [HSD]). All tests were two-tailed with results considered significantly different at $p < 0.01$ using Statistica (v. 6.0, StatSoft, 2003).

3. Results and discussion

3.1. In vitro metabolism of N-EtFOSA and FOSA

As already reported in McKinney et al. (2011), the CYP1A-mediated EROD activity in the liver microsomes of the present Arctic wildlife species was highly variable, with rates from 200 to 2200 pmol mg^{-1} min^{-1} (Table 1). These rates fell within the expected ranges for beluga whale (McKinney et al., 2006), polar bear (Letcher et al., 1996) and ringed seal (Routti et al., 2008). This CYP1A-mediated EROD activity was a general indicator of the overall CYP isoform catalytic activity, and thus indicated that the enzyme activity was sufficiently viable in these liver microsomes for use in our present study.

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^{-1} mg^{-1} protein (Figs. 1 and 2). In fact, after only 10 min, *N*-EtFOSA metabolism and FOSA formation was >90% complete. As also shown in Figs. 1 and 2, 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. (2009) who reported no FOSA depletion or PFOS formation in assays using human microsomes and recombinant human cytochrome P450 enzymes (CYPs) 2C9 and 2C19. Xu et al. (2004) 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. PFOS has been shown to be formed through the biotransformation of perfluorooctane

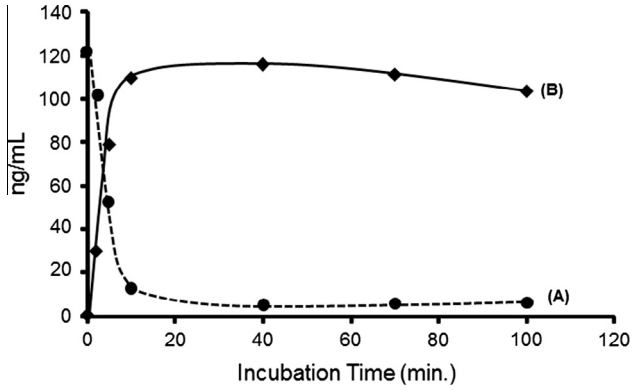

Fig. 1. Time course incubation of (A) *N*-EtFOSA depleted and (B) FOSA formed concentrations (based on $n = 3$ replicate assays for each time point) in an *in vitro* liver assay using adult male Wistar–Han rat microsomes. The initial *N*-EtFOSA concentration was 300 nM.

sulfonamide alcohols (FOSEs) and FOSAs in dosed rainbow trout (*Onchorhynchus mykiss*) *in vitro* (Tomy et al., 2004b). Xu et al. (2006) reported FOSA-glucuronides detected in rat, human, dog and monkey microsomal assays with *N*-EtFOSA as substrate. However, FOSA-glucuronide metabolites were possible because uridine diphosphate glucuronic acid (UDPGA) co-factor was added to the microsomal assays. At the functional cell level UDPGA co-factor can be produced.

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 (Parsons et al., 2008; Martin et al., 2010; Liu and Avendaño, 2013; Wang et al., 2013). FOSEs have been shown to degrade via atmospheric oxidation to PFOS (Stock et al., 2007). FOSEs and the related FOSAs have also been detected in Canadian Arctic air (Butt et al., 2010). The key intermediate in the abiotic or biotic formation of PFOS appears to be FOSA (Houde et al., 2011).

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 (Fig. 3a). Concomitantly, the corresponding FOSA formation *in vitro* was also quantitatively in the order of rat ≈ polar bear > ringed seal >>> beluga whale (Fig. 3b), and followed the same change trend of *N*-EtFOSA depletion (Fig. 3a). 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 proportionally the formation of the only measureable metabolite, FOSA. Note that the *in vitro* incubation parameters were not specifically optimized for maximal enzyme-mediated activity for *N*-EtFOSA depletion (and FOSA formation) as it was assumed that such parameters for polar bear, ringed seal and beluga whale would be the same as for the optimized parameters for the rat, given that they are all mammalian species. Regardless, interpretive caution needs to be exercised in comparing the absolute quantitative differences in the degree of *N*-EtFOSA depletion (and FOSA formation) among the species.

