Perfluoroalkyl Substances in Arctic Polar Bear and Ringed Seal From East Greenland and Hudson Bay: Comparisons of Adipose and Liver Levels and Patterns, and *In Vitro* Depletion and Metabolite Formation of PFAS Precursors

by

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ABSTRACT

The levels of 22 perfluorinated alkyl substances (PFASs) were examined in the livers of polar bears from Southern Hudson Bay (SHB) (n= 10) and Western Hudson Bay (WHB) (n= 9) and in the livers and adipose tissues of East Greenland (EG) polar bears (n= 10) and EG ringed seals (n=16), all harvested in recent years (2011-2012). EG polar bears livers contained greater concentrations of ΣPFAS than SHB and WHB bears, while having greater proportions of perfluorooctane sulfonic acid (PFOS) and lesser proportions of perfluorooctane sulfonamide (FOSA), and long-chained perfluoroalkyl carboxylic acids (PFCA). ΣPFAS concentrations in EG polar bears were greater than in ringed seals both in liver and in adipose, while no PFOS was detected in ringed seal blubber. Perfluoro-4-ethylcyclohexane (PFEtCHxS), perfluobutane carboxylic acid (PFBA), perfluorohexane carboxylic acid (PFHxA) and perfluorobutane sulfonamide (FBSA) were reported for the first time in Arctic wildlife. The biotransformation of N-ethyl-perfluorooctane sulfonamide (N-Et-FOSA) was measured in extracted microsomes of EG polar bears and ringed seals. The degradation of N-Et-FOSA was significant ($p > 0.01$) over a 40-minute incubation for high enzymatic activity polar bears, while FOSA formation was significant ($p > 0.01$) for all tested samples, except for low enzymatic activity ringed seals. Overall, the findings in this thesis underlined the importance of studying the fate of PFASs in the Arctic marine foodweb, as well as the necessity to consider short-chained PFASs and precursors when establishing PFAS exposure and levels.

LIST OF WORKS ASSOCIATED WITH THE THESIS

Journal Publications

Boisvert, G., Letcher, R. J., Dyck, M., Sonne, C. and Dietz, R., Polar bears From Two Arctic Pollution Hot Spots: Comparison of Recent Levels of Established and New Perfluorinated Sulfonic and Carboxylic Acids and Precursors, *Sci. Total Environ.*, In preparation (Chapter 3).

Boisvert, G., Letcher, R. J., Sonne, C., Rigét, F. F. and Dietz, R., Predator-Prey Biomagnification: Perfluoroalkyl Acids and Their Precursors in East Greenland Ringed Seals and Polar Bears, *Environmental Pollution*, In preparation (Chapter 4 and 5).

Conference Abstracts

Boisvert, G., Letcher, R. J., Sonne, C., Rigét, F. F. and Dietz, R., Novel and established per-/poly-fluoroalkyl substances and bioaccumulation and biomagnification in East Greenland ringed seals and polar bears, CREATE-REACT Summer school, University of Alberta, July 27th 2015 (Oral).

Boisvert, G., Letcher, R. J., Sonne, C., Rigét, F. F. and Dietz, R., Novel and established per-/poly-fluoroalkyl substances and bioaccumulation and biomagnification in East Greenland ringed seals and polar bears, Society of Environmental Toxicology and Chemistry (SETAC)-North America, Novembre 1-5, Salt Lake City, Utah, USA (Poster).

Boisvert, G., Letcher, R. J., Dyck, M., Sonne, C. and Dietz, R., Perfluorinated sulfonic and carboxylic acids and precursors in East Greenland and compared to subpopulations of polar bears from Hudson Bay, Canada, SETAC-North America, Novembre 1-5, Salt Lake City, Utah, USA (Poster).

Boisvert, G., Letcher, R. J., Sonne, C., Rigét, F. F. and Dietz, R., Novel and established per-/poly-fluoroalkyl substances and bioaccumulation and biomagnification in East Greenland ringed seals and polar bears, Northern Contaminants Program (NCP) Results Workshop, December 7-8, Vancouver, BC, Canada (Poster)

Boisvert, G., Letcher, R. J., Dyck, M., Sonne, C. and Dietz, R., Perfluorinated sulfonic and carboxylic acids and precursors in East Greenland and compared to subpopulations of polar bears from Hudson Bay, Canada, NCP Results Workshop, December 7-8, Vancouver, BC, Canada (Poster)

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BFR	Brominated Flame Retardant
BMF	Biomagnification Factor
BSA	Bovine Serum Albumin
CYP	Cytochrome P450
d-EtFOSA	N-ethyl-d_5-perfluoro-1-octanesulfonamide
d-MeFOSA	N-methyl-d_3-perfluoro-1-octanesulfonamide
DDT	Dichlorodiphenyldichloroethane
ECF	Electrochemical Fluorination
EG	East Greenland
EROD	7-ethoxyresorufin-O-deethylase
ESI-	Negative Electrospray Ionization
EtFOSE	Ethyl Perfluorooctane Sulfonamidoethanol
FASA	Perfluoroalkyl Sulfonamide
FOSA	Perfluorooctane Sulfonamide
FOSE	Perfluorooctane Sulfonamidoethanol
FTOH	Fluorotelomer Alcohol
GLG	Growth Layer Groups
HB	Hudson Bay
HCH	Hexachlorocyclohexane
HPLC	High-Performance Liquid Chromatography
ILOD	Instrumental Limit of Detection
LOAEL	Lowest No-adverse-effect Level
$LogK_{OW}$	Partition Coefficient Between Octanol and Water
M8FOSA	Perfluoro-1-[$^{13}C_8$]octanesulfonamide
MeFOSE	Methyl Perfluorooctane Sulfonamidoethanol
MLOD	Method Limit of Detection
MLOQ	Method Limit of Quantification
MPFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid
MPFDA	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid

MPFDoA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid
MPFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid
MPFHxS	Perfluoro-perfluoro-1-hexane[$^{18}O_2$]sulfonate
MPFNA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid
MPFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid
MPFOS	Perfluoro-perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate
MPFUdA	Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid
MRM	Multiple Reaction Monitoring
N-Et-FOSA	N-Ethyl-Perfluorooctane Sulfonamide
N-Et-FOSE	N-Ethyl-Perfluorooctane Sulfonamidoethanol
N-Me-FOSA	N-methyl-perfluorooctane sulfonamide
N/Q	Not Calculated
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NOAEL	No-adverse-effect Level
OHC	Organohalogen Compound
PB	Polar Bear
PBDE	Polybrominated Diphenyl Ether Congeners
PCB	Polychlorinated Biphenyl
PFAA	Perfluoroalkyl Acid
PFAS	Perfluorinated Alkyl Substance
PFBA	Perfluorobutanoic Acid
PFBS	Perfluorobutane Sulfonic Acid
PFCA	Perfluoroalkyl Carboxylic Acid
PFDA	Perfluorodecanoic Acid
PFDoA	Perfluorododecanoic Acid
PFDS	Perfludecanoic Acid
PFEtCHxS	Perfluoro-4-ethylcyclohexane Sulfonic Acid
PFHpA	Perfluoroheptanoic Acid
PFHxA	Perfluorohexanoic Acid
PFHxDA	Pefluorohexadecanoic Acid
PFHxS	Perfluorohexane Sulfonic Acid

PFNA	Perfluorononanoic Acid
PFOA	Perfluorooctanoic Acid
PFODA	Perfluorooctadecanoic Acid
PFOS	Perfluorooctane Sulfonic Acid
PFPeA	Perfluoropentanoic Acid
PFSA	Perfluoroalkyl Sulfonic Acid
PFTeDA	Perfluorotetradecanoic Acid
PFTrDA	Perfluorotridecanoic Acid
PFUdA	Perfluoroundecanoic Acid
pKa	Acid Dissociation Constant
POP	Persistent Organic Pollutant
POSF	Perfluorooctane Sulfonyl Fluoride
PreFOS	PFAS Precursor
QC	Quality Control
RS	Ringed Seal
SE	Standard Error
SHB	Southern Hudson Bay
SPE	Solid Phase Extraction
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography-Mass Spectrometry
WHB	Western Hudson Bay
ww	Wet Weight
$\delta^{13}C$	Stable Isotope Carbon 13
$\delta^{15}N$	Stable Isotope Nitrogen 15
Σ	Sum of

Chapter 1

General Introduction

The industrialization and scientific development of mankind has led to the unavoidable contamination and pollution of the environment. Persistent organic pollutants (POPs) were synthesized for specific applications and subsequently found to persist in the environment and bioaccumulate and have deleterious effects on exposed biota. Among POPs, organohalogen (chlorinated, brominated or fluorinated) compounds (OHCs) comprise a very large class of chemicals (Letcher et al., 2010). As of 2009, 12 chemicals were considered as legacy POPs by the Stockholm Convention: polychlorinated biphenyls (PCBs), Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex, Toxaphene, Polychlorinated dibenzo-p-dioxins and Polychlorinated dibenzofurans. However newer emerging POPs have and continue to be reported over the past years. For example, newer POPs include polybrominated diphenyl ether congeners (PBDE) and poly- and perfluorinated alkyl substances (PFASs). As of 2009, Penta- and Octa- PBDE formulations were added to the Annex A (Elimination) of the Stockholm convention, while Perfluorooctane sulfonic acid (PFOS) and its salts were added to the Annex B (Restriction) (Stockholm Convention, 2009).

1.1 Perfluorinated Alkyl Substances (PFASs)

PFASs encompass a wide grouping of chemicals that includes perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). They were first synthesized in 1920 and their structure was elucidated for the first time in 1926 (Banks et al., 1994; Lebeau et al., 1926). Later, with the discovery of Teflon, the commercialization of PFASs led to massive production by the 3M company from 1947 to 2002 (Jiang et al., 2015).

1.1.1 PFAS Structure and Chemical Properties

PFASs are comprised of a central carbon chain of varying length depending on the product. This carbon chain is either polyfluorinated (i.e. substituted with multiple fluorine atoms) or perfluorinated (i.e. fully substituted with fluorine atoms). At the end of the carbon chain a functional group completes the molecule. Depending on the PFAS class, the functional group is different (i.e. a sulfonic acid for PFSAs, a carboxylic acid for PFCAs and a neutral group for neutral PFASs) (See Table 1.1 for PFASs structure). Finally, PFASs come in two different isomer forms (i.e. linear and branched forms) (Ng et al., 2014).

The physical and chemical properties of PFASs vary greatly with their carbon chain length and functional groups (i.e. acids, sulfonamides, alcohols, aldehydes). Using perfluoroalkyl acids (PFAAs) that have been reported in environmental samples as examples, the molecular weight varies greatly from 300 g/mol (PFBS) to 600 g/mol (PFDS) for PFSAs g/mol and from 214 (PFBA) to 914 g/mol (PFODA) for PFCAs (Ng et al., 2014). Solubility in water goes up to approximately 1000 mg/L at 4 carbon-chain length and down to 0.01 mg/L at 10 carbon-chain length (Liu et al., 2007). Similarly, vapour pressure varies in a similar manner, reaching 200 Pa at 4 carbon-chain length and 0.2 Pa at 10 carbon-chain length (Bhhatarai et al., 2011). There is a broad spectrum of PFASs that are PFAA precursors. For example, perlfuorooctanesulfonyl fluoride, perfluorooctane sulfonamide (FOSA), perfluorooctane sulfinate, N-Ethyl perfluorooctane sulfonamide, perfluorooctane sulfonamidoehtanol (FOSE), FOSE phosphates, FOSE Acrylate Esters and perfluorooctane sulfonamidoacetate are a few known percursors to PFAAs (Martin et al., 2010). However, relative to PFAAs, chemical volatility is greater for neutral precursors such as fluorotelomer alcohols (FTOHs) which as major precursors to PFCAs (Stock et al., 2004). A physico-chemical property that is important in the environment and to

exposed biota is the partition coefficient between octanol and water (logK_{ow}) (Hidalgo et al., 2016). LogK_{OW} values for PFASs vary greatly; they range from being 4 times as high for perfluoro decanoic acid (PFDA) (4.65) compared to perfluorobutanoic acid (PFBA) (1.01) (Hidalgo et al., 2016). A physico-chemical property, pKa, is also extremely low for PFSAs and PFCAs as they are all acids, and can range from 0 for PFCAs and even lower for PFSAs, meaning that they are present as their conjugate base anions in aqueous solutions under neutral pH conditions (Ng et al., 2014). The important phyco-chemical properties important for this study are summarised in Table 1.2.

Table 1.1 Names, acronyms and structures of 22 PFASs that are found in biota in the environment and were studied in this project

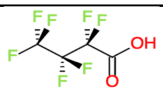
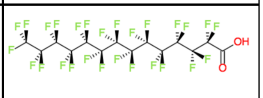
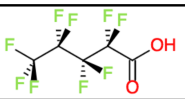
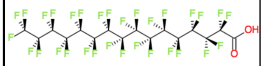
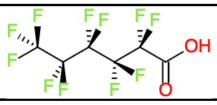
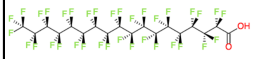
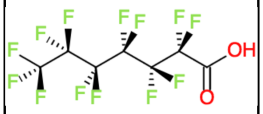
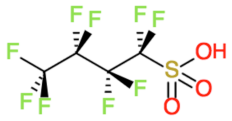
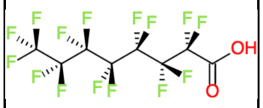
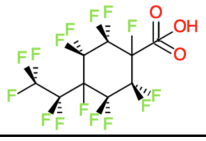
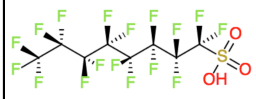
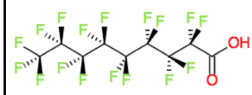
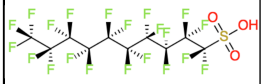
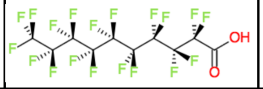
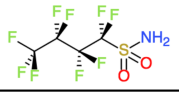
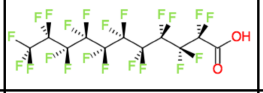
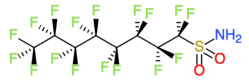
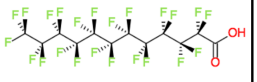
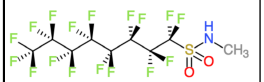
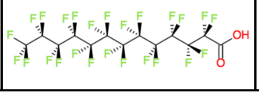
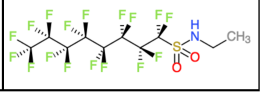
Compound name	Acronym	Structure	Compound name	Acronym	Structure
Perfluoro-n-butanoic acid	PFBA		Perfluoro-n-tetradecanoic acid	PFTeDA	
Perfluoro-n-pentanoic acid	PFPeA		Perfluoro-n-hexadecanoic acid	PFHxDA	
Perfluoro-n-hexanoic acid	PFHxA		Perfluoro-n-octadecanoic acid	PFODA	
Perfluoro-n-heptanoic acid	PFHpA		Perfluoro-1-butanesulfonic acide	PFBS	
Perfluoro-n-octanoic acid	PFOA		Perfluoro-1-hexanesulfonic acid	PFHxS	
Perfluoro-4-ethylcyclohexane sulfonic acid	PFEtCHxS		Perfluoro-1-octanesulfonic acid	PFOS	
Perfluoro-n-nonanoic acid	PFNA		Perfluoro-1-decanesulfonic acid	PFDS	
Perfluoro-n-decanoic acid	PFDA		Perfluoro-1-butanesulfonamide	FBSA	
Perfluoro-n-undecanoic acid	PFUdA		Perfluoro-1-octanesulfonamide	FOSA	
Perfluoro-n-dodecanoic acid	PFDoA		N-methylperfluoro-1-octanesulfonamide	N-Me-FOSA	
Perfluoro-n-tridecanoic acid	PFTrDA		N-ethylperfluoro-1-octanesulfonamide	N-Et-FOSA	

Table 1.2 Physicochemical properties of a suite of PFASs important in this study. Values are reported at 25°C.

Compound[a]	Log K_{ow}	Log K_{aw}	Vapour Pressure (Pa)	Compound[a]	Log K_{ow}	Log K_{aw}	Vapour Pressure (Pa)
PFBA	-0.62	0.30	251	PFHxDA	**6.84**	**7.42**	**0.45**
PFPeA	-0.02	0.86	151	PFODA	**8.09**	**8.50**	**0.16**
PFHxA	0.59	1.43	91	PFBS	0.14	1.02	132
PFHpA	1.19	2.00	55	PFHxS	1.35	2.15	48
PFOA	1.79	2.57	32	PFEtCHxS[b]	0.47	-9.83	2.09E-7
PFNA	2.40	3.14	19	PFOS	2.56	3.29	17
PFDA	3.00	3.70	12	PFDS	**3.77**	**4.43**	**6.13**
PFUdA	3.51	4.18	7.6	FBSA	**1.04**	**1.86**	**62**
PFDoA	4.21	4.84	4.2	FOSA	2.70	3.42	15
PFTrDA	5.30	5.87	1.7	N-Me-FOSA	3.17	3.86	10
PFTeDA	**5.59**	**6.14**	**1.31**	N-Et-FOSA	3.53	4.21	7.4

Values calculated by Kim et al., 2015.
Values in bold were calculated using the equations proposed by Kim et al., 2015
[a]Full compound names listed in Table 1.1
[b]Values reported by Howard and Muir 2010

1.1.2 PFAS Production and Uses

Perfluorinated alkyl substances are not naturally occurring in the environment as they are synthesized and thus anthropogenic compounds (Krafft and Riess, 2015). The two main products that were the first to be commercialized (i.e. PFOS and PFOA) were initially produced by an electrochemical fluorination (ECF) process by the 3M company (Jiang et al., 2015). The production method has since changed to a telomerisation process (Jiang et al., 2015). The method of production of PFASs determines the branched isomer ratios. As ECF produces around 70-80 % of the linear isomer, telomerisation produces only linear isomers (Jiang et al., 2015).

Up until 2002, the 3M company was the main PFOS producer as they produced approximately 3700 tons of PFOS per year at the peak production time (1990-2000) (Alexander et al., 2009). Even though PFOS was voluntarily phased out by the 3M Company in 2002, it is

still produced in China (3M, 2000; Jiang et al., 2015). Currently, most of the environmental release of PFOS comes from industrial use and production (99.3 %) (Li et al., 2015). Additionally, precursor degradation also generates PFASs in the environment (Martin et al., 2010). These precursors (e.g. fluorotelomer alcohols (FTOHs) and perfluorinated alkyl sulfonamides) can undergo hydrolysis and photolysis (Martin et al., 2010; Rayne et al., 2010). Hydrolysis mostly happens in acidic environment and is caused by an intramolecular nucleophilic attack on the S-N bond allowing the change from sulfonamide to sulfonic acid (e.g. FOSA to PFOS) (Rayne et al., 2010). Another abiotic transformation process, photolysis, takes place in aqueous environments when the precursor is irradiated by UV light in the presence of hydrogen peroxide (Martin et al., 2010). Most observed photolysis experiments have reported N-ethyl perfluorooctane sulfonamidoethanol (N-Et-FOSE) degradation and N-ethyl perfluorooctane sulfonamide (N-Et-FOSA) and perfluorooctane sulfonamide (FOSA) formation (Plumlee et al., 2009). Even though the reaction does not go to completion by producing the terminal acids such as PFOS, these sulfonamides can then be hydrolyzed easily to form the sulfonic acid either by abiotic hydrolysis or by biotransformation processes (Xu et al., 2004)

With respect to PFCAs, PFOA is a major one as it was also produced in great quantities. Major companies used to release between 1000 and 100 000 kg of PFOA per year, before the 2010/15 PFOA Stewardship program initiated in 2006, aiming to reduce the emissions by 95 % by 2010 for these companies (USEPA, 2006). Just as with PFOS, the direct release of PFOA is not the only source. It is also released as a side product of fluoropolymer production and processing (Li et al., 2015). Liquid and gaseous PFOA is emitted in the process of drying and heating of fluoropolymers (Li et al., 2015). Using an aqueous dispersion of fluoropolymers (i.e. a mixture of fluoropolymer powder in an aqueous solution used for coating) can also disperse PFOA in the environment (Li et al., 2015). The use and production of PFOA precursors (i.e.

perfluorooctane sulfonyl fluoride (POSF) and FTOHs)) are also major PFOA sources since it is a major side product in their production (Li et al., 2015). Additionally, these precursors can be degraded to PFOA through abiotic hydrolysis and photolysis as well as biotransformation (Li et al., 2015). Up to this day, China is still producing and releasing PFOA in the environment, mainly through the synthesis and release of PFOA precursors (Li et al., 2015).

PFASs are used in a wide variety of ways, some industrial, some domestic. Firstly, they are used as emulsifiers for fluoropolymer production (Li et al., 2015). The surfactant properties of PFASs are the basis of their use in detergents, dispersing agents, emulsifiers and foaming agents (Prevedouros et al., 2006). PFASs are also excellent repellents for water, oil and stains (Prevedouros et al., 2006). In fact, they were the main component of the textile-coating product ScotchGard (Prevedouros et al., 2006). Domestic uses of PFASs include coating carpets, furniture, clothing, shower curtains, bags and food packaging to make them water, oil and stain resistant (Kharitonov et al., 2005). Special textile products also include PFASs to allow the water vapour to be released from the textile (i.e. waterproof sportswear) (Kharitonov et al., 2005). The addition of PFOS and its precursors to the Annex B of the Stockholm convention on POPs in 2009 banned most of these uses, however, some uses are still allowed (OECD, 2014). One of the permitted PFOS uses is in the aviation industry, where it is used as a hydraulic fluid, a lubricant and a sealer because of its high temperature resistance (OECD, 2014). Another tolerated use for PFOS is in flame retardants and firefighting foam (OECD, 2014). Finally, PFASs are also used as protective films for strong acids and highly corrosive products (OECD, 2014).

1.2 PFAS Arctic Transport and Distribution

1.2.1 PFAS Long Range Transport

As stated before, the majority of the PFAS production was in the U.S.A. from 1947 to 2002, followed by an increase in production in the developing countries, notably in China (Li et al., 2015). Several PFASs including PFAAs are found in environmental compartments throughout the Arctic. One of the hypotheses regarding the Arctic long-range transport of PFASs is the atmospheric transport of volatile precursors (e.g. FTOHs, FOSA and perfluorooctane sulfonamidoethanol (FOSE)) followed by the degradation of those precursors to their corresponding terminal acids (Shoeib et al., 2006; Li et al., 2015; Liu and Avendaño, 2013; Wang et al., 2013b). In fact, these PFAS precursors have been detected in both the vapour phase and the particle phase of Arctic air (Shoeib et al., 2006). FTOH has been detected at 5.8 to 26 pg/m^3 in the Arctic air and at 40 to 150 pg/m^3 in North American air (i.e. considered as a major source region) (Shoeib et al., 2006). As for FOSE and its variations (MeFOSE and EtFOSE), they were also detected in the Arctic in concentrations of 2.6 to 31 pg/m^3 for MeFOSE and 8.9 pg/m^3 for EtFOSE, as opposed to concentrations ranging from 3 to 300 pg/m^3 in source regions (Shoeib et al., 2006). It was observed that the dominant contaminant in the air varied depending on the region of the collected sample (MeFOSE for North Atlantic region and FTOH for West Greenland) (Shoeib et al., 2006). As stated before, these PFAS precursors are known to undergo abiotic and biotic transformation to e.g. PFOS and PFOA (Xu et al., 2004; Li et al., 2015; Rayne et al., 2010; Plumlee et al., 2009).

Another known transport pathway for PFASs to the Arctic is the long-range direct oceanic transport. Using PFASs in aqueous conditions leads to the compounds to leak and get washed in bodies of water, leading to the ocean (Li et al., 2015). The low pKa of PFCAs and PFSAs ensures that they stay in ionic form, increasing their solubility (Ng et al., 2014). As PFASs are highly

stable at environmental conditions, they can travel for extended periods of time without being degraded, allowing them to eventually travel to the Arctic waters (Butt et al., 2010).

Direct release of PFASs in the atmosphere is a final, less important, form of transport allowing the compounds to reach the Arctic region. Indeed, small particles of ammonium perfluorooctanoate have been shown to be adsorbed to larger particles in the waste of fluoropolymer manufacturing industries (Prevedouros et al., 2006). Ammonium perfluorooctanoate, once in the air, can sublime to gaseous PFOA, increasing its longer-range transport potential (Prevedouros et al., 2006).

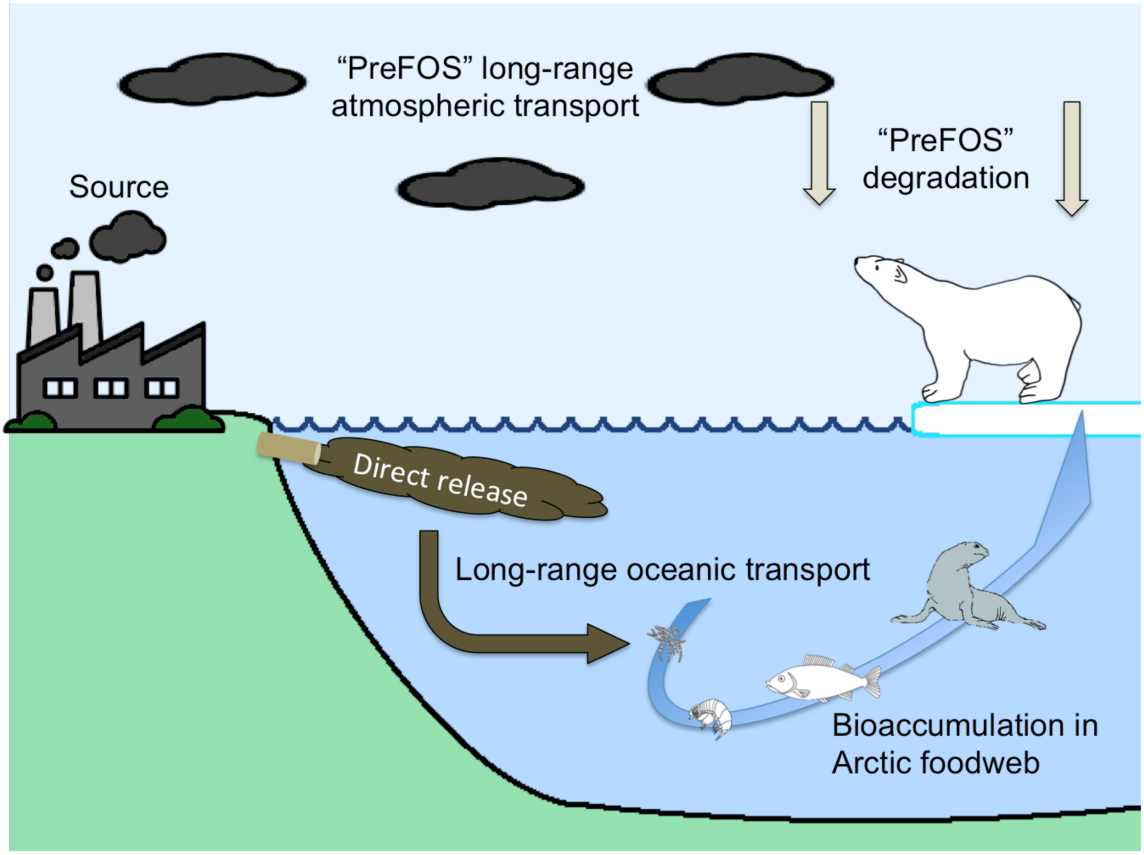


Figure 1.1 Graphical representation of PFAS transport to the Arctic and marine food web accumulation with respect to the polar bear.

1.2.2 PFASs and Environmental Sinks

PFSAs and PFCAs being acids and thus water soluble under environmental conditions are found in bodies of water in every part of the globe including in the Arctic (Prevedouros et al., 2006). PFOA for example has been reported mostly in samples from ocean waters, with high disparities for coastal waters (0.2 ng/L to 450 ng/L in South-East Asia) (Prevedouros et al., 2006). The variation can be associated with the proximity of some of these bodies of water to source regions (Prevedouros et al., 2006). Using those concentrations, the total content of PFOA in the oceans was estimated to be between 110 and 10 000 tons (Prevedouros et al., 2006). In freshwater bodies, the concentration was estimated between 0.1 and 10 ng/L depending on the proximity to source regions (Prevedouros et al., 2006). The total content was then estimated to be between 4 and 800 tons, representing a lesser portion of the world content (Prevedouros et al., 2006). As for sediments, observed concentrations ranged from 24 pg/g to 18 000 pg/g (Prevedouros et al., 2006). The highest concentrations were excluded from the total content estimation because they were collected in source regions and the total amount of PFOA in sediments was estimated to be between 3 and 340 tons (Prevedouros et al., 2006). The air compartment was determined to be negligible compared to the amounts in the oceans and in the sediments even if PFOA and PFOS have been detected in precipitations, confirming their presence in the atmosphere (Kallenborn et al., 2004; Scott et al., 2003; Pevedouros et al., 2006). Finally, the biota is also considered to be a negligible compartment for PFOA even if PFASs are known to bioaccumulate in most biota (Prevedouros et al., 2006). By using PFOA as an example, PFASs environmental sinks are most likely the oceans and less importantly, fresh water and sediments.

1.2.3 PFASs in the Arctic Biota and Polar Bears

As mentioned, PFASs travel to the Arctic via long-range transport and subsequently accumulate in biota (Prevedouros et al., 2006). Even if biota is considered as a negligible environmental sink for PFASs, it is still the most concerning compartment due to the toxic effects of PFASs (see 1.3.1). Arctic biota has been monitored over the years due to the vulnerability of Arctic biota and exposure to PFASs (Reviewed in Letcher et al., 2010).

Polar bears (*Ursus maritimus*), as a top predator in the Arctic marine food web, is by far the biota and wildlife species accumulating and exposed to the greatest concentrations of PFASs in the Arctic (Table 1.3). As a comparison, ringed seals (*Pusa hispida*) (i.e. the main prey species of polar bears) have approximately 20 to 100 times less PFASs (in the liver) than in the liver of polar bears (Table 1.3). Generally, mammals accumulate more PFASs than pinnipeds, who accumulate more than whales (Table 1.3). Therefore, polar bears were the main focus of this study along with their prey. Since PFCAs and PFSAs are able that bind to serum albumin and interact with the liver fatty acid binding protein, the main focus of most Arctic biota studies on PFASs focus on liver (Bischel et al., 2010 ; Luebker et al., 2002).

Table 1.3 Summary of mammalian studies in the Arctic for mean PFAS concentrations in the liver. Concentrations are in ng/g ww. ± 1 SE, SD in brackets where available.

Species	Reference	Location	Mean ΣPFSA	Mean ΣPFCA
Polar Bear	Smithwick et al. 2005 Dietz et al., 2008	East Greenland	3000	500
	Gebbink et al., 2016	East Greenland	1825 ± 219	506 ± 55
	Greaves et al., 2012	East Greenland	3310 ± 300	1160 ± 90
	Rigét et al., 2013	East Greenland	1067 (278)	299 (141)
	Smithwick et al., 2005	Canadian Arctic	1200-1430	304-630
	Smithwick et al., 2005	Southern Hudson Bay	2800	515
	Smithwick et al., 2005	Alaska	850	285
Ringed Seal	Bossi et al., 2005a	East Greenland	95	13
	Gebbink et al., 2016	East Greenland	94 ± 6	43 ± 2
	Rigét et al., 2013	East Greenland	112 (37)	36 (14)
	Bossi et al., 2005a	West Greenland	28	7
	Rigét et al., 2013	West Greenland	16 (10)	13 (5.7)
	Martin et al., 2004 Butt et al., 2007, 2008	Canadian Arctic	10-88	9-83
	Powley et al., 2008	Beaufort Sea	18-34	10-16
	Quakenbush and Citta 2008	Alaska	8.2 (1.6)	14 (7.6)
Walrus	Tomy et al., 2004	South East Baffin Island	2.4 ± 0.4	-
Narwhal	Tomy et al., 2004	South East Baffin Island	11 ± 2.3	-
Beluga Whale	Tomy et al., 2004	South East Baffin Island	13 ± 1.1	-
Arctic Fox	Martin et al., 2004	Western Hudson Bay	6.1-1400	4.9-227
Mink	Martin et al., 2004	Yukon Territory	1.3-20	2.7-58

1.3 PFAS Toxicity and Metabolism

1.3.1 PFAS Toxicity in Biota

First, PFASs are reported to cause immunotoxicity in multiple species including in mammals (DeWitt et al., 2012). PFASs were revealed as immunosuppressing agents, by affecting the adaptive immune response (Andersen et al., 2008). Additionally, PFOS seems to be able to suppress the production of the pro-inflammatory interleukin-6 (DeWitt et al., 2012). These two immunosuppressive paths lead to an overall lesser protection from infection, meaning a lower chance of survival (DeWitt et al., 2012).

PFASs were shown to elicit toxicity in rat offspring from an exposed mother (Lau et al., 2003). Exposing pregnant female rats showed mortality in the offspring while the survivors showed a significantly slower development (Lau et al., 2003). As for PFOA, the same developmental effects were seen in the offspring (Lau et al., 2006). It is thought that the offspring toxicity is caused by oxidative damage in the brain and liver, as PFOS is able to suppress the superoxide dismutase (an antioxidant enzyme) in those specific organs (Liu et al., 2009).

Both PFSA and PFCA have been shown to be able to cross the blood-brain barrier and accumulate in certain parts of the brain in the polar bear (Pedersen et al., 2015 ; Greaves et al., 2012 and 2013). Both of these chemical classes were shown to correlate with neurochemical enzymes or neuroreceptors, which are known to affect the dopamine synthesis (Pedersen et al., 2015 ; Rutledge et al., 1967). Therefore, it is thought that PFASs affect the behaviour of affected animals, as the dopaminergic system is an important regulator of the motivational system, the learning abilities and the general behaviour (Girault et al., 2004).

1.3.2 Biotransformation Pathways and Metabolism of PFASs

There is a dearth of information regarding PFAS biotransformation, however, the known precursors, which are affected by the abiotic degradation described above, are known to be metabolized in the liver, increasing the exposure to bioaccumulable PFASs (Ng et al., 2014; Xu et al., 2004). In rat, N-Et-FOSE was shown to be degraded into FOSE through dealkylation, which was then subsequently transformed into FOSA by deethylation (Xu et al., 2004). Further FOSA degradation was only observed in complete liver slices (Xu et al., 2004). In the rat, these detoxification pathways are all mediated by cytochrome P450 (CYP) isomers, most notably by CYP 2C11 (Xu et al., 2004) (See figure 1.2 for the degradation pathways).

The N-Et-FOSA deethylation, thought to lead to PFOS formation, takes place in varying metabolic rates depending on the species (Letcher et al., 2014). Over a 90-minute reaction time, rat and polar bear microsomes are able to degrade 95 % of initial 300nM of N-Et-FOSA, while ringed seal microsomes only degrade 65 % of the initial N-Et-FOSA and beluga whale microsome show negligible degradation (Letcher et al., 2014). The ability of complete liver slices to transform FOSA in PFOS suggests that the reaction requires more machinery than what is in the extracted microsomes (Xu et al., 2004; Letcher et al., 2014).

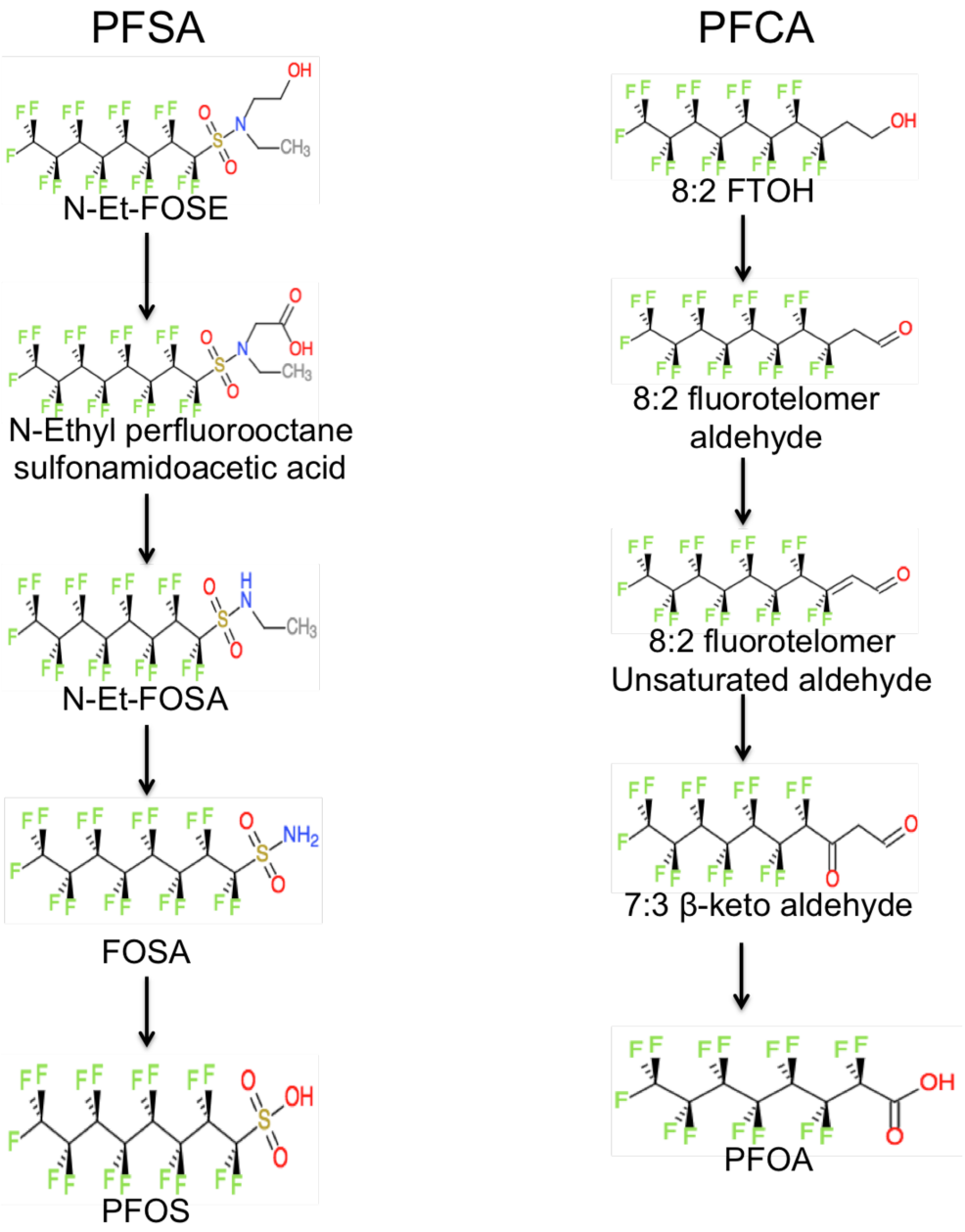


Figure 1.2 Metabolic pathways of PFASs observed in laboratory-based rat studies. The PFSA pathway was observed in extracted rat liver microsomes, with the exception of FOSA -> PFOS, which was observed in complete rat liver slices (Xu et al., 2004, Benskin et al., 2009). The PFCA pathway was observed *in vivo* (Butt et al., 2014)

1.3.3 Bioaccumulation and Biomagnification of PFASs

PFSA and PFCA are highly persistent and have been shown to accumulate notably in marine mammals including those from the Arctic (Kannan et al., 2001). As PFSAs and PFCAs are highly recalcitrant and basically not metabolized, they are chemically "terminal" PFASs and they accumulate mostly in the liver, whereas neutral PFASs are more akin to lipid rich tissues (Ng et al., 2014; Greaves et al., 2011, 2012 and 2013). PFCAs and PFSAs are protein-associated as that bind to e.g. serum albumin (Bischel et al., 2010). These compounds are also known to be able to interact with the liver fatty acid binding protein (Luebker et al., 2002). This explains in part the accumulation of PFASs seen in the liver and in the kidneys, where the protein is also present (Ng et al., 2014). The fact that PFCAs and PFSAs are found mainly in in the conjugate base anion form at physiological pH, they interact with phospholipids, and thus hindering excretion and increasing their persistency in the body (Ng et al., 2014).