Our *N*-EtFOSA *in vitro* depletion results were consistent with that of Benskin et al. (2009), who reported similar isomer-specific *in vitro* biotransformation rates of *N*-EtFOSA in assays based on human microsomes and recombinant human cytochrome P450 enzymes (CYPs) 2C9 and 2C19. They also reported that starting at about 6 min of microsomal or protein incubation, human microsomes and CYP isozymes depleted branched as well as linear *N*-EtFOSA, and with concomitant formation of isomer-specific FOSAs. Similarly, Ross et al. (2012) examined the isomer-specific fate of FOSA in male Sprague–Dawley rats exposed to commercial FOSA via food. The elimination half-lives of the two major branched FOSA isomers were higher than for linear FOSA (5.9 ± 4.6 d), resulting in a depletion of branched FOSA isomers in blood and tissues relative to the dose. They concluded that their results confirmed that *in vivo* exposure to commercially relevant PFOS precursors can result in PFOS formation and a distinct PFOS isomer profile.

3.2. In vitro metabolism of N-EtFOSA and comparison to PFOS and precursor residues in Arctic wildlife tissues

Biomonitoring in humans, wildlife and fish continues to show tissue concentrations of FOSA, which may be biotransformed to PFOS (Tomy et al., 2004a, 2004b; Xu et al., 2004; Houde et al., 2011; Wang et al., 2013). The present study on "PreFOS" *in vitro* metabolism, as shown by *N*-EtFOSA to FOSA transformation, was