Additionally, PFCAs and PFSAs have been shown to biomagnify in the Arctic marine food web (Tomy et al., 2009). In fact, biomagnification factors (BMF) (the ratio between contaminant burden between a predator and its prey) higher than 1 have been reported for PFOS, PFOA and other PFASs, following a positive correlation between the BMF and the carbon-chain length (Tomy et al., 2009). A clear increase in concentration across the trophic levels (i.e. one order of magnitude per trophic level for PFOS in an Arctic marine food web) is an indication of the ability to biomagnify (Tomy et al., 2004b).

1.4 PFAS in the Arctic Top Predator: Polar Bears

1.4.1 PFASs in East Greenland Polar Bear and Spatio-Temporal Trends

A substantial amount of data has been gathered over the years for East Greenland polar bears and the most recent temporal trend analysis revealed a decreasing trend for PFOS, PFHxS,

FOSA, PFDA and PFUdA in the last years (2006-present) (Rigét et al., 2013). Prior to 2006 (1984 to 2006), PFASs in polar bears showed a constant increasing temporal trend with variable yearly increases (4.7 % per year for PFOS, 2.5 % for PFOA, 6.1 % for PFNA, 4.3 % for PFDA, 5.9 % for PFNA, and 8.5 % for PFTrDA) (Butt et al., 2010). There seems to be a steeper increase for PFOS between 2000 and 2006, which could be attributed to a time lag of the transport of PFOS to the Arctic (Butt et al., 2010). Past 2006, a decreasing trend is noticeable in most PFASs (Rigét et al., 2013). The fact that decreasing trends are only now noticeable after the 2002 phase-out could be tied to the same reason explaining the steeper increase between 2000 and 2006, a serious lag in the PFAS long-range transport (Rigét et al., 2013; Butt et al., 2010). Even if decreasing trends in PFASs concentration is encouraging, the emergence of replacing PFAS compounds, including PFBS, PFEtCHxS and PFHpA, might affect said trend (Gebbink et al., 2016).

1.4.2 Hudson Bay Polar Bears and PFAS Trends

Temporal data for PFASs on polar bears from Hudson Bay is scarce compared to that of East Greenland bears. In the scientific literature PFAS (i.e. PFOS and PFCAs) temporal trend analysis is known for polar bears from the eastern Arctic (i.e. samples collected near Baffin Island, Canada) (Butt et al., 2010). In those bears, between 1972 and 2002, significant increases were noticeable for PFOS, PFOA, PFNA, PFDA and PFUdA (Smithwick et al., 2006). On the contrary, a decreasing trend for FOSA could be observed in those bears over the 30-year period (Smithwick et al., 2006). The doubling time for PFASs during that period was estimated at 5.8 years. As the most recent evaluation on PFASs concentration in Hudson Bay polar bears dates back to 2005 an update is much needed (Smithwick et al., 2005).

1.4.3 East Greenland Ringed Seals and PFAS Trends

Temporal trends for PFASs in East Greenland ringed seals were documented between 1986 and 2006 (Bossi et al., 2005a) and then up to 2010 (Rigét et al., 2013). From 1986 to 2006, an increasing trend was noticed for most PFASs although not significant (annual increases were 12.1 % for PFOS, 6.4 % for PFDA and 7.8 % for PFUdA) (Bossi et al., 2005a; Butt et al., 2010). A decreasing trend was then noticed between 2006 and 2010 for ringed seals for PFOS, PFHxS, FOSA and PFOA (Rigét et al., 2013). PFNA, PFDA and PFUdA are not part of the decreasing trend either because their concentrations increased (PFDA and PFUdA) or plateaued (PFNA) after 2006 (Rigét et al., 2013).

1.5 Predator-Prey Relationship: Polar Bears and Ringed Seals

In an effort to better understand the exposure patterns of polar bears, an increasing interest has risen in examining predator and prey relationships of POPs and especially emerging POPs in the Arctic (Letcher et al., 2009). As East Greenland polar bears mainly consume ringed seal blubber (47.5 ± 2.1 % of their total food consumption) and since it is mostly expected that polar bears are exposed to PFASs through the food chain, analyzing ringed seals and polar bears that are relatable is necessary (McKinney et al., 2013). Much data is available for both ringed seals and polar bears regarding PFASs, but little has been done in order to truly understand the behaviour of PFASs in this predator / prey relationship due to the little availability of relatable samples (i.e. different collection time periods or different collection regions) (Letcher et al., 2010; Bossi et al., 2005a; Butt et al., 2010; Rigét et al., 2013; Gebbink et al., 2016).

1.6 PFASs Analysis

1.6.1 Ultra-High-Performance Liquid Chromatography-Mass Spectrometry

Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS/MS) is an updated version of the High-Performance Liquid Chromatography (HPLC). A UHPLC-MS/MS based approach was used for the separation and mass spectral determination of the present PFASs (Chu et al., 2015). The UHPLC guard and analytical columns used were comprised of chemically bonded polysiloxane beads coated with 18-carbon alkyl chains. Therefore, the UHPLC columns used were non-polar for the necessary reverse-phase chromatography of the PFAS analytes. Via a mobile phase gradient over time, the proportion of the polar organic solvent, methanol was increased relative to water (polar solvent). Thus, the organic aspect of the methanol mobile phase became increasingly competitive relative to the non-polar stationary phase the non-polar fluorinated carbon chain of the PFAS molecules. Greater proportions of the methanol in the mobile phase are required with the increasing chain length of the PFASs. It has been well documented that such UHPLC parameters, and with the use of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) are ideal for the separation and ionization of PFASs (Letcher et al., 2014; Chu et al., 2015).

1.6.1.1 Negative Electrospray Ionization

ESI in the negative ionization mode was presently used for the perfluorinated acids, PFCAs and PFSAs (including PFOS). Being acids, PFCAs and PFSAs are readily to deprotonates to generate negative ion conjugate bases. Subsequently, the PFCA and PFSA negative ions are mass-to-charge (m/z) separated by the MS/MS mass analyzers. For the various PreFOS

sulfonamides (e.g. FOSA), which are weak bases, ESI(+) was used. That is, the amines of the PreFOS sulfonamides are readily protonated to generate positively charges conjugate acids.

1.6.1.2 Triple-Quadrupole Mass Spectrometry

Once ESI(-) or ESI(+) analyte ions are generated, they are potential accelerated into the first quadrupole MS, which separates the focused ions based on their mass to charge (m/z) ratio. In triple-quadrupole mass spectrometry, the first and last quadrupoles both serve as mass selective analyzers, while the second quadrupole serves as a collision chamber for production of secondary ions exiting from the first quadrupole MS. From the first MS, the primary ions collide with neutral atoms (argon gas) breaking the molecule into daughter ions. The third quadrupole MS then separates the daughter ions generated based also on selective potential modulation in relation to the m/z ratio of the daughter ions. Thus, for PFAS quantification, abundant ions comprising the primary-daughter ion, multiple reaction monitoring (MRM) channel (unique for each PFAS) is determined for the most sensitive and specific quantification of a given PFAS.

1.7 Thesis Objectives

The objectives and hypotheses for this project go as follows :

1) Measure the PFAS levels in the liver and adipose tissues of polar bears and ringed seals from East Greenland using UHPLC-MS/MS and compare the levels between individuals, sexes and species.
    a. Based on the current knowledge (Letcher, 2010) (Butt, 2010), it is hypothesized that the levels of PFAS in polar bears and ringed seals are not sex or age dependent.

b. Based on the known levels of PFAS (Letcher, 2010), it is thought that the ringed seals have lower tissue levels of PFAS than polar bears.

c. According to the biomagnification effect, the known levels of PFAS (Letcher, 2010) and polar bears' eating habits (McKinney et al., 2013), it is hypothesized that most PFASs will have biomagnification factors higher than 1.

2) Geographic Subpopulation Comparisons of PFAS levels and patterns in polar bears from East Greenland and Western and Southern Hudson Bay.

   a. Based on current knowledge on Hudson Bay polar bears (Smithwick et al., 2005), current temporal trends (Rigét et al., 2013) and East Greenland polar bears (Letcher et al., 2010; Gebbink et al., 2016), Hudson Bay polar bears will be less PFAS contaminated than East Greenland polar bears.

3) Determine the biomagnification factors and further investigate what they mean for exposure

4) Measure the biotransformation capacity of (East Greenland) polar bears and ringed seals to transform FOSA precursors to the highly bioaccumulative PFOS, using a hepatic microsomal in vitro assay.

   a. Based on previously observed data (Letcher et al., 2014) and the known pathway of biotransformation (Xu et al., 2004), it is hypothesized that N-Et FOSA is converted rapidly and completely to FOSA in polar bears while it is not completely transformed to FOSA in ringed seals.

   b. Based on previous tests (Xu et al., 2004) (Letcher et al., 2014), no PFOS formation is observed as a product of the enzymatic assay with extracted microsomes. PFOS formation can only be observed by an enzymatic assay with complete liver tissue.

c. Based on previously observed data (Letcher et al., 2014), it is thought that the polar bears have a higher metabolite formation rate from precursors than ringed seals

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Chapter 2

Materials and Methods

2.1 Sampling

All of the East Greenland ringed seal and polar bear harvesting and sampling (Ittoqqortoormiit/Scoresby Sound area) was done in cooperation with local Inuit subsistence hunters and in accordance with the regulated quota of 35 polar bears per year. Fresh liver and fat/blubber samples were collected from polar bears (Feb. – March 2011) and ringed seals (Feb. – March 2012) harvested from East Greenland, Scoresby Sound (70°-71°N, 20°-21°W). Liver and blubber tissues were obtained from 16 ringed seals (9 males and 7 females) and comprised of 5 fetuses, 4 subadults and 10 adults. Liver and fat (subcutaneous rump) tissues were from 10 polar bears (5 males and 5 females) and comprised 2 adults and 8 subadults. See Table 2.1 for all individual seal and bear sample and sampling information. Frozen samples were shipped to and stored at -20°C at Department of Bioscience Specimen Bank at Aarhus University, Roskilde, Denmark and subsamples later shipped to Environment and Climate Change Canada's, National Wildlife Specimen Bank at Carleton University in Ottawa, ON, Canada.

As reported in Rigét et al. (2013), the individual age estimation of the East Greenland ringed seals and bears was done by counting the cementum Growth Layer Groups (GLG) of the canine or lower right incisor (I3), respectively, after decalcification, thin sectioning (14 lm) and staining with Toluidine Blue.

Table 2.1 Sample and sampling information for polar bears harvested from East Greenland (n=10), Southern Hudson Bay (n=10) and Western Hudson Bay (n=9) and ringed seals harvested from East Greenland (n=16).

USOX	Species	ID	Age	Sex	Date of Collection	Latitude	Longitude	Location
East Greenland Subpopulation								
K12-30894	Polar Bear	43101	5-6y	Male	2011/02/25	7071	02021	South of Kap Tobin
K12-30895	Polar Bear	43102	2-3y	Male	2011/02/25	7071	02021	South of Kap Tobin
K12-30896	Polar Bear	43103	2y	Female	2011/02/25	7071	02021	South of Kap Tobin
K12-30897	Polar Bear	43104	2-3y	Male	2011/03/02	7071	02021	1 km south of Scoresbysound
K12-30898	Polar Bear	43105	2-3y	Female	2011/03/10	7071	02021	South of Kap Tobin
K12-30899	Polar Bear	43106	2-3y	Female	2011/03/10	7071	02021	South of Kap Tobin
K12-30900	Polar Bear	43107	2y	Female	2011/03/11	7071	02021	South of Kap Tobin
K12-30901	Polar Bear	43108	2y	Female	2011/03/11	7071	02021	Kap Hope
K12-30902	Polar Bear	43109	10y	Male	2011/03/11	7071	02021	Hurry Fjord
	Polar Bear	43110	Unknown sample					
K13-34174	Ringed Seal	43169	Adult	Female	2012-02-22	7071	02021	Scoresby Sound
K13-34175	Ringed Seal	43168	Adult	Male	2012-02-24	7071	02021	Scoresby Sound
K13-34176	Ringed Seal	43167	Adult	Male	2012-02-24	7071	02021	Scoresby Sound
K13-34177	Ringed Seal	43166	Subadult	Male	2012-02-24	7071	02021	Scoresby Sound
K13-34178	Ringed Seal	43181	Adult	Female	2012-02-24	7071	02021	Scoresby Sound
K13-34179	Ringed Seal	43182	Foetus	Male	2012-02-24	7071	02021	Scoresby Sound
K13-34180	Ringed Seal	43183	Adult	Male	2012-02-26	7071	02021	Scoresby Sound
K13-34181	Ringed Seal	43184	Subadult	Female	2012-02-27	7071	02021	Scoresby Sound
K13-34182	Ringed Seal	43185	Adult	Female	2012-02-27	7071	02021	Scoresby Sound
K13-34184	Ringed Seal	43187	Adult	Male	2012-02-27	7071	02021	Scoresby Sound
K13-34185	Ringed Seal	43188	Subadult	Male	2012-02-27	7071	02021	Scoresby Sound
K13-34186	Ringed Seal	43189	Adult	Male	2012-02-29	7071	02021	Scoresby Sound
K13-34187	Ringed Seal	43190	Subadult	Male	2012-02-29	7071	02021	Scoresby Sound
K13-34188	Ringed Seal	43192	Adult	Female	2012-03-01	7071	02021	Scoresby Sound
K13-34189	Ringed Seal	43193	Foetus	Female	2012-03-01	7071	02021	Scoresby Sound
K13-34190	Ringed Seal	43194	Adult	Female	2012-03-05	7071	02021	Scoresby Sound

Table 2.1 (continued)

USOX	Species	ID	Age	Sex	Date of Collection	Latitude	Longitude	Location
Southern Hudson Bay Subpopulation								
K12-31612	Polar Bear	12276	Sub-adult	Male	2012/01/23			West of Kugong Island, Sanikiluaq, Nunavut
K12-31613	Polar Bear	12277	Sub-adult	Male	2012/01/23			West of Kugong Island, Sanikiluaq, Nunavut
K12-31614	Polar Bear	12279	Sub-adult	Male	2012/01/23			East of Johnson Island, Sanikiluaq, Nunavut
K12-31615	Polar Bear	12280	Sub-adult	Male	2012/01/23			East of Johnson Island, Sanikiluaq, Nunavut
K12-31616	Polar Bear	12281	2 yr	Female	2012/01/24			West of Kugong Island, Sanikiluaq, Nunavut
K12-31617	Polar Bear	12282	Sub-adult	Male	2012/01/26			South-West of Kata'u Island, Sanikiluaq, Nunavut
K12-31618	Polar Bear	12283	Adult	Male	2012/01/26			West of Split Island, Sanikiluaq, Nunavut
K12-31619	Polar Bear	12284	Sub-adult	Male	2012/01/26			West of Split Island, Sanikiluaq, Nunavut
K12-31620	Polar Bear	12285	Sub-adult	Male	2012/01/26			West of Split Island, Sanikiluaq, Nunavut
K12-31621	Polar Bear	12286	Adult	Male	2012/01/30			East of Joy Island, Sanikiluaq, Nunavut
Western Hudson Bay Subpopulation								
K12-32688	Polar Bear	12475	Adult	Male	2011/11/02			Arviat, Nunavut
K12-32675	Polar Bear	12476	Adult	Male	2011/11/01			Arviat, Nunavut
K12-32689	Polar Bear	12478	Sub-adult	Female	2011/11/02			Arviat, Nunavut
K12-32691	Polar Bear	12479	Sub-adult	Female	2011/11/02			Arviat, Nunavut
K12-32694	Polar Bear	12238	Adult	Female	2011/11/15			Arviat, Nunavut
K12-32693	Polar Bear	12239	Adult	Male	2011/11/27			Arviat, Nunavut
K12-32677	Polar Bear	12368	Yearling	Female	2011/11/23			Rankin Inlet, Nunavut
K12-32676	Polar Bear	12369	Sub-adult	Male	2011/11/22			Rankin Inlet, Nunavut
K12-32692	Polar Bear	12370	Adult	Male	2011/12/02			Rankin Inlet, Nunavut

Figure 2.1 A map of North-Eastern Canada and Greenland representing the sampling locations for the polar bears and ringed seals.

2.2 Chemical Standards

The linear PFSA (perfluoro- 1-butanesulfonic acid (PFBS), perfluoro-1-hexanesulfonic acid (PFHxS), perfluoro-1-octanesulfonic acid (PFOS), perfluoro-1-decanesulfonic acid (PFDS)) and cyclic PFSA (PFEtCHxS) standards, PFCA standards (perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUdA), perfluoro-n-dodecanoic acid (PFDoA), perfluoro-n-tridecanoic acid (PFTrDA), perfluoro-n-tetradecanoic acid (PFTeDA), perfluoro-n-hexadecanoic acid (PFHxDA) and perfluoro-n-octadecanoic acid (PFODA)), perfluorinated sulfonamide standards (perfluoro-1-octane sulfonamide (FOSA), N-methyl-

perfluoro-1-octane sulfonamide (N-Me-FOSA) and N-ethyl-perfluoro-1-octane sulfonamide (N-E-tFOSA)), as well as all internal standards (two labelled PFSAs (C_6 and C_8), six labelled PFCAs (C_6, C_8, C_{10}, C_{11} and C_{12}) and three labelled FASAs ($^{13}C_8$-FOSA, d_3-N-MeFOSA and d_5-N-EtFOSA)), were purchased from Wellington Laboratories (Guelph, ON, Canada). The full names of all of the compounds are listed in Table 1.1.

All solvents were HPLC grade and purchased from Fisher Scientific (Ottawa, ON, Canada). Phosphate buffers (0.1M, pH 7.4 and 0.05M, pH 8.0) were prepared on site using potassium phosphate monobasic and sodium phosphate dibasic both purchased from Fisher Scientific (Ottawa, ON, Canada). Resorufin sodium salt, 7-ethoxyresorufin, Bovine serum albumin (BSA) and Fluorescamine were purchased from Sigma-Aldrich (Oakville, ON, Canada). Wistar-Han male rat liver microsomes were purchased from Corning Gentest (Corning, NY, USA). The NADPH regenerative system and the 0.5M potassium phosphate pH 7.4 buffer were purchased from BD Gentest (Woburn, MA, USA). Deionized water was prepared on site.

Table 2.2 Suite of 22 PFASs with their acronyms, MLOQ (ng/g ww), MLOD (ng/g ww) and their specific UHPLC-MS/MS operating parameters and the suite of 12 internal standards with the positive control recovery (%).

	Target compounds	Positive Control Recovery (%)	MLOQ (ng/g ww)	MLOD (ng/g ww)	Parent ion (Da)	Daughter ion (Da)	Cone Voltage (V)	Collision Energy (eV)
1	PFBA	100%	0.59	0.04	213	169	6	9
2	PFPeA	112%	0.07	0.04	263	219	9	9
3	PFHxA	98%	0.07	0.04	313	269	9	10
4	PFHpA	108%	0.04	0.02	363	319	9	10
5	PFOA	99%	0.02	0.01	413	369	9	10
6	PFNA	103%	0.05	0.01	463	419	10	11
7	PFDA	102%	0.05	0.02	513	469	10	12
8	PFUdA	102%	0.04	0.03	563	519	10	12
9	PFDoA	108%	0.31	0.01	613	569	10	13
10	PFTrDA	81%	0.05	0.01	663	619	10	14
11	PFTeDA	96%	0.07	0.01	713.0	669.0	11	14
12	PFHxDA	129%	0.08	0.04	812.9	769.0	12	15
13	PFODA	138%	0.04	0.01	912.9	869.0	18	17
14	PFBS	102%	0.06	0.01	298.9	80.0	50	27
15	PFHxS	102%	0.08	0.03	398.9	80	50	35
16	PFEtCHxS	58%	0.06	0.04	460.9	99.0	61	24
17	PFOS	103%	0.08	0.02	498.9	80	59	40
18	PFDS	71%	0.17	0.04	598.9	80	75	50
19	FBSA	73%	0.13	0.10	298	78	50	20
20	FOSA	97%	0.07	0.04	498	78	55	29
21	N-MeFOSA	100%	0.13	0.09	512	169	55	27
22	N-EtFOSA	96%	0.11	0.09	526	169	55	27

Table 2.2 (continued)

	Internal Standard compounds	Acronyms	% Recovery (%)	Parent ion (Da)	Daughter ion (Da)	Cone Voltage (V)	Collision Energy (eV)
1	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	MPFBA	99%	217	172.0	6	9
2	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	MPFHxA	98%	315	270	9	10
3	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	MPFOA	99%	417	372	9	10
4	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	MPFNA	103%	468	423.0	10	11
5	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	MPFDA	102%	515	470	10	12
6	Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	MPFUdA	102%	565	520	10	12
7	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA	108%	615	570.0	10	13
8	Perfluoro-perfluoro-1-hexane[$^{18}O_2$]sulfonate	MPFHxS	102%	403.0	84	50	35
9	Perfluoro-perfluoro-1-[1,2,3,4-	MPFOS	103%	502.9	80	59	40
10	Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	97%	505.97	78	55	29
11	N-methyl-d_3-perfluoro-1-octanesulfonamide	d-MeFOSA	100%	515	169	55	27
12	N-ethyl-d_5-perfluoro-1-octanesulfonamide	d-EtFOSA	96%	531	169	55	27

2.3 PFAS Determination in Seal and Bear Samples

The extraction and determination of the target PFASs (Table 2.2) was done according to our previously published methods and described in detail elsewhere (Chu et al., 2015, 2016; Letcher et al., 2015). Liver (0.3-0.5g) and fat (1.0-1.6g) samples were weighed precisely in VWR 15 mL plastic tubes and then spiked with 10 ng (100 ng for MPFOS) of PFAS internal standards (MPFBA, MPFHxA, MPFOA, MPFNA, MPFDA, MPFUdA, MPFDoA, MPFHxS, MPFOS, M8FOSA, d-MeFOSA and d-EtFOSA). A volume of 3 mL of a solution of formic acid in acetonitrile (0.2 % v/v) was added to the samples, which were then homogenized for 1 min with a IKA T10 Basic UltraTurrax. The samples were centrifuged for 5 min at 5000 rpm (2906g) and the supernatant was transferred into clean and weighed glass tubes. The extraction was repeated two additional times. SPE WAX cartridges (60 mg x 3 cc) were attached to a SPE vacuum manifold and were conditioned with 3 mL of methanol and then 3 mL of water. The samples were filtered through the SPE cartridges and then washed with 1 mL of formic acid in water (2 % v/v) and 2 x 1 mL of water. The first fraction containing the FASAs was eluted with 1 mL of methanol. The cartridges were washed with an additional 1 mL of methanol before eluting the second fraction containing the PFSAs and PFCAs with 2 x 1 mL of 1 % ammonium hydroxide in methanol. 20 mg of active carbon was added to 500 µL of the first fraction. The mixture was then filtered through a VWR centrifugal filter, conditioned with 0.5 mL of methanol, at centrifuged at 6000 rpm (2939g) for 5 min. The second SPE fraction was evaporated just to dryness and then reconstituted in methanol, and filtered the same way as for the first fraction.

The PFASs in the extracted fractions were determined by ultra-high-performance liquid chromatography-tandem quadrupole mass spectrometry (UHPLC-MS/MS). A 5 μL volume of the sample was injected in the UHPLC system (Waters Acquity I-Class ultra-performance liquid chromatograph) by an auto-injector. The separation was done on a Cortecs UHPLC C18 column (1.6 μm particle size, 2.1 x 50 mm column) at 50°C. The mobile phases for the chromatography were water and methanol both containing 2 mM of ammonium acetate and filtered through a 0.45 μm membrane. The flow rate was set at 0.6 mL/min. The gradient of mobile phases started at 98 % water and 2 % methanol and went to 5 % water, 95 % methanol at 3 min through 5 min and returned to 98 % water and 2 % methanol at 5.1 min.

The MS/MS used was a Waters Xevo TQ-S mass spectrometer system and was operated in negative electrospray (ESI-) mode using a multiple reaction monitoring scanning mode (MRM). High purity argon and nitrogen were used as collision and nebulizing gas, respectively. The capillary voltage was set at 0.5 kV. The desolvation temperature was set at 400°C. The desolvation gas, cone gas and collision gas flow were at 800 L/h, 15 L/h and 0.2 mL/min respectively. The compound specific settings are listed in Table 2.2.

The concentrations were determined using the internal standard method. The calibration curves of PFASs contained 5 different concentrations (between 0 ng/mL and 100 ng /mL). The correlation coefficient (r^2) was higher than 0.99 for all compounds.

2.4 Microsome Extraction and Enzymatic Activity Determination

To maintain microsome viability, all manipulations were done on ice, between 0-4°C. All buffers, glassware and apparatus were chilled at 4°C before use. Method is described in McKinney et al., 2011. Liver tissues were thawed on ice and were later precisely weighed (approximately 200 mg per sample). The tissue was transferred in a Petri dish and washed with the phosphate buffer (0.1M, pH 7.4). The buffer was discarded and the tissue was minced, and then transferred in a Wheaton homogenizing tube to which was added 800 µL of the phosphate buffer. The liver tissue was homogenized with 10 ups and downs of a IKA T10 Basic UltraTurrax. The homogenate was then transferred in a 1.8 mL Eppendorf microcentrifuge tube and centrifuged at 9000 g for 15 min at 4°C. The supernatant was then decanted and transferred in a Beckman 3.2mL polyallomor thick wall ultracentrifuge tube and centrifuged at 100 000g for 60 min at 4°C. The supernatant was decanted and the pellet was resuspended in 100µL of phosphate buffer. The microsome solution was pooled in cryovials and separated per individuals.

The enzymatic activity of the microsomes was estimated by the 7-ethoxyresorufin O-deethylase (EROD) activity using the method reported by Kennedy and Jones (1994). Briefly, phosphate buffer (0.05M, pH 8.0) was added in a 48 wells Falcon plate. To the buffer was added BSA (2mg/mL in a 0.05M pH 8.0 phosphate buffer) and Resorufin (3µM in a 0.05M pH 8.0 phosphate buffer)(to the standards only), 7-ethoxyresorufin (9.4µM in a 0.05M pH 8.0 phosphate buffer) and microsomes. The reaction was initiated by adding 50µL of NADPH regenerative system. After 7 minutes at 37°C, the reaction was interrupted by adding 100µL of Fluorescamine (2.16 mM in acetonitrile). See Table

2.3 for the plate preparation details and volumes used. Fluorescence was then read at 465 nm (protein content) and 595 nm (Resorufin concentration) with a multiwall plate reader (Millipore). The enzymatic activity was determined as pmole of resorufin formed per minute per mg of total protein. Based on their EROD activity, polar bear and ringed seal individuals were combined in two groups (high activity and low activity). The microsome pools were then used for the *in vitro* biotransformation assay.

Table 2.3 Preparation of the 48 well-plate containing the 5-point standard (Std), samples and controls.

Order of addition ->	1		2		3		4	5		Start Rx	End Rx
	PBS Buffer (µL)	[BSA] (µg/mL)	Vol. BSA (µL)	[R] (µM)	Vol. R (µL)	[ER] (µM)	Vol. ER (µL)	Vol. Micro. (µL)	[NADPH] (mM)	Vol. NADPH (µL)	Vol. Fluorescamine (µL)
Std 0	135	0	0	0	0	2	50	-	0.39	50	100
Std 1	120	59	10	0.045	5	2	50	-	0.39	50	100
Std 2	105	119	20	0.090	10	2	50	-	0.39	50	100
Std 3	75	239	40	0.179	20	2	50	-	0.39	50	100
Std 4	50	358	60	0.224	25	2	50	-	0.39	50	100
Std 5	15	477	80	0.358	40	2	50	-	0.39	50	100
Control Blank	185	-	-	-	-	2	50	0	0	0	100
Negative Control	135	-	-	-	-	2	50	0	0.39	50	100
Sample Blank	175	-	-	-	-	2	50	10	0	0	100
Sample	125	-	-	-	-	2	50	10	0.39	50	100

- R : Resorufin
- ER : 7-ethoxyresorufin
- Micro. : Microsome suspension

2.5 *In vitro* Metabolism Assay

The *in vitro* biotransformation assay was done according to McKinney et al. (2011). The biotransformation reaction was done in a total volume of 1mL. The incubation solution contained potassium phosphate buffer (0.5 M, pH 7.4), 3 µL of N-Et-FOSA (50 mg/mL) and 60 µL of NADPH regenerative system (50 µL of solution A and 10 µL of solution B). The solution was incubated at 37°C for 5 minutes. The reaction was then initiated by adding 1mg of microsomes to the incubation solution. The incubation was done at 37°C in a water bath with shaking (120 rpm). The positive controls were Wistar-Han rat liver microsomes and negative controls were the same microsomes, which

were heat deactivated at 100°C for 5 minutes. The negative controls were used to assess for any non-enzymatic reactions with the substrate (N-Et-FOSA). A buffer blank did not contain any substrate and was prepared to ensure there was no background contamination. At each time point (2, 5, 10, 20 and 40 minutes), 100 µL of the incubation solution was extracted and transferred in a quenching tube, containing 900 µL of acetonitrile and 10 ng of d5-N-Et-FOSA, M8FOSA and MPFOS. The quenching tubes were then sonicated for 30 minutes and transferred in VWR centrifugal filters (modified nylon, 0.2 µm) and centrifuged at 5000 rpm (2041 g). The filtrate was then transferred in a UHPLC vial for quantitative UHPLC-MS/MS analysis. For each sample (Low ringed seal, High ringed seal, Low polar bear and High polar bear) there were n = 6 replicates (3 intra-day replicates and 2 inter-day experiments). Each experiment included n = 1 blank, n = 1 positive control, n = 1 negative control and 2 x n = 3 samples.

2.6 Quality Control and Assurance

Data is presented on a wet weight basis instead of a lipid weight basis since PFASs are known to bind to proteins and are not related to lipid weight (Jones et al., 2003; Luebker et al., 2002; Greaves et al. 2012).

The method limit of quantification (MLOQs) was determined by analyzing 8 replicates of matrix samples (1g of pork liver), calculating the standard deviation and multiplying it by the student value at 99 %. The method limit of detection (MLODs) was estimated by the analyte signal to noise ratio of 3 in fortified matrix samples. The PFAS MLODs and MLOQs are listed in Table 2.2. The instrument limit of detection (ILODs) was determined by the analyte signal to noise ratio of 3 in standard solution.

Each batch contained the 5-point calibration curve, both a positive and a negative QC, a blank, and a total of 6 samples, including a duplicate. The positive QC matrix was pork liver samples spiked with the internal standard solution and standard solution, both at 10ng, whereas the negative QC matrix was a pork liver sample spiked only with 10 ng of the internal standard. The blank in each batch assessed for the method contamination. A background correction was applied (background level was subtracted from sample concentration) where the blank concentration reached 5 % of the sample concentration. If the blank concentration exceeded 50 % of the sample concentration, the sample was coded as N/Q and was not quantified. The background was applied to polar bear liver samples for PFEtCHxS PFDS, PFHxDA, PFODA, FBSA and N-Et-FOSA. It was also applied to polar bear fat samples for PFBA, PFPeA, PFOA, PFNA, PFDA and PFUdA. For ringed seal liver samples, it was applied to PFBA, PFHxS, PFOA, PFEtHxS PFDS, PFTeDA, PFHxDA, PFODA, FOSA and N-Et-FOSA. For the ringed seal fat samples, background correction was applied to PFHxS, PFHxA, PFUdA, PFDoA and PFTrDa, Positive control recoveries are reported in Table 2.2 and ranged from 58 % for PFEtCHxS to 138 % for PFODA. The samples were randomized to ensure there were no biases in data analysis.

2.7 Data Analysis

Arithmetic mean concentrations were only determined for individual PFASs with >50 % detection in the liver and fat/blubber samples per species. Concentrations below the MLOQ were replaced with half the MLOQ value for statistical purposes. Shapiro–Wilk's *W* test indicated a normal distribution of PFAS concentrations. To determine significant

differences in PFAS concentration as a function of sex, tissue and species, a one-way analysis of variance (ANOVA) was performed separately, with results considered significantly different at $p < 0.05$. Statistical analyses were done with Microsoft Excel.

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Chapter 3

Polar bears From Two Arctic Pollution Hot Spots: Comparison of Recent Levels of Established and New Perfluorinated Sulfonic and Carboxylic Acids and Precursors[1]

3.1 Introduction

Marine feeding polar bears are one of the Arctic wildlife species that have the highest tissue PFAS concentrations (Letcher et al. 2010). In particular, the East Greenland polar bear subpopulation has been the focus of many studies on PFASs (Ahrens et al., 2009; Bossi et al., 2005; Dietz et al., 2008; Greaves et al., 2011, 2012 and 2013; Letcher et al., 2010; Rotander et al., 2012). Concentrations for PFOS have been reported as high as 2966 ng/g ww for East Greenland polar bears in 2006 (Rigét et al. 2013). The concentrations of PFOS, PFDA and PFTrA had been on an increasing trend over a 19-year period (1984 to 2006) with a faster increase between 2000 and 2006 (Rigét et al. 2013). However, for PFOS, PFHxS, FOSA, PFDA and PFUdA from 2006 to 2010, there were decreasing concentration trends in the same bears (Rigét et al., 2013). The temporal trends for PFAS concentrations in East Greenland polar bears will be addressed in more detail in the following chapter (Chapter 4).

Other PFASs, which have been shown to be of relevance in the Arctic environment in air water and biota (notably the marine wildlife), are also precursors to established PFCAs and PFSAs (Butt et al., 2010; Houde et al., 2011; Shoeib et al., 2006). Those include fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides (FASAs)(e.g. FOSA) (Butt et al., 2010; Houde et al., 2011; Shoeib et al., 2006). Shorter

[1] Based on a paper in preparation to be submitted to Science of the Total Environment.

chain PFASs such as PFHxS and PFBS have also been reported in rather low concentrations in Arctic wildlife, including the liver of East Greenland polar bears (Gebbink et al., 2016).

East Greenland and the Hudson Bay (HB) region are known as two "hotspots" of persistent organic pollutant (POP) contamination in the Arctic (Letcher et al., 2010). However, there is currently little published information on PFASs in polar bears from Canadian subpopulations and specifically on animals from the HB region. The HB region is affected by global climate change as it is heating twice as fast as temperate regions due to sea ice loss (McKinney et al., 2015). As Arctic wildlife species do not have the option to shift their habitat further to the North, global climate change heavily affects their food webs and diets (McKinney et al., 2015). One of the impacts on polar bears is the growing introduction of subarctic seal species (e.g. harbor seal (*Phoca vitulina*)) in their diet, leading to an increase in the biomagnification of contaminants (McKinney et al., 2015). The little published information that does exist is now over a decade old (Smithwick et al., 2005). As reported for bears harvested over a decade ago, PFOS concentrations were very high in polar bears reaching 2730 ng/g ww in Southern Hudson Bay (Smithwick et al., 2005). Following the voluntary phase-out of PFOS in 2002 by the 3M Company (at the time 3M produced around 80 % of all C8 PFOS chemistry for commercial use), Rigét et al. (2013) showed decreasing trends for some PFASs, and mostly for PFOS. Therefore, based on these temporal trends, it is hypothesized that PFOS and related PFAS contamination in HB polar bears will be lower in polar bears then was reported by Smithwick et al. in 2005.

Polar bears from the HB region and from East Greenland have much in common, where their diets are mostly composed of ringed seal blubber (McKinney et al., 2013). However, recent studies, using dietary trackers (e.g. nitrogen and carbon stable isotope ratio and fatty acid profiles), indicate that the diets of those two polar bear subpopulations are somewhat different, where Hudson Bay bears feed more on marine wildlife (e.g. bearded seal and beluga) relative to East Greenland bears (Galicia et al., 2015; McKinney et al., 2010). Those diet differences could mean major differences in patterns and levels of bioaccumulative PFASs between East Greenland polar bears and Hudson Bay polar bears.