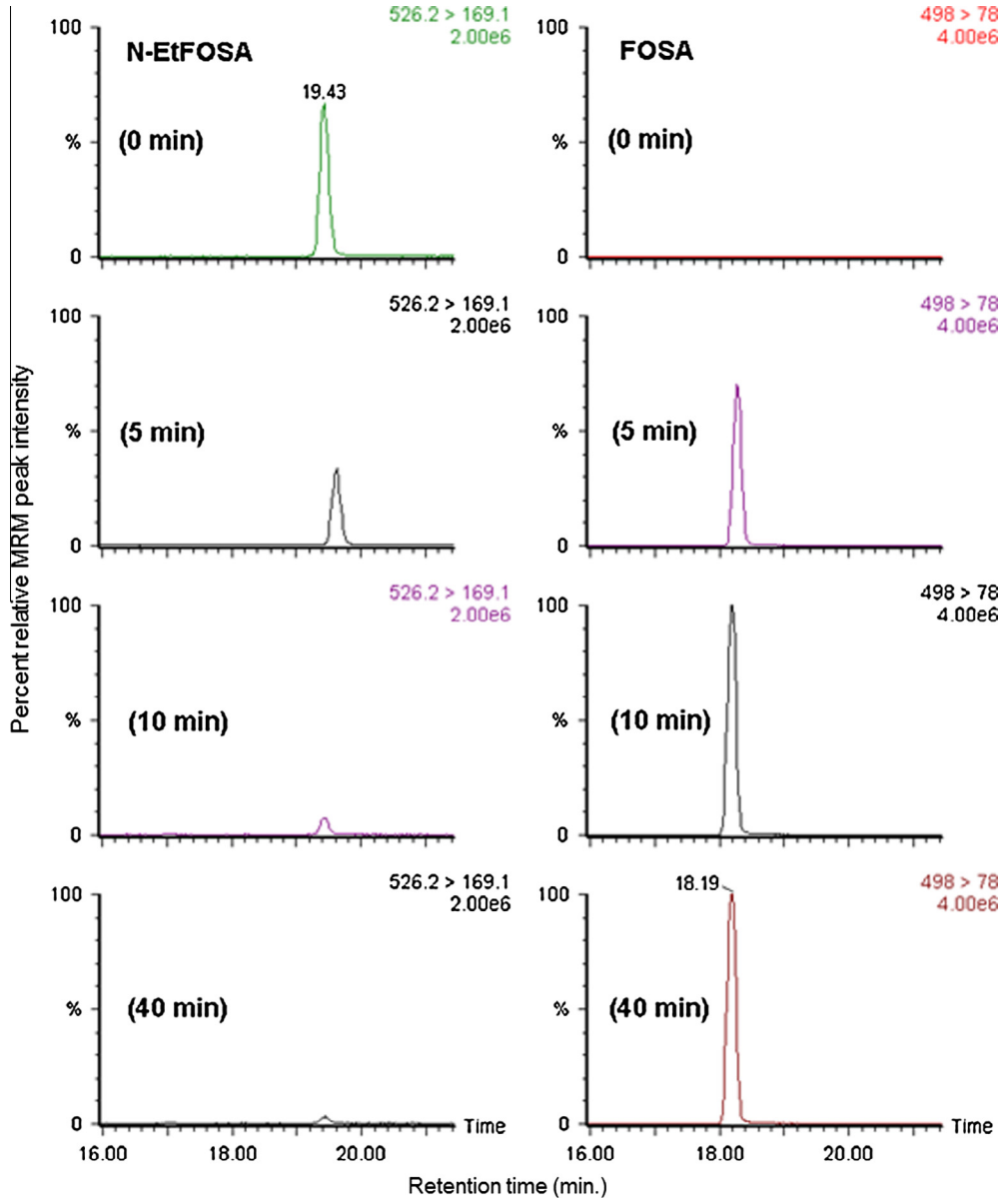

Fig. 2. LC-ESI(−)-MS/MS multiple reaction monitoring (MRM) (see Table 2) mass chromatograms of the time course incubations with Wister–Han rat liver microsomes at 0, 5, 10 and 40 min, showing the time-dependent depletion of *N*-EtFOSA and formation of FOSA. The percent relative MRM peak intensity (*y*-axis) of the *N*-EtFOSA (left column) and FOSA (right column) peaks at each time point are normalized as a percentage relative the *N*-EtFOSA and FOSA responses at time zero.

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. Mean PFOS:FOSA concentration ratios in the livers of Canadian polar bears collected in 2007–2008 were found to be consistently very high at 463 for southern Hudson Bay, 387 for Foxe Basin/Gulf of Boothia, and 488 for Beaufort Sea, where the mean PFOS concentrations in liver were 2970, 550 and 770 ng g^{-1} wet weight, respectively (Houde et al., 2011; R. Letcher, unpublished data). *N*-EtFOSA was also not detectable in these same bear liver samples. These PFOS:FOSA concentration ratios correspond well with results and reviewed literature values conducted by Galatius et al. (2013) 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. In the livers of polar bears from East Greenland collected in 2006, the mean PFOS level was reported to be 3270 ng g^{-1} ww, and the PFOS:FOSA concentration ratio averaged 21 (Greaves et al., 2012), and where this PFOS:FOSA ratio was substantially lower relative to bears from Canadian subpopulations (Houde et al., 2011; R. Letcher, unpublished data). Greaves et al. (2012) also reported that for these East Greenland polar bears the mean PFOS:FOSA concentration ratios ranged from 11 to 36 for liver, blood, brain, muscle and adipose tissue and thus were very similar. For East Greenland bears harvested in 2006, Rigét et al. (2013) also reported a mean PFOS levels of 2966 ng g^{-1} ww, and a mean PFOS:FOSA concentration ratio of 134 in liver samples.

Rigét et al. (2013) reported on PFAS levels in liver tissue of West and East Greenland ringed seals, however, FOSA was either in the order of 1 ng g^{-1} ww or was not detectable, although the mean PFOS concentrations were around 350 ng g^{-1} ww. In harvested (2002–2005) ringed seal liver from Cumberland Sound and surrounding Baffin Bay (Pangnirtung, Pond Inlet and Qikiqtarjuaq), PFOS concentrations were reported to be 53–138 ng g^{-1} ww (Butt et al., 2008), and generally lower than that of polar bears from subpopulations across the Canadian Arctic (387–488 ng g^{-1}