Considering the dearth of recent PFAS data for Hudson Bay polar bears, the reported and more recent decreasing trends in East Greenland polar bears, and the emergence of newly identified PFAS contaminants, the present study investigated the levels and patterns and comparative differences of 22 PFASs, including new and established PFSAs and PFCAs and some of their precursors, in polar bear livers from Western Hudson Bay, Southern Hudson Bay and East Greenland. This investigation elucidates how exposure to PFASs for these three "hotspot" subpopulations of polar bears differs by means of concentrations and patterns, and provides a much-needed update on the status of PFASs in Canadian polar bear subpopulations.

3.2 Results and Discussion

3.2.1 PFASs and Precursors in EG, SHB and WHB Polar Bears

PFASs levels were determined in all East Greenland polar bears (n=10) and Hudson Bay polar bears (n=10 southern Hudson Bay bears; n=9 western Hudson Bay bears)

(Tables 2.1, 2.2 and 3.1). Since there were no significant differences between the males and females (ANOVA, $p>0.05$), sex was not considered as a confounding factor for the three subpopulations (see figure 4.1 in chapter 4). Also, since the sample set contained a wide variety of ages and the standard error was less than 10 % of the means, age was also deemed an insubstantial confounding factor and thus was not considered further in the present discussion.

The ΣPFAS was significantly different (ANOVA, $p<0.05$) between East Greenland and Southern Hudson Bay. In East Greenland bears, the mean concentration was 3546 ± 275 ng/g ww, with PFOS at 2583 ± 199 ng/g ww and representing 73% of the ΣPFAS. For SHB bears, the mean concentration was 1955 ± 226 ng/g ww, with PFOS at 1273 ± 171 ng/g ww and representing 65 % of the ΣPFAS concentration. Mean FOSA levels were 9.5 ± 1.4 ng/g ww and 0.27 % of ΣPFAS concentrations for the Greenland bears and were significantly lower than the mean FOSA level of 25 ± 9 ng/g ww and representing 1.26 % for the Southern Hudson Bay bears. Western Hudson Bay bears had PFAS concentrations similar to Southern Hudson Bay bears (ANOVA, $p<0.05$) with ΣPFAS at 1682 ±198 ng/g ww. PFOS represented 64 % of the liver contamination at 1069 ±122 ng/g ww. FOSA was again higher at 38 ± 9 ng/g ww representing 2.24 % of the liver burden. For EG bears, the ΣPFCA was 924 ± 71 ng/g ww and the most abundant contaminant was PFNA at 364 ± 28 ng/g ww representing 39 % of that sum. For SHB bears, ΣPFCA reached 649 ± 48 ng/g ww and the most abundant contaminant was also PFNA at 298 ± 22 ng/g ww which represents 46 % of ΣPFCA. Finally, for WHB bears, the ΣPFCA was 568 ± 65 ng/g ww and the most abundant PFCA was PFNA at 247 ± 26 ng/g ww, representing 43 % of ΣPFCA. The differences between the two Hudson Bay

subpopulations and the East Greenland subpopulation were also noticeable in the bioaccumulation patterns of the PFASs (Figure 1). In both the Hudson Bay bears subpopulations, PFCAs (PFOA, PFNA, PFDA, PFUdA and PFDoA) comprised a greater proportion of the ΣPFAS concentrations relative to East Greenland bears.

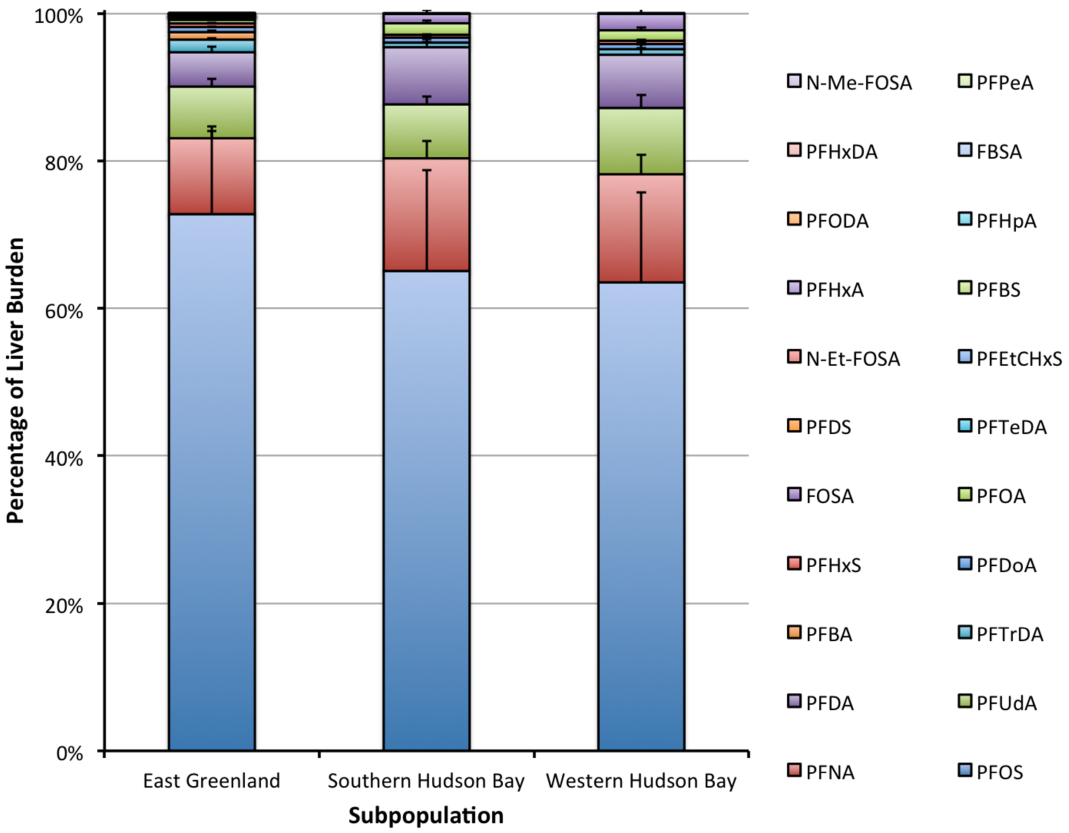

Figure 3.1 The mean (± standard error) percent composition of the concentration of each perfluoroalkyl substance (PFAS) relative to the sum-PFAS concentration in polar bear livers from East Greenland (n=10), southern Hudson Bay (n=11) and western Hudson Bay (n=10) polar bears.

Table 3.1 Arithmetic mean and range of concentrations of 22 PFASs (ng/g wet weight) in the liver of East Greenland (n=10), southern Hudson Bay (n=11) and western Hudson Bay (n=10) polar bears. Shaded areas represent data that was not measured.

		Carboxylic Acids													
		PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA	PFTeDA	PFHxDA	PFODA	ΣPFCA
East Greenland	Mean (ng/g ww)	36	0.0	0.5	0.3	17	364	165	249	24	61	7.0	0.2	0.3	924
	± SE	4.9		0.1	0.1	2.6	28	13	17	1.5	3.0	0.4	0.0	0.0	71
	Range (ng/g ww)	14 – 59		0.2 – 0.8	0.0 – 0.8	12 – 38	248 – 508	112 – 238	174 – 338	16 – 31	37 – 68	3.8 – 8.5	0.1 – 0.2	0.2 – 0.4	
Southern Hudson Bay	Mean (ng/g ww)					30	298	152	143	14	12				649
	± SE					3.9	22	9.9	9.2	1.2	1.4				48
	Range (ng/g ww)					14.2 – 52	156 – 447	92 – 213	83 – 199	6.0 – 20	5.7 – 20				
Western Hudson Bay	Mean (ng/g ww)					24	247	121	151	12	13				568
	± SE					4.8	26	14	18	1.6	1.8				65
	Range (ng/g ww)					5.2 – 59	127 – 339	57 – 180	73 – 245	5.2 – 22	5.5 – 23				

Mean was not calculated (N/Q) when more than 50 % of the samples were <MLOD. Refer to table 2.2 for compounds MLOD

Table 3.1 (continued)

		Sulfonic Acids						Neutral				
		PFBS	PFHxS	PFEtCHxS	PFOS	PFDS	ΣPFSA	FBSA	FOSA	N-Me-FOSA	N-Et-FOSA	ΣPFAS
East Greenland	Mean (ng/g ww)	0.7	18	3.1	2583	5.7	2610	0.436	9.5	N/Q	1.5	3544
	± SE	0.1	1.6	0.1	199	1.1	202	0.107	1.4		0.4	275
	Range (ng/g ww)	0.3 – 1.0	7.2 – 29	2.3 – 3.9	1500 – 3373	2.6 – 11		0.145 – 0.889	2.4 – 16		0.2 – 3.5	
Southern Hudson Bay	Mean (ng/g ww)	0.6	7.6		1274	0.1	1282		25			1955
	± SE	0.1	0.7		169	0.2	169		9			226
	Range (ng/g ww)	0.1 – 1.3	4.0 – 12		653 – 2411	0.0 – 1.1			3.4 – 92			
Western Hudson Bay	Mean (ng/g ww)	<MLOD	7.7		1069	N/Q	1076		38			1682
	± SE		0.9		122		123		9			198
	Range (ng/g ww)		3.7 – 13		549 – 1694	<MLOD – 0.3			0.01 – 92			

Mean was not calculated (N/Q) when more than 50 % of the samples were <MLOD. Refer to table 2.2 for compounds MLOD

Previously, a circumpolar study compared now-established PFCA and PFSA contaminant levels in polar bear liver tissues harvested between 1999 and 2002 from different subpopulations (i.e. Chukchi Sea (2001), Northwest Territories (2001), High Arctic (2002), South Hudson Bay (2002), South Baffin Island (2002), East Greenland (1999-2001) and Svalbard (2002)) (Smithwick et al. 2005). PFOS and PFHxS were much greater in 2002 at 2730 and 62.3 ng/g ww in Southern Hudson Bay bears, respectively, compared to the 1274 ±169 and 7.6 ± 0.7 ng/g ww found in the present bears (Table 3.1, Smithwick et al. 2005). In contrast, the PFOS precursor, FOSA, was more concentrated in the present bears at 25 ng/g ww compared to 6.2 ng/g ww in bears harvested in 2002 (Table 3.1, Smithwick et al. 2005). As for East Greenland, most of the PFASs measured previously were at lesser concentrations compared to the present bears with the major exception of PFHxS at 80 ng/g ww in 2005 versus 18 ± 2 ng/g ww in the current data (Table 3.1, Smithwick et al. 2005). The high PFOS and PFHxS concentrations in Southern Hudson Bay in 2002 were previously attributed to the proximity of this subpopulation to major PFAS sources in Eastern North America (Smithwick et al. 2005). The difference noticed between 2002 and 2011-2012 suggests that the phasing out of long chained perfluorinated chemistry in the United States of America helped in decreasing the concentration of legacy PFASs (i.e. PFOS and PFHxS) in polar bears of the Southern Hudson Bay (Wang et al. 2013b). However, at the same time, the increasing interest towards alternate PFASs, such as FOSA, also seems to have an effect on the FOSA concentrations (Wang et al. 2013b). Even though long-chained PFASs are known to be bioaccumulative, regulations apparently have had an effect on their concentrations since 2002. As for Greenland, the high PFOS concentration from 2002 was attributed to

the proximity of this subpopulation to sources in Europe (Smithwick et al. 2005). The similarity between the concentrations of 2002 and 2011 polar bears, could partially be attributed to the fact that China, to which East Greenland is much more exposed than Hudson Bay has increased its PFOS production since the phase out in 2002 and has not stopped producing since then (Lim et al. 2011).

There are several explanations that could possibly justify the PFAS pattern and concentration differences between bears from the Greenland Southern Hudson Bay and Western Hudson Bay populations. EG polar bears were generally more contaminated than both HB bear subpopulations. As stated before, the difference in atmospheric circulation between EG and HB could explain the differences between the levels of PFASs in the two subpopulations (Bilello, 1973). As China remains the only country with a massive PFOS and PFOS related products production (Carloni, 2009, Lim et al. 2011), the atmospheric circulation, with the dominant winds leading from China towards EG could enhance the quantity of PFASs present in EG. Indeed, one of the main sources of PFASs in the Arctic is the long-range atmospheric transport of volatile precursors such as FTOH, FOSA and N-Et-FOSA (Prevedouros et al. 2006, Li et al. 2015). During PFOS production, a variety of volatile PFOS salts are produced (i.e. perfluorooctane sulfonamidoethanols (FOSE) and FOSA) and expelled in the atmosphere (Lim et al. 2011). Those products are then transported by the air (i.e. dominant winds) until they reach colder environment like the Arctic and accumulate or transform in their less volatile metabolites (Prevedouros et al. 2006 ; Li et al., 2015). A more direct transport can occur where particulate PFASs are released from fluoropolymer manufacturing facilities (Prevedouros et al. 2006 ; Li et al., 2015). Those particulates can then travel for

weeks in the air before reaching the Arctic by wet or dry deposition (Prevedouros et al. 2006).

Another phenomenon that could explain the discrepancy between the bioaccumulation patterns of the EG subpopulation and the SHB and WHB subpopulations is the transport patterns themselves. Indeed, short-lived species (i.e. volatile precursors like FOSA) are more easily transported to Arctic locations near 60°N (i.e. Hudson Bay) than other locations further north (i.e. East Greenland) (Klonecki et al., 2003). By introducing a short-lived tracer (i.e. 7-day decay time) and a long-lived tracer (chemically passive) in a regional chemical transport model, Klonecki et al. were able to reveal that most of the short-lived species (e.g. FOSA) present in the Arctic were from North-American origin (Klonecki et al., 2003). Also, by using a long-lived tracker, Eurasia south of 45° and east of 40°E (most notably China) was revealed as the most important contributor for long-lived species (e.g. PFOS) (Klonecki et al., 2003). Therefore, not only does the production patterns of the surrounding regions, but the transport patterns themselves could explain the major discrepancy between the amount of FOSA and PFOS between EG and SHB and WHB.

Differences in the food web structure and the diet for bears from the three subpopulations can explain PFAS exposure differences. Stable isotope ratios of carbon and nitrogen and fatty acid signatures are routinely used as ecological/dietary tracers (Jardine et al. 2006). The trophic position of an animal can be inferred by its $\delta^{15}N$ values since $\delta^{15}N$ increases by trophic levels in a food chain. Similarly, the particular food chain can be inferred by the $\delta^{13}C$ values since different photosynthesis mechanisms result in a typical $\delta^{13}C$ pattern that remains largely unchanged throughout the food chain,

differentiating between feeding strategies (e.g., nearshore/offshore, benthic/pelagic, freshwater/marine and terrestrial/aquatic) (Jardine et al., 2006). As has been reported for 2007-2008 sampled bears from circumpolar populations, WHB and SHB signatures have been characterized by depleted $\delta^{15}N$ and $\delta^{13}C$, lower proportions of C20 and C22 monounsaturated fatty acids and higher proportions of C18 and longer chain polyunsaturated fatty acids. In contrast, EG signatures were reversed relative to Hudson Bay (McKinney et al., 2010; McKinney et al. 2015). Since ringed seals are mainly pelagic foragers, $\delta^{13}C$ values in the Hudson Bay bears suggested that ringed seal are feeding more on freshwater or terrestrially associated prey relative to bears from other subpopulations including EG (McKinney et al., 2010). Consumption of harbor seals in Hudson Bay could also contribute to depleted $\delta^{13}C$ in these bears, as these seals inhabit rivers and areas of fresh (and tidal) flowing waters. As Hudson Bay bears and EG bears show differing food structure/dietary tracer signatures, this likely accounts in part for the differing PFAS concentrations and patterns in the liver, most notably for PFOS (2583 ±199 , 1274 ± 169 and 1069 ± 122 ng/g ww for EG, SHB and WHB respectively) and FOSA (9.5 ± 1.4, 25 ± 9 and 38 ± 9 ng/g ww for EG, SHB and WHB respectively) (Table 3.1). Since PFOS biomagnifies through the aquatic-marine food chain (Butt et al., 2010, Houde et al., 2011 and Tomy et al., 2004), eating prey at a lower trophic level would mean a reduced exposure compared to eating at higher trophic levels. EG bears would therefore have an increased exposure to highly biomagnifiable PFASs (i.e. PFOS) when compared to Hudson Bay bears who feed on lower trophic level prey again a contributing factor to the difference in PFOS concentration between the bears from EG and Hudson Bay.

3.2.2 PFOS and its Known Precursor FOSA in EG, SHB and WHB Polar Bears

As the FOSA concentration difference between (the livers) of the two subpopulations of bears was the greatest, the ratio of the metabolite (PFOS) to the precursor (FOSA) was examined (Figure 2). There was a major difference between the three mean concentration ratios at 271 ± 62 for EG, 51 ± 25 for SHB and 28 ± 10 for WHB bears (Figure 2). The ratio was significantly lower for SHB than for EG (ANOVA, $p<0.05$) while there was no significant difference between SHB and WHB (ANOVA $p<0.05$). The same trend was found for the mean concentration ratios for the metabolite (FOSA) to the precursor (N-Et-FOSA). The mean concentration ratio was 6.4 ± 2.5 for EG bears and could not be calculated for HB bears as no N-Et-FOSA was detected. Due to the differences in diet as previously explained, PFOS biomagnifies through the marine food web (Butt et al., 2010, Houde et al., 2011 and Tomy et al., 2004). The EG polar bear diet has been shown to have a greater proportion of a benthic food web signature relative to HB polar bears. Thus, it has been hypothesized that EG bears bioaccumulate more PFOS and less FOSA (McKinney et al., 2010). Another hypothesis regarding the difference in metabolite to precursor ratio is that there is a metabolic capacity difference between bears from HB and EG. Further to this hypothesis is that because of greater POP exposure of EG bears (relative to HB bears) greater enzyme induction facilitates a greater biotransformation capacity and the more rapid metabolism of N-Et-FOSA to FOSA and FOSA to PFOS. It is therefore plausible that such metabolism to PFOS is added source of PFOS accumulation in the bear.

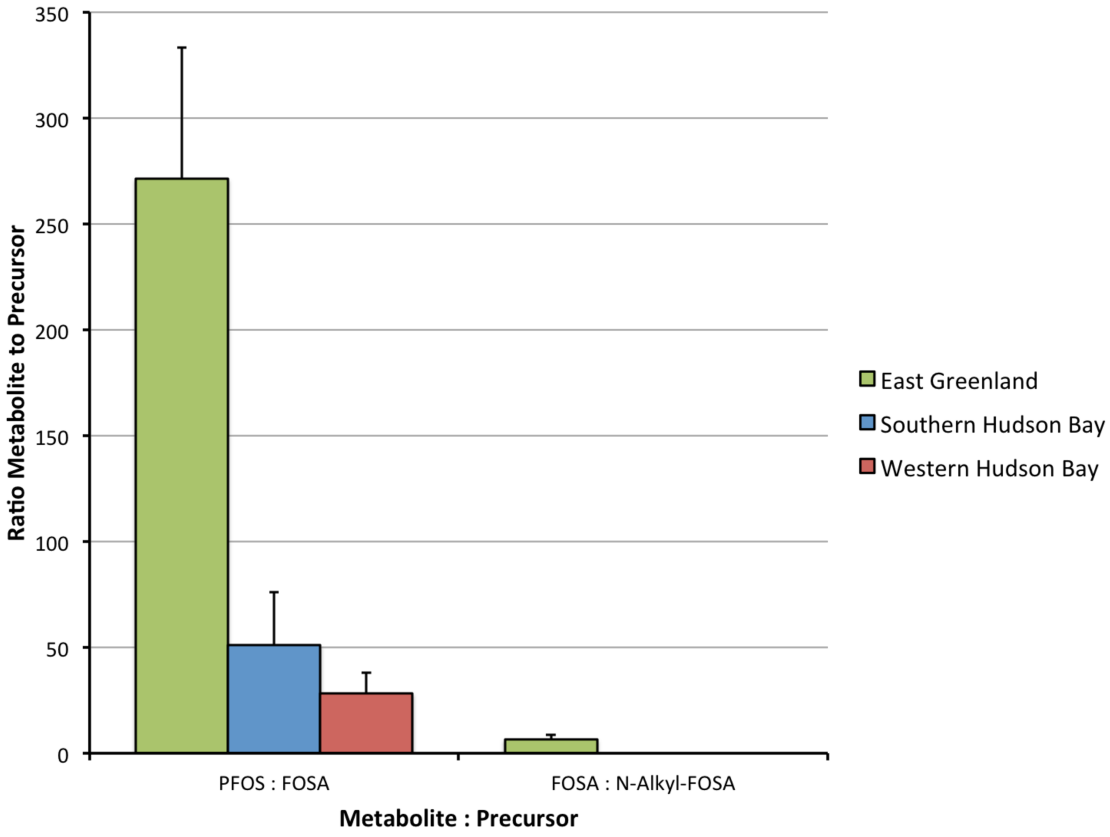

Figure 3.2 Arithmetic means (± standard error) of the perfluorooctane sulfonate (PFOS) to perfluorooctane sulfonamide (FOSA) concentration ratios, and the FOSA to N-alkyl-FOSA concentration ratios in polar bear livers from East Greenland (n=10), southern Hudson Bay (n=11) and western Hudson Bay (n=10) polar bears.

3.2.3 Conclusions

Since the last report on PFASs in polar bears was by Smithwick et al. (2005) based on collected samples from over a decade again, this study updates the PFAS exposure situation for bears from Arctic "hot spot" subpopulations. There are major differences in PFAS concentrations and patterns of exposure between the polar bears from subpopulations in East Greenland and Hudson Bay. EG polar bears were generally

more PFAS contaminated than HB polar bears. Based on bioaccumulation patterns, EG polar bears accumulate more PFSAs and less PFCAs relative to HB bears. Whether due to differences in PFAS exposure or metabolism, these differences affected FOSA levels (a precursor to PFOS) and resulting in greater concentrations in the livers of polar bears from both Hudson Bay subpopulations. The metabolite (PFOS) to precursor (FOSA) ratio suggested a slower metabolic degradation of FOSA to PFOS in HB bears as the concentration ratio was one order of magnitude lower for the HB bears relative to the EG bears.

This study shows that different polar bear subpopulations are exposed differently to PFASs are a function contaminant sources and transport, and the food web structure, predator-prey relationships and diet and most likely metabolism. The latter will be studied in more detail in Chapters 4 and 5. The behaviour of PFASs in polar bears should be further studied to better understand the reasons behind the differences in concentrations and bioaccumulation patterns across circumpolar subpopulations.

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Chapter 4

Predator-Prey Bioaccumulation and Biomagnification of PFASs and their Precursors in East-Greenland Polar Bears and Ringed Seals[2]

4.1 Introduction

PFASs are known to bioaccumulate, mostly in protein rich tissues such as liver and kidney (Jones et al., 2002; Luebker et al., 2002; Greaves et al., 2012 and 2013). Indeed, Jones et al. showed the ability of PFOS to bind to serum albumin with very high affinity in fish and birds. Luebker et al. (2002) showed the ability of some PFASs (PFOS, PFOA, N-Et-FOSA and N-Et-FOSE) to bind to liver fatty acid binding proteins in male rats. Additionally, Greaves et al. showed a clear distribution where approximately 90 % of the PFASs were located in the liver in East Greenland polar bears. However, the longer the carbon chain (i.e. 10 and more carbons) the more affinity the PFAS has towards lipophilic environments due to their lower solubility (Jones et al., 2002). As such, long carbon-chain PFASs were shown to have concentrations correlated to extractable lipids in East Greenland polar bear brains (Greaves et al., 2013).

As polar bears mainly consume ringed seal blubber, it is expected that the bioaccumulation pattern of PFASs in the prey will affect the predator through the biomagnification process (McKinney et al., 2010, 2011 and 2013). However, different bioaccumulation patterns have been observed between the two species, notably, longer chained PFCAs (PFUnDA) are more abundant in ringed seals than in polar bears (Rigét et al. 2013). In addition, biomagnification is known to vary for PFASs depending on the

[2] Based on a paper in preparation to be submitted to Environmental Pollution

carbon chain length and reaches a maximum at 9 fluorinated carbons (PFNA) (Müeller et al. 2011). Indeed, multiple studies have shown that long carbon chain lengths (i.e. >6 carbons) PFASs biomagnify in Arctic marine food webs most notably in the Ringed Seal / Polar Bear food web (Letcher et al. 2010; Rigét et al. 2013; Gebbink et al. 2016). In fact, it is known that the sum of PFASs is approximately 15 times higher in the predator (i.e. polar bear) than in its prey (i.e. ringed seal) in East Greenland (Gebbink et al. 2016).

In 2015, Dietz et al. revealed that PFOS was the second-highest contributor for genotoxic effects in polar bears behind PCBs, with risk quotients (estimated exposure divided by estimated health effect) ranging from 0.35 to 2.5 across the Arctic (> 1 represents an actual risk). According to their physiologically-based pharmacokinetic modelling, East Greenland polar bears had the highest total risk quotient regarding reproductive effects, immune effects and genotoxic effects compared to the 10 other subpopulations studied (Dietz et al., 2015). As PFOS seems to represent the highest exposure risk for polar bears, its concentration, along with PFCAs (i.e. PFDA and PFTrA), increased significantly in polar bears and ringed seal liver tissues between 2000 and 2006 (Dietz et al., 2008; Rigét et al., 2013). Concentrations of PFASs have been increasing from 1984 to 2006 with steeper increases after 1990 for FOSA and after 2000 for PFOS, PFDA and PFTrA (Dietz et al., 2008). Following the trends, Dietz et al., estimated that the lowest no-adverse-effect level (NOAEL) and the lowest-adverse-effect level (LOAEL) for rats and monkeys would be exceeded between 2014 and 2024. However, following the PFOS phase-out in 2002, decreasing trends were observed in ringed seals and polar bears liver tissues for the same PFASs between 2006 and 2010 (Rigét et al., 2013). Indeed, annual decreases in East Greenland polar bears ranged from 6

% for PFNA to 57.6 % for FOSA (Rigét et al., 2013). In ringed seals, however, some PFASs still increased between 2006 and 2010, PFNA increased by 21.6 % per year, PFDA by 4.2 % per year and PFUnDA by12.4 % per year (Rigét et al., 2013). At the same time, replacement chemicals for PFOS (i.e. F-53B, PFBS, PFHxS and PFHpA) were manufactured and were reported recently in both East Greenland polar bears and ringed seals (Gebbink et al., 2016). F-53B, a chlorinated polyfluorinated ether sulfonic acid with chemical properties similar to PFOS, PFHxS and PFHpA were detected in higher concentrations in polar bears than in ringed seals, while PFBS was only detected in polar bears (Gebbink et al., 2016).

Additionally to direct release, PFAS exposure can be sourced to the release and subsequent degradation of said precursors, notably, N-Et-FOSA and FOSA (Liu and Avendaño, 2013; Wang et al., 2013). More recently, the contribution of FOSA to PFOS exposure in wildlife remains unclear. However, FOSA concentrations are much lower than PFOS concentration in Arctic wildlife. As a consequence, East Greenland polar bear and ringed seal liver tissues are very high in PFOS : FOSA concentration ratio (Dietz et al., 2008; Greaves et al., 2012, 2013; Rigét et al., 2013).

Given the high PFAS exposure in East Greenland polar bears, their eating habits (i.e. consuming almost exclusively ringed seal blubber) and the concern raised by emerging PFOS replacement products, in this study, we determined the concentrations a suite of 22 PFASs including established and new bioaccumulation PFSAs and PFCAs along several of their PFAS precursors in adipose / blubber and liver tissues in East Greenland polar bears and ringed seals. Using this data, we assessed the predator / prey

relationship for many of these PFASs to better understand the contributions of PFASs accumulation and precursor degradation in polar bear.

4.2 Results and Discussion

4.2.1 Sex, Age and PFASs Concentrations

After evaluating the concentrations for the 22 PFASs in both ringed seals (n=19) and polar bears (n=10), the sample group was split by sex and compared (Figure 4.1 and 4.2). There were no statistical differences between male and female for either species (ANOVA, $p > 0.05$). Therefore, sex was not considered as a confounding factor and male and female individuals were used together to calculate mean concentrations and allow species comparison.

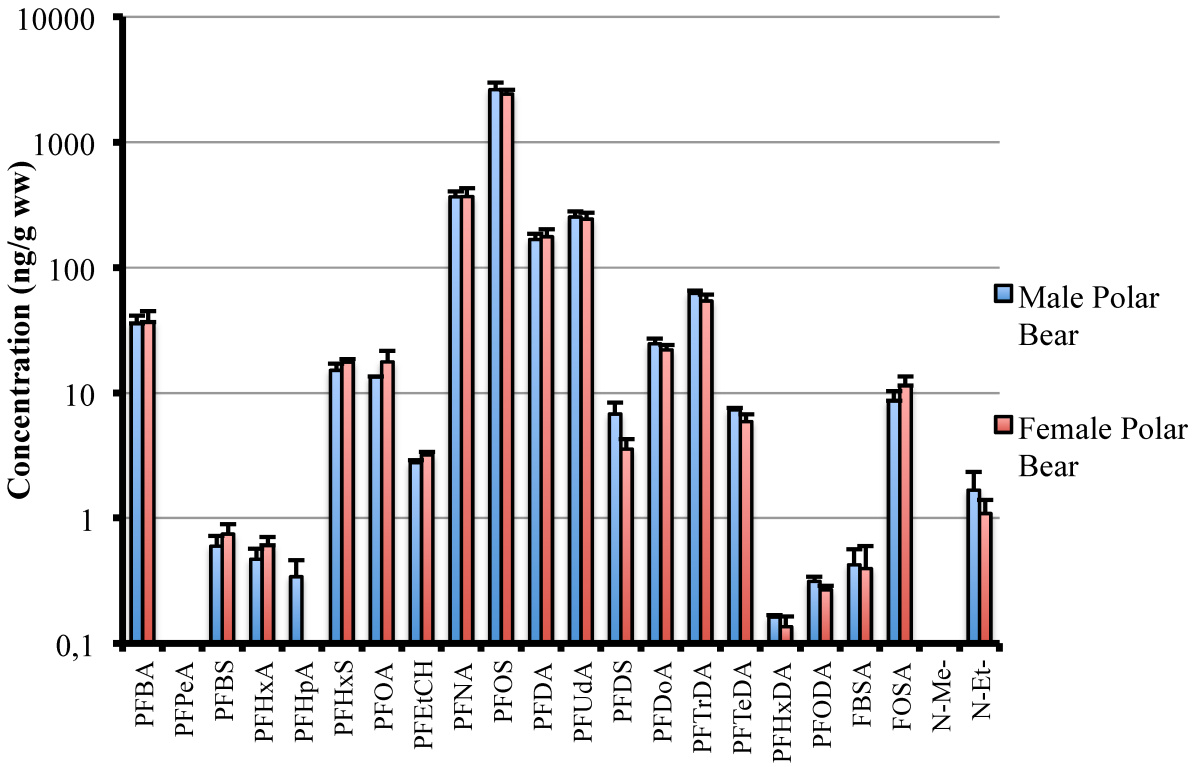

Figure 4.1 Concentration (ng/g wet weight) of a Suite of PFASs in Male (n=5) and Female (n=3) Polar Bear Livers. Error bars : SE.

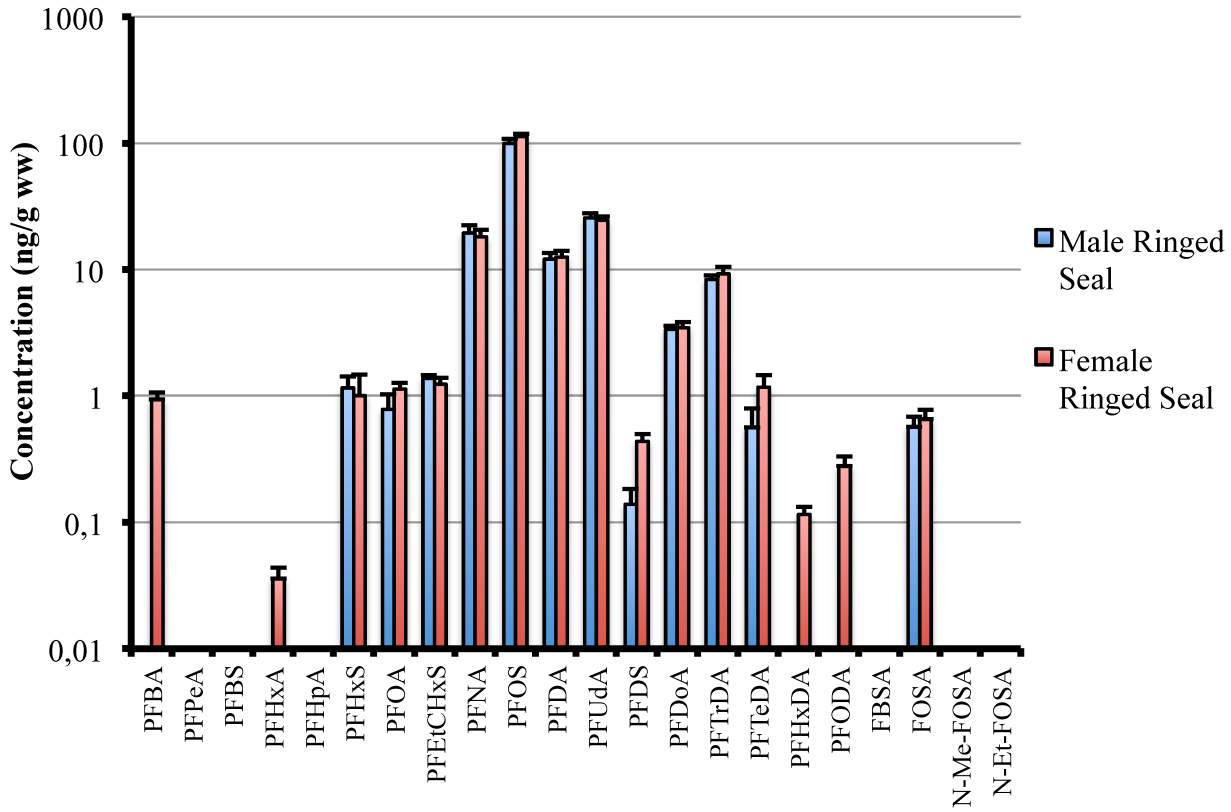

Figure 4.2 Concentration (ng/g wet weight) of a Suite of PFAS in Male (n=9) and Female (n=8) Ringed Seal Livers. Error bars : SE.

As for age, based on the high variation of the ages (fetuses to adults for ringed seals and sub adults to adults for polar bears) and the low variations of the means (see error bars in figures 4.1 and 4.2), it was also not considered as a confounding factor. Therefore, the data set was not corrected for age and was used without further modifications.

4.2.2 PFSAs and Precursors in Ringed Seals and Polar Bears

In polar bears, the mean ΣPFSA concentration in liver was 2611 ± 202 ng/g. Among the PFASs, the mean PFOS concentration in liver was 2583 ± 199 ng/g and

accounted for 98 % of the ΣPFSA concentration (Table 4.1). In polar bear adipose tissue, the mean ΣPFAS concentration was 20 ± 3.2 ng/g and two orders of magnitude lower than in liver. The mean PFOS concentration in polar bear adipose tissue was 18 ± 2.8 ng/g and accounted for 89 % of the ΣPFSA concentration (Table 4.1). As previously shown in a larger tissue distribution study, the liver contained the greatest PFAS concentrations in East Greenland polar bears (Greaves et al., 2012). PFOS is more associated with proteins rather than lipids (Jones et al., 2003 ; Luebker et al., 2002), which may explain the higher PFOS concentration in the high-protein liver relative to the low-protein adipose tissue and blubber of polar bears and ringed seals.

Significant yearly increases were documented in East Greenland polar bears between 1984–2006 (Dietz et al. 2008; Rigét et al. 2013). When comparing present study results with those of bears sampled in 2005 (Greaves et al., 2012), no significant differences were found for PFOS or other PFASs. This indicates that PFOS did not decrease over the 6-year period, 2005-2011, despite the phase-out of PFOS by the 3M Company in 2002. In contrast, PFOS concentrations showed a decreasing trend in East Greenland polar bears (liver) from 2006 to 2010 as reported by Rigét et al. (2013). Rigét et al. (2013) reported PFOS concentrations over a time span of 1984-2010, which may explain the decreasing trend observed by the authors. In East Greenland polar bears, PFSA concentrations were reported to be highest in liver followed by blood > brain > muscle ≈ adipose (Greaves et al., 2012). Also, in different brain region samples for the same bears, PFOS was found to be significantly and positively correlated with lipid content for all brain regions (Greaves et al., 2013). PFBS (0.7 ± 0.1 ng/g), PFHxS (18 ±1.6 ng/g) and PFEtCHxS (3.1 ± 0.1 ng/g), replacement products for PFOS, are

bioaccumulating in these bears. They were all quantifiable at low levels in polar bear liver, but only PFHxS (2.2 ± 0.4 ng/g) was quantifiable in polar bear fat. As expected from the tendency of PFSAs to accumulate in high-protein tissues, the three PFOS replacement products were more abundant in the liver than in the fat. PFBS and PFHxS were reported for these bears in similar concentrations (Gebbink et al., 2016). PFBS was detected at 0.7 ± 0.1 ng/g an order of magnitude higher than previously reported at 0.032 ± 0.008 ng/g (Gebbink et al. 2016). PFHxS was detected at 18 ±1.6 ng/g, a similar concentration reported recently at 13 ± 2 ng/g (Gebbink et al., 2016). The major difference in concentrations between the two PFSAs could be linked to the fact that PFEtCHxS is exclusively used in aircraft hydraulic fluids as an erosion inhibitor, therefore being released significantly less in the atmosphere than the widely used PFOS (De Silva et al. 2011). PFEtCHxS has been reported in the Great Lakes biota and surface waters (Letcher et al. 2015; De Silva et al. 2011; de Solla et al. 2012 and Howard et al. 2010) but to our knowledge, this is the first report on PFEtCHxS in polar bears or any other Arctic wildlife species. Known PFSA precursors, FBSA, FOSA, N-Me-FOSA and N-Et-FOSA were detected in concentrations ranging from 0.4 ± 0.1 ng/g for FBSA to 10 ± 1.4 ng/g for FOSA in polar bear liver samples whereas the only one quantified in polar bear fat was N-Et-FOSA at 1.2 ± 0.3 ng/g. As observed previously, FOSA concentrations are significantly higher in liver tissues than adipose tissue (Greaves et al. 2012). N-Et-FOSA does not seem to share that behaviour as it was quantified in similar concentrations in the liver and in the fat tissues at 1.5 ± 0.4 ng/g and 1.2 ± 0.3 ng/g respectively. To our knowledge, FBSA (precursor to PFBS) has never previously been reported in polar bears or any other compartment in the Arctic environment.