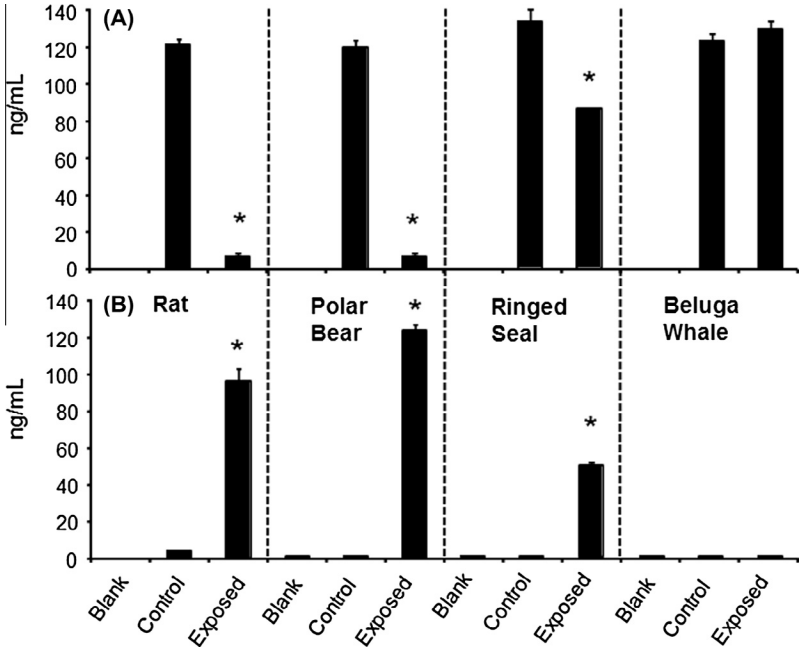

Fig. 3. The mean concentration (±SD) of (A) *N*-EtFOSA depleted and (B) FOSA formed in an *in vitro* liver assay using adult male Wistar–Han rat (RAT), polar bear (PB), ringed seal (RS) and beluga whale (BW) microsomes. There were $n = 1$ blank, $n = 4$ inactive control replicate and $n = 4$ active replicate *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. See Table 1 for details about the wildlife liver samples.

ww). Furthermore, for these same Canadian ringed seals, the mean FOSA levels for the various subpopulations was 0.05–2.7 ng g^{-1} www, and much lower than the mean PFOS levels.

In contrast to ringed seals and polar bears, for Cumberland Sound (Baffin Bay) beluga whales, Tomy et al. (2004a) reported mean PFOS, FOSA and *N*-EtFOSA liver concentrations of 12, 21 and 4 ng g^{-1} ww, respectively, and thus the PFOS:FOSA concentration ratio in liver was in the order of 0.6. Reiner et al. (2011) also reported PFOS:FOSA concentration ratios of 0.1–1.0 in the liver of Alaskan beluga whale. Such low PFOS:FOSA concentration ratios (in liver tissue) have been consistently reported in the literature for other cetacean species, with 1.8 reported for narwhal, 0.7–5.4 for long-finned pilot whales (*Globicephala melas*), 0.6 for bottlenose dolphins (*Tursiops truncatus*), 1.1 for common dolphin (*Delphinus delphis*), 0.9 for melonheaded whale (*Peponocephala electra*), and 0.2–12.3 for minke whale (*Balaenoptera acutorostrata*) (Kannan et al., 2002; Bossi et al., 2005; Hart et al., 2008; Moon et al., 2010). The present *in vitro* studies do not shed further light on whether *N*-EtFOSA metabolism is a source of FOSA tissue residues in free-ranging beluga whale. However, we stress that the lack of observed *N*-EtFOSA to FOSA transformation in the present beluga whale, *in vitro* liver microsomal assay may be due to (1) a very slow rate of transformation, and (2) being based on available liver from only individual beluga whale. Thus, in free-ranging beluga one reason for the lower PFOS:FOSA concentration ratios may be due to the (slow) rate of *N*-EtFOSA to FOSA transformation still being more rapid that the rate of FOSA to PFOS transformation. Regardless, our *in vitro* results showing a lack of *N*-EtFOSA to FOSA transformation in beluga whale is consistent with lower PFOS versus "preFOS" concentrations in cetaceans versus carnivora (Galatius et al., 2013).

4. Conclusions

Although the number of individual animals was limited, 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 accumulation FOSA. Overall, our study adds further evidence to the growing importance of "PreFOS" (Martin et al., 2010; Wang et al., 2013) degradation to more terminal PFSAs in biota (Butt et al., 2010; Houde et al., 2011).

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