For ringed seals, overall ΣPFSA concentrations were significantly lower compared to the polar bears. Mean liver concentration of ΣPFSA was 111 ± 5.3 ng/g, and the mean PFOS concentration was 108 ± 4.9 ng/g and 98 % of the mean ΣPFSA concentration. PFAS concentration in ringed seal blubber was either very low or not detectable. The mean ΣPFSA concentration was 0.05 ± 0.01 ng/g. PFOS was not detectable in any seal blubber sample. PFOS replacement products, PFHxS (1.1 ± 0.2 ng/g) and PFEtCHxS (1.3 ± 0.1 ng/g), were both detected in ringed seal liver samples, but, similarly to polar bear fat, only PFHxS (0.05 ± 0.01 ng/g) was quantifiable in blubber samples. As PFEtCHxS can be considered less hydrophobic than PFOA, the absence of PFEtCHxS in blubber could mean that it is even less hydrophobic than PFHxS since they are in comparable concentrations in liver (De Silva et al. 2011). For the known precursors, only FOSA was quantified at 0.6 ± 0.1 ng/g in ringed seal liver. In the blubber, none of the precursors could be quantified due to high background. However, FOSA and N-Et-FOSA were both detected in some of the samples in concentrations as high as 0.2 ng/g for N-Et-FOSA. Again, concentrations in blubber were lower than in the liver with the exception of N-Et-FOSA, which was detected in the same range of concentrations both in liver and blubber.

Rigét et al. (2013) recently reported on a time-series of PFASs in East Greenland ringed seals in order to see whether a response to the major reduction in perfluoroalkyl production in the early 2000s had occurred. In ringed seal liver samples collected up until 2010, PFOS was still by far the most predominant compound and constituting 88 % of the ΣPFAS concentration. PFOS concentrations also increased up to 2006 with doubling times of approximately 6 years for the ringed seal populations; however, rapid decreases

were reported after 2006. In the present study, PFOS did indeed represent most of the ΣPFAS concentration (57.7 %), but to a lesser extent. More compounds were measured in this study (22 compared to 7), which could explain the difference for the PFOS proportion.

Table 4.1 Arithmetic mean (± standard error) and range of concentrations of 22 PFASs (ng/g wet weight) in the liver and adipose/blubber of polar bears (n=10) and ringed seals (n=19) from East Greenland from 2011-2012.

	Polar Bear						Ringed Seal					
PFAS	Liver			Adipose			Liver			Blubber		
	Mean	± SE	Range	Mean	± SE	Range	Mean	± SE	Range	Mean	± SE	Range
PFBA	36	4.9	14-59	NQ		1.3-21	0.7	0.1	<MLOD-1.6	NQ		<MLOD-0.636
PFPeA	<MLOD			NQ		0.3-4.8	NQ		0.1	NQ		<MLOD-0.139
PFHxA	0.5	0.1	0.2-0.8	NQ		0.1-1.1	NQ	0.0	<MLOD-0.1	0.08	0.04	<MLOD-0.4
PFHpA	0.3	0.1	<MLOD-0.8	NQ		<MLOD-0.2	NQ			<MLOD		
PFOA	17	2.6	12-38	1.2	0.0	<MLOD-0.8	1.0	0.1	<MLOD-2.0	<MLOD		
PFNA	364	28	248-508	3.0	0.5	0.5-6.7	20	1.9	3.2-35	0.07	0.01	<MLOD-0.2
PFDA	165	13	112-238	1.2	0.2	0.2-3.0	13	1.0	3.0-19	0.10	0.01	<MLOD-0.2
PFUdA	249	17	174-338	4.3	0.6	0.8-9.2	26	1.5	12-37	0.37	0.03	0.2-0.5
PFDoA	24	1.5	16-31	1.1	0.1	0.5-1.8	3.5	0.2	1.7-5.9	0.06	0.01	<MLOD-0.2
PFTrDA	61	3.0	37-68	3.7	0.4	1.1-6.1	8.7	0.6	4.3-17	0.22	0.03	<MLOD-0.4
PFTeDA	7.0	0.4	3.8-8.5	0.5	0.1	0.2-1.0	0.8	0.2	<MLOD-3.2	<MLOD		
PFHxDA	0.2	0.0	0.1-0.2	<MLOD		0.0	0.1	0.0	<MLOD-0.2	<MLOD		
PFODA	0.3	0.0	0.2-0.4	<MLOD		0.0	0.3	0.0	0.1-0.5	<MLOD		
ΣPFCA	924	71		15	1.9		74	5.6		0.9	0.1	
PFBS	0.7	0.1	0.3-1.0	<MLOD		0.0	<MLOD	0.0	0.2-0.2	<MLOD		
PFHxS	18	1.6	7.1-29	2.2	0.4	0.7-5.8	1.1	0.2	0.3-4.5	0.05	0.01	<MLOD-0.1
PFEtCHxS	3.1	0.1	2.3-3.9	NQ		0.0	1.3	0.1	0.7-1.8	<MLOD		
PFOS	2583	199	1500-3373	18	2.8	8.3-40	108	4.9	51-143	NQ		<MLOD-0.082
PFDS	5.7	1.1	2.6-11	<MLOD		0.0	0.2	0.1	<MLOD-0.7	<MLOD		
ΣPFSA	2611	202		20	3.2		111	5.3		0.05	0.01	
FBSA	0.4	0.1	0.1-0.9	<MLOD		0.0	<MLOD			<MLOD		
FOSA	10	1.4	2.4-16	NQ		<MLOD-0.1	0.6	0.1	<MLOD-1.3	NQ		<MLOD-0.052
N-Me-FOSA	<MLOD			NQ		<MLOD-0.1	<MLOD			<MLOD		
N-Et-FOSA	1.5	0.4	0.2-3.5	1.2	0.3	<MLOD-3.3	NQ	0.0	<MLOD-0.2	NQ		<MLOD-0.2
ΣPFAS	3546	274		36	5.4		185	11		1.0	0.1	

N/Q indicated that the PFAS could not be quantified (N/Q), if 50 % of the samples were <MLOQ or the blank represented >50 % of their concentration.
Refer to Table 2.1 for the MLOD and MLOQ values for each PFAS.

4.2.3 PFCAs in Ringed Seals and Polar Bears

In polar bear livers, mean ΣPFCA concentration was 924 ± 71 ng/g. The most concentrated PFCA was PFNA at 364 ± 28 ng/g representing 39 % of the PFCA burden. In the fat, mean ΣPFCA concentration reached 15 ± 1.9 ng/g with PFUdA being the most concentrated at 4.3 ± 0.6 ng/g representing 29 % of the PFCA burden. In ringed seal livers, mean ΣPFCA concentration was 74 ± 5.6 ng/g. The most concentrated product was PFUdA at 26 ± 1.5 ng/g representing 35 % of the PFCA burden. In the blubber, mean ΣPFCA was 0.9 ± 0.1 ng/g and the most concentrated PFCA was PFUdA at 0.37 ± 0.03 ng/g representing 41 % of ΣPFCA. Replacement shorter-chained PFCA PFBA and PFHxA were both detected in polar bear liver at 36 ± 4.9 ng/g and 0.5 ± 0.1 ng/g respectively. Only PFBA was detectable in ringed seal liver at 0.7 ± 0.1 ng/g, whereas, only PFHxA was detectable in ringed seal blubber at 0.08 ± 0.04 ng/g. None of them were detected in polar bear fat. This is also, to our knowledge the first report of PFBA and PFHxA in ringed seals and in polar bears.

Differences in the bioaccumulation patterns across tissue and species become apparent when organized in percentage of the total adipose/blubber and liver burden (Figure 4.3). As stated before, PFOS represents the major part of the total burden in polar bear liver and fat and in ringed seal liver at 72.8 %, 49.1 % and 57.7 % of the total burden respectively. First of all, between liver and fat, there seems to be the same shift from the medium-chained PFASs (PFOS and PFNA) towards the longer-chained PFASs (PFUdA, PFTrDA and PFDoA). As explained before, PFOS has the ability to bind to protein, explaining its decrease in low-protein content tissues, such as adipose tissue and blubber (Jones et al., 2003; Luebker et al., 2002). Moreover, long-chained PFCAs (C_{10}-C_{15}) are

correlated to the amount of extractable lipid content in the polar bear brain (Greaves et al., 2013). Therefore, this explains the increase in long-chain PFCAs in the fat tissues as their longer lipophilic chain allows them to penetrate the highly lipophilic environment that is the fatty tissues of the animals.

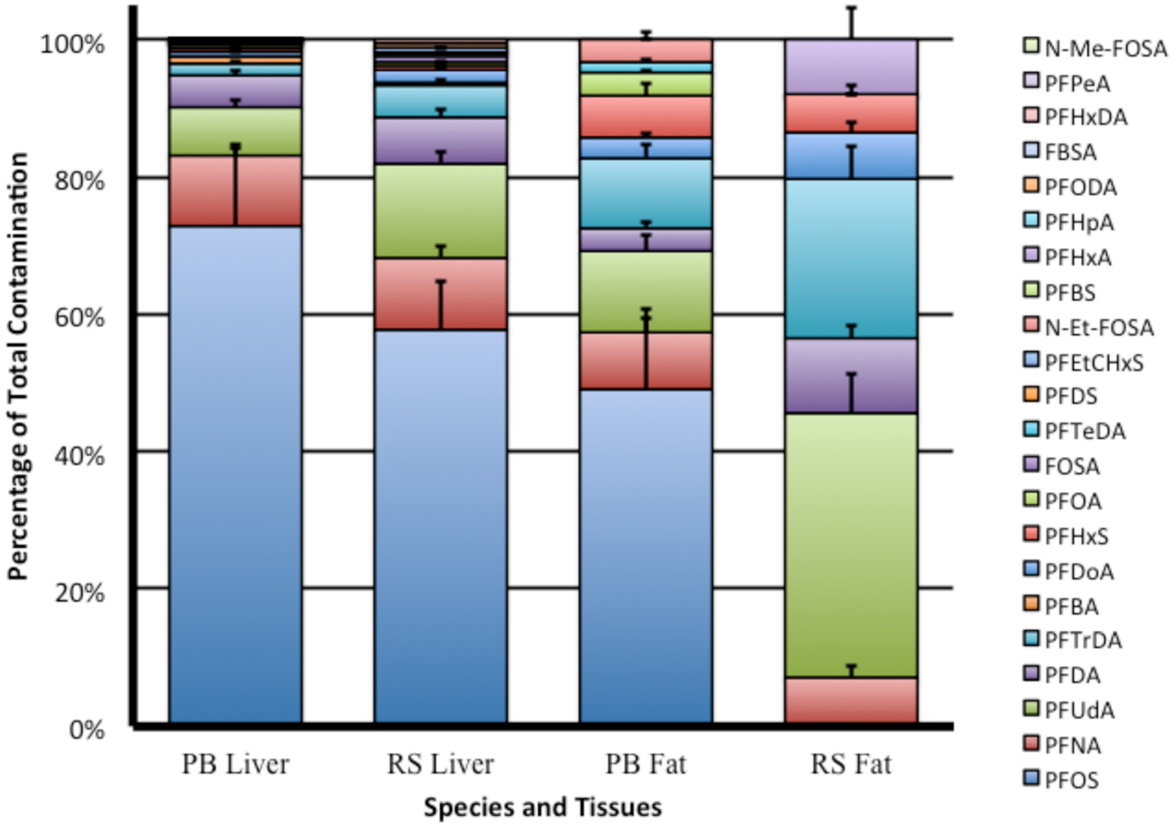

Figure 4.3 The percent composition of the concentration of each perfluoroalkyl substance (PFAS) relative to the sum-PFAS concentration in polar bear liver and fat and ringed seal liver and blubber from East Greenland, Scoresby Sound.

4.2.4 Ringed Seal to Polar Bear – PFOS and Precursors Bioaccumulation and Biomagnification

In the present study, there were major differences between the bioaccumulation of PFOS and precursors in ringed seals and polar bear blubber/fat and liver. In the liver, PFOS concentration represented a smaller portion of the ΣPFAS concentration in ringed seals (57.7 %) than in polar bears (72.8 %). At the same time, the FOSA concentration accounted for a higher portion in ringed seals than in polar bears of 0.83 % and 0.27 % of the ΣPFAS concentration, respectively (Figure 1). This suggests that, in their livers, ringed seals have more precursors and less metabolite, which would point to a slower biotransformation metabolism. Letcher et al. (2014) reported that N-Et-FOSA to FOSA biotransformation happened faster in a single polar bear than in two ringed seals, and that general microsomal enzymatic activity estimated by the ethoxyresorufin-O-deethylase activity was also higher in polar bears. Also, carnivora species including Pinnipedia are known to have a much higher metabolic capacity for transforming FOSA to PFOS than cetacean species (Galatius et al., 2013; Letcher et al., 2014). This suggests that the biotransformation of the PFOS precursors accounts for a large portion of the PFOS concentrations found in polar bears. Indeed, the precursor metabolism by the bear could enrich the tissue concentrations of PFOS as it depletes its precursors. The in vitro metabolism of a major PFOS precursor, N-Et-FOSA, is investigated in Chapter 5 for the present ringed seals and polar bears. Undetectable PFOS in ringed seal blubber, the main source of the diet of the polar bear, indicates that dietary uptake of PFOS is not the major source of PFOS exposure in these polar bears or that polar bears eat ringed seal muscle to a larger extent than previously suggested. Arguably, for polar bears to accumulate

substantial PFOS via the diet, they would have to ingest a considerable amount of seal blubber or muscle. However, since FOSA, N-Et-FOSA and N-Me-FOSA can be detected in the bears, this suggests that the dietary uptake of PFOS precursors and their subsequent biotransformation by the bears could account for more PFOS exposure than via direct PFOS uptake from the seal blubber diet.

Biomagnification factors (BMFs between ringed seal liver and polar bear liver) ranged from 1.2 ± 0.3 for PFODA to 49 ± 21 for PFBA and PFOS had a BMF of 24 ± 6.2 (Figure 4.4). However, applying the traditional BMF calculation to polar bears seems to be inaccurate as polar bears do not eat the liver of the seals they hunt, and they almost exclusively eat their blubber, which also constitutes a much larger percentage of the seals (McKinney et al., 2013). Another explanation, as stated before could be that polar bears ingest more muscle, blood and liver than previously suggested. In this regard, BMFs were calculated using the seal blubber (Ringed seal blubber vs Polar bear liver) burden instead the liver burden. The new BMFs ranged from 6.9 ± 5.9 for PFHxA to 5356 ± 2236 for PFNA. PFOS and PFOA BMFs could not be calculated as their concentration was below the method limit of detection in ringed seal blubber for most of the individuals. As for PFOS precursors, BMFs could not be established because of high background. The new established BMFs were significantly higher than the ones calculated previously indicating that the biomagnification potential of PFASs has been underestimated in polar bears by not taking into account their eating habits. PFASs BMF presented a bell-shaped distribution when organized by carbon chain length, reaching a maximum at 9 (PFNA) (Figure 4.4). The same observation was made in a terrestrial food chain (caribou:wolf) where the maximum BMF was for PFNA at 9 fluorinated carbons (Müller et al., 2011).

Similarly, the same conclusion was found in an Arctic marine model (fish:beluga whale), that the biomagnification of PFASs is chain-length dependent and reaches its maximum at 9 fluorinated carbons (Kelly et al., 2009).

PFASs, due to their ability to accumulate in protein-rich environment, show a different behaviour than other persistent organic pollutants, like the conventional POPs and brominated flame retardants (BFRs). These substances are measured in fatty tissues (i.e. blubber for seals and adipose of bears) and are corrected for lipid percentage (Letcher et al., 2010). The BMFs of POPs are quite easier to determine, as the main sink of the contaminants in the animals is also the main food source of the predator (Letcher et al., 2010 and McKinney et al., 2013). Calculated BMFs for BFRs are quite lower than those for PFASs when considering the liver-fat BMF, reaching 33 for Σ-OH-PBB (Letcher et al., 2010). Other halogenated organic contaminants also present lower BMFs such as polychlorinated biphenyl congeners (PCBs) at 5.7, dichlorodiphenyldichloroethylene and dichlorodiphenyldichloroethane (DDTs) at 0.26, hexachlorocyclohexane (HCH) at 3.9 and Toxafen at 13 (Reviewed by Letcher et al. 2010). In comparison, the BMFs reached 5356 ± 2236 for PFNA.

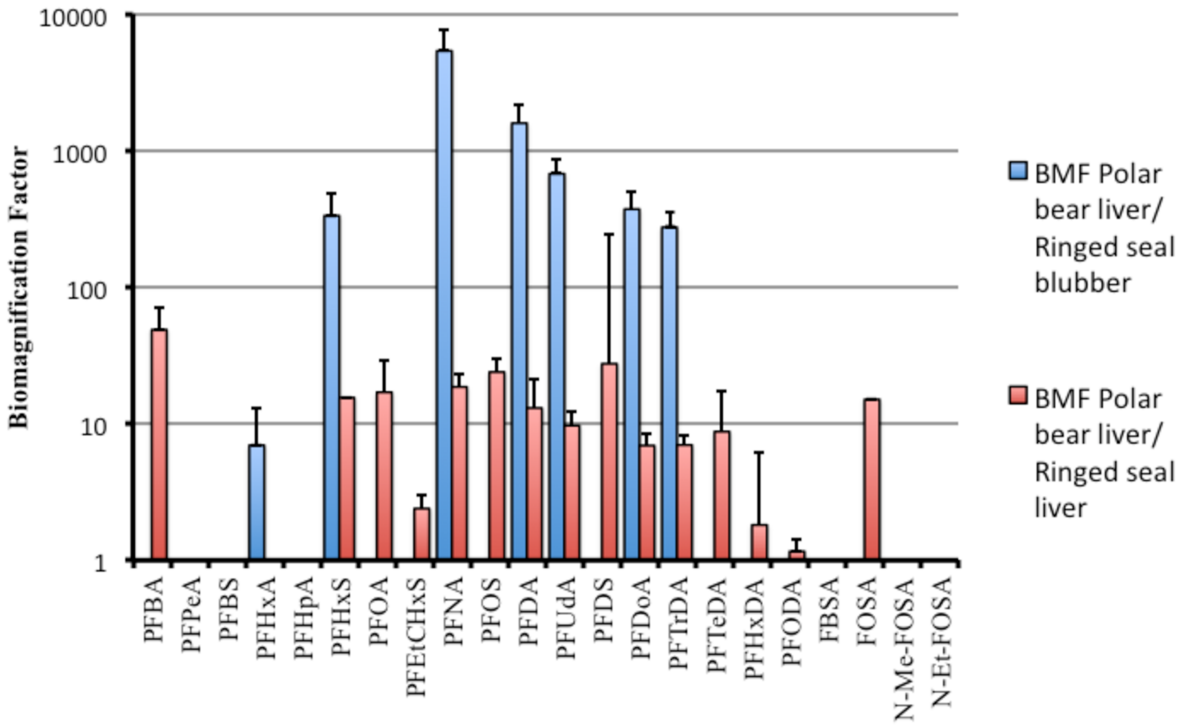

Figure 4.4 Arithmetic means (± standard error) of the biomagnification factors of perfluoroalkyl substance concentrations of polar bear liver to ringed seal liver and polar bear liver to ringed seal blubber.

The concentration ratio of PFOS to its known precursors (i.e. FOSA, N-Et-FOSA and N-Me-FOSA) was largely different between the two studied species. In the polar bears, the ratio of PFOS to FOSA reached 363 ± 82 and ratio of PFOS to its three precursors reached 296 ± 80. For the ringed seal, both of the ratios were inferior, reaching 210 ± 28 for PFOS to FOSA and 189 ± 23 for PFOS to its three precursors (Figure 4.5). As polar bears are known to more rapidly deplete N-Et-FOSA and N-Me-FOSA relative to ringed seals (Letcher et al. 2014), it is strongly suggested that bears indeed consume PFOS precursors and subsequently metabolize them to PFOS more rapidly than by ringed seal.

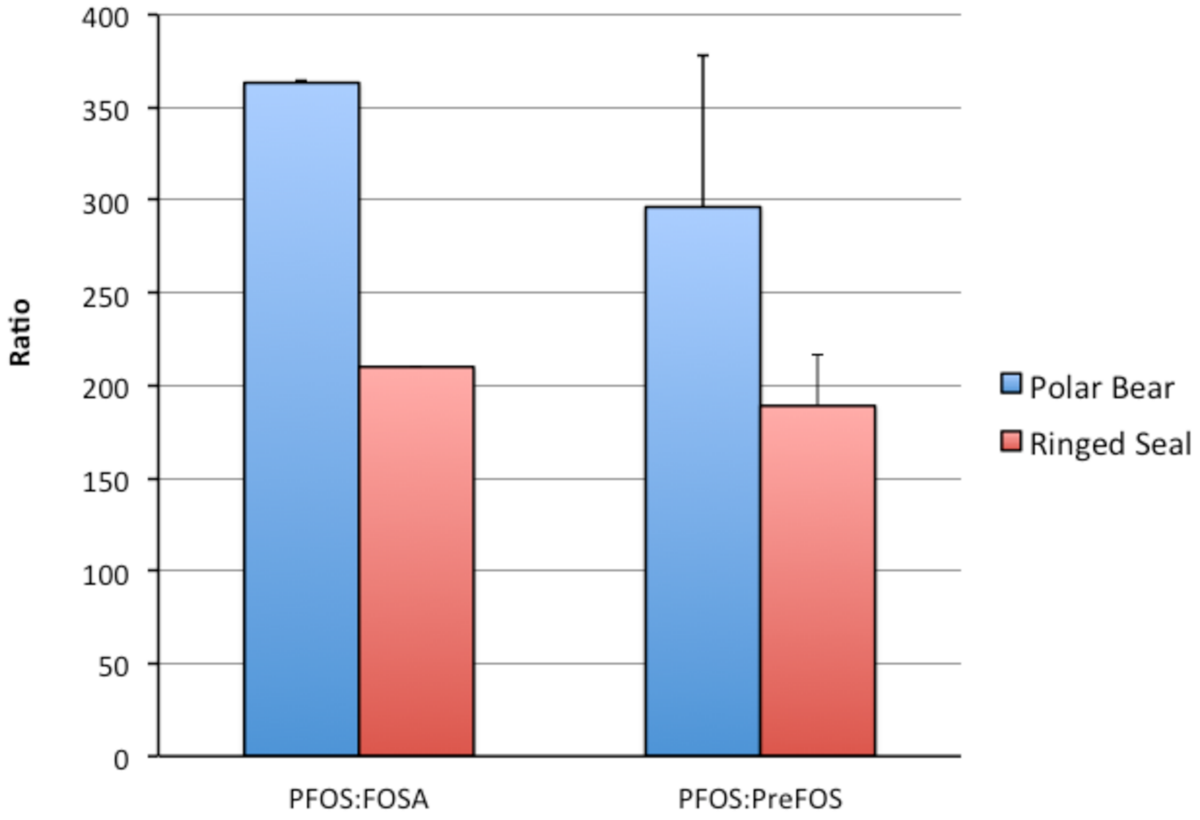

Figure 4.5 Arithmetic means (± standard error) of the perfluorooctane sulfonate (PFOS) to perfluorooctane sulfonamide (FOSA) concentration ratio, and the PFOS to PreFOS concentration ratio in ringed seal liver (n=19) and polar bear liver (n=10).

4.2.5 Conclusions

Replacement chemicals (i.e. C_4-C_6) for the C_8 chemistry were detectable in ringed seals and polar bears. We also, for the first time, to our knowledge, report that PFBA, PFHxA and PFEtCHxS are present in detectable concentration in both polar bears and ringed seals, while FBSA was only detectable in polar bear liver. PFASs mainly accumulate in the liver and their composition shifts to longer carboxylic acids in the fatty

tissues. The differences noticed in bioaccumulation patterns and PFOS to preFOS ratio between ringed seals and polar bears suggests that biotransformation of precursors could account for a large portion of the exposure to PFOS or that the polar bear diet includes more muscle, blood and liver than previously thought. All in all, as biotransformation of preFOS to PFOS seems to have an important impact on the exposure of the polar bears to PFOS it will be further investigated (Chapter 5).

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Chapter 5

Polar Bear and Ringed Seal Hepatic Biotransformation of PFOS Precursors

5.1 Introduction

As presented and discussed in Chapter 4, a source of PFSAs and PFCAs as environmental contaminants can be traced to the degradation of PFAS precursors (Liu and Avedaño, 2013; Wang et al., 2013). As fluorotelomer alcohols (volatile polymers used in surfactant synthesis) undergo different degradation mechanisms (including reaction with hydroxyl radicals, photolysis and biodegradation) they eventually form PFCAs (Wang et al., 2013 ; Butt et al., 2014). PFAS precursors such as perfluoroalkyl sulfonamides (FASAs) can be degraded in the environment to PFSAs. For example, PFOS can be formed from the microbial degradation of N-Et-FOSA and perfluorooctane sulfonamidoethanol (N-Et-FOSE) (Liu and Avedaño, 2013).

For PFSA precursors specifically, biotransformation processes have been shown for multiple biotic species. Earthworms (*Eisenia fetida*) were shown to be able to absorb N-Et-FOSE and transform *in vivo* some of it over a 2-day period into FOSA and with further metabolism of FOSA to PFOS (Zhao et al., 2016). Rainbow trout (*Onchorhynchus mykiss*) was also shown to be able to metabolize N-Et-FOSA and produce both FOSA and PFOS (Tomy et al., 2004b). In that experiment, the trout were able to degrade approximately 50 % of the N-Et-FOSA over 30 hours and produce approximately equal amounts of PFOS and FOSA (Tomy et al., 2004b). In male Sprague-Dawley rat (*Rattus norvegicus*), the biotransformation pathway of N-Et-FOSE is thought

to take place mostly in the liver and involve multiple cytochrome P450 (CYP) enzyme isoforms (Xu et al., 2004). The first reaction of the N-Et-FOSA -> FOSA -> PFOS metabolic pathway (i.e. N-Et-FOSA dealkylation) was observed *in vitro* using extracted rat liver microsomes (Xu et al., 2004) and has been attributed to the isomers CYP2C9 and CYP2C19 using human microsomes (Benskin et al., 2009). However, the complete reaction was only shown to occur *in vitro* using complete Sprague-Dawley rat liver slices (Xu et al., 2004). Recently, using extracted liver microsomes (i.e. organelles containing cytochrome P450 enzymes), a comparison between the three top mammalian predator in the Arctic, polar bear, beluga whale and ringed seal, revealed that *in vitro* polar bears have the highest N-Et-FOSA to FOSA biotransformation activity, followed by ringed seal and then beluga whale (Letcher et al., 2014). Again, FOSA to PFOS was not observed in polar bear or in ringed seal (Letcher et al., 2014). In Chapter 4, it was shown that the PFOS : FOSA ratio of polar bear livers was much higher than ringed seal livers suggesting that the ability to transform N-Et-FOSA to FOSA in these Arctic species could correlate to the ability to transform FOSA to PFOS. Polar bears are known to have greater cytochrome P450 monooxygenase capacity and activity (an estimation of the detoxification ability) than other marine mammals (i.e. ringed seals and beluga whales) (Bandiera et al., 1995). As polar bear's express similar cytochrome P450s to rats, (i.e. CYP1A, 2B, 2C and 3A isozymes) they are expected to behave in a similar way (Bandiera et al., 1995 and 1997). As rats are able to transform FOSA to PFOS, polar bears are expected to do the same (Xu et al., 2004).

Considering the major difference between the PFOS : FOSA ratio between polar bear and ringed seal livers (Chapter 4), the known difference in N-Et-FOSA degradation between polar bears and ringed seals, this study examined the ability of polar bears and ringed seals to biotransform N-Et-FOSA to FOSA using isolated microsomes from enzymatically viable liver tissue.. This data allowed for a better understanding of the role of metabolism in determining tissue concentration of PFOS in this important Arctic predator / prey relationship. That is that PFOS precursor accumulation from ringed seal prey to polar bear, and subsequent biotransformation in polar bear, is an important source of accumulated PFOS in polar bear tissues.

5.2 Results and Discussion

5.2.1 EROD Activity and Pool Preparation

In this study, ethoxyresorufin-O-deethylase (EROD) activity is an assay that specifically determines the activity of the CYP1A isoform, and was presently used as an estimate of the general CYP450 monooxygenase activity in polar bear and ringed seal liver microsomes, and thus as an estimation of biotransformation capacity. EROD activity as a measure of CYP1A activity and has been used extensively over the years to evaluate how enzymatically active liver samples are and including for polar bears (Letcher et al., 1996, 2014; McKinney et al., 2010).

In the present study, the EROD activity was highly variable among individual animals with values ranging from 282 ± 8.4 to 110 ± 4.0 pmol min^{-1} mg^{-1} for polar bears and 146 ± 0.8 to 37 ± 0.2 pmol min^{-1} mg^{-1} for ringed seals (Table 5.1). These EROD activities were much lower than was previously reported for other individual polar bears

(2008, Iceland) and ringed seals (2001, Cumberland Sound, Canada) (i.e. 2167 ± 99 pmol min^{-1} mg^{-1} for polar bear and 298 ± 13 pmol min^{-1} mg^{-1} for ringed seal) (Letcher et al. 2014, 1996; Routti et al., 2008). However, the individual sample set size of these previous studies were extremely small, i.e. n=1 polar bear and n=2 ringed seals. The present bear and seal liver were shown to be enzymatically viable based on EROD activity measurements. To have a sufficient amount of liver microsomes to carry out the present study, microsomes of individual animals (polar bears (PB) and ringed seals (RS)) were pooled into four groups based on their EROD activity (i.e. high activity group and a comparatively lower activity group) and their species (Table 5.1). The PB and RS microsomal pools were adjusted to a protein concentration of approximately 20 mg/mL, i.e. 19 ± 1.7, 16 ±0.2, 21 ± 0.8, 23 ± 0.8 mg/mL for High PB, Low PB, High RS and Low RS, respectively (Table 5.1). The EROD activities obtained for all four groups were 200 ± 36, 164 ± 4.2, 148 ± 6.1 and 79 ± 3.9 pmol min^{-1} mg^{-1} for High PB, Low PB, High RS and Low RS, respectively (Table 5.1).

Table 5.1 Arithmetic mean of East Greenland polar bear (PB) and ringed seal (RS) and Winstar-Han Rat liver microsomal protein content (n=3 replicates) and ethoxyresorufin-O-deethylase (EROD) activity (n=3 replicates)

Pool	Sample ID	Protein Content (mg/mL) ± SD	EROD Activity (pmol min^{-1} mg^{-1})) ± SD	Pooled Protein Content (mg/mL) ± SD[b]	Pooled EROD Activity (pmol min^{-1} mg^{-1})) ± SD
High PB	43101	74 ± 7.2	221 ± 2.4	19 ± 1.7	200 ± 36
	43104	61 ± 8.5	203 ± 28		
	43105	42 ± 4.4	282 ± 8.4		
	43106	44 ± 5.0	241 ± 3.4		
	43171	53 ± 3.4	226 ± 15		
Low PB	43107	128 ± 1.6	120 ± 0.9	16 ± 0.2	164 ± 4.2
	43108	70 ± 1.0	118 ± 1.5		
	43109	35 ± 0.5	110 ± 4.0		
High RS	43188	73 ± 3.3	146 ± 0.8	21 ± 0.8	148 ± 6.1
	43192	69 ± 4.0	135 ± 4.3		
Low RS	43183	68 ± 3.0	37 ± 0.2	23 ± 0.8	79 ± 3.9
	43184	67 ± 1.7	45 ± 0.3		
	43187	88 ± 3.4	60 ± 0.5		
Wistar-Han Rat[a]		25 ± 2.4	38 ± 1.9		

[a] Commercially available liver microsomes from Wistar-Han rats were used as control, n=10 inter day assays (n=3 replicates per assay) [b] Pools were diluted to approximately equal concentrations of 20 mg/mL.

5.2.2 *In vitro* Metabolism of N-Et-FOSA

In the current experiment, Wistar-Han rat liver microsomes were used for control metabolism assays based on their use elsewhere for examination of PFAS precursor metabolism (Letcher et al., 2014). The rat control metabolism assays showed rapid depletion of N-Et-FOSA with > 98 % depletion after 20 minutes of microsomal incubation, and with the concomitant and rapid formation of FOSA metabolite (Figures 5.1 and 5.2). As a negative control, heat inactivated Wistar-Han rat microsomes were used and no significant ($p > 0.01$) N-Et-FOSA depletion or FOSA formation was observed (Figures 5.1 and 5.2). Metabolism assays based on the high PB microsomal pool showed that the depletion of N-Et-FOSA was < 35 % after 40 minutes, as compared to the slower rate of depletion for the Low PB pools as well as both the High RS and Low RS pools. The Low PB, High RS and Low RS depletion assays showed no significant ($p > 0.01$) depletion of N-Et-FOSA after 40 minutes of incubation (Figures 5.1 and 5.2). The greatest FOSA formation was attributed to High PB at 68 ± 2 ng/mL followed by Low PB at 16 ± 2 ng/mL and High and Low RS at 6.2 ± 0.6 ng/mL and 6.9 ± 1.1 ng/mL respectively at 40 minutes (Figure 5.1 and 5.2).

Studies by Letcher et al. (2014) used the same metabolic depletion assay but based on liver microsomes of only n=1 polar bear and n=2 ringed seals from East Greenland. With respect to N-Et-FOSA depletion and concomitant FOSA formation, they obtained similar results where the rate order was rat ≈ polar bear > ringed seal. In contrast to the present study where Low RS and High RS assay showed no significant N-Et-FOSA depletion, Letcher et al. (2014) reported 65 % N-Et-FOSA depletion after a 90 minute incubation period. As the EROD activity is used as an estimate of the liver's CYP

monooxygenase activity, the N-Et-FOSA degradation was expected to take place slower than previously reported (Letcher et al., 2014). Letcher et al. (2014) reported EROD activities of 199 and 397 pmol min^{-1} mg^{-1} for the two seals used. As the highest EROD activity in this present study was 148 pmol min^{-1} mg^{-1} for the seals, an EROD activity > 150 pmol min^{-1} mg^{-1} could be necessary in order to observe a rapid depletion of N-Et-FOSA over a 90 minute incubation. For the polar bears, the same argument could be expressed as the High PB pool (200 ± 36 pmol min^{-1} mg^{-1}) did significantly degrade N-Et-FOSA over the 40-minute incubation period, while the Low PB pool did not (164 ± 4.2 pmol min^{-1} mg^{-1}).

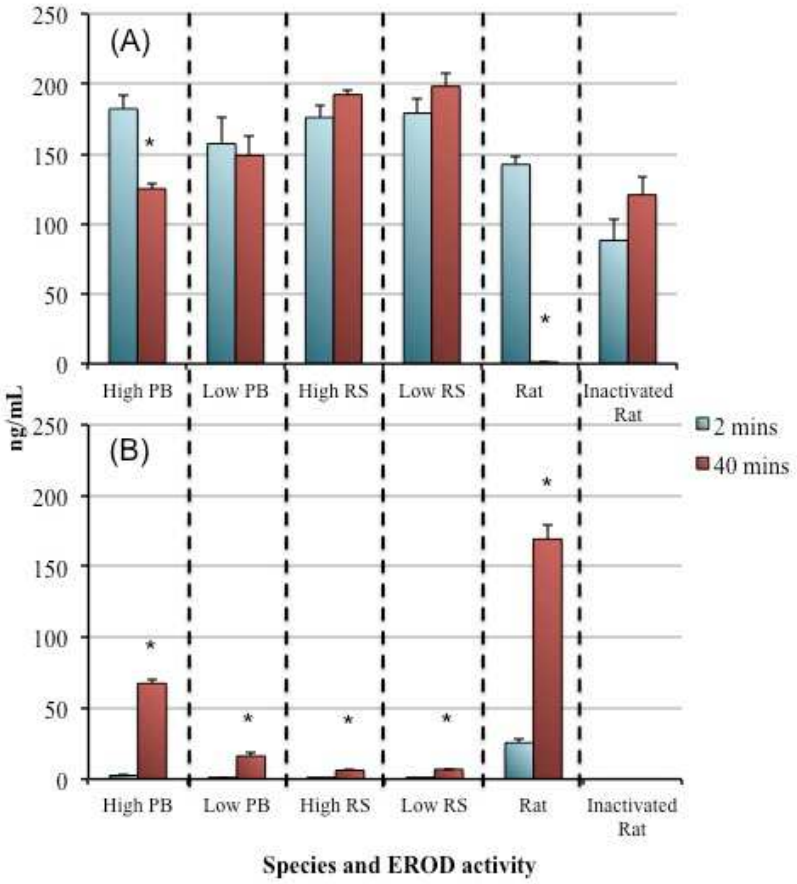

Figure 5.1 The mean concentration (±SE) of (A) N-Et-FOSA depleted and (B) FOSA formed in an *in vitro* using polar bear (PB), ringed seal (RS), Wistar-Han rat (Rat) extracted liver microsomes. Samples are separated by EROD activity (High and Low) and were realized as duplicate inter day (n=3 per assay). Asterisk indicates a significant ($p < 0.01$) difference between the start (2 mins) and the end (40 mins) of the experiment. The starting N-Et-FOSA concentration was 300 nM.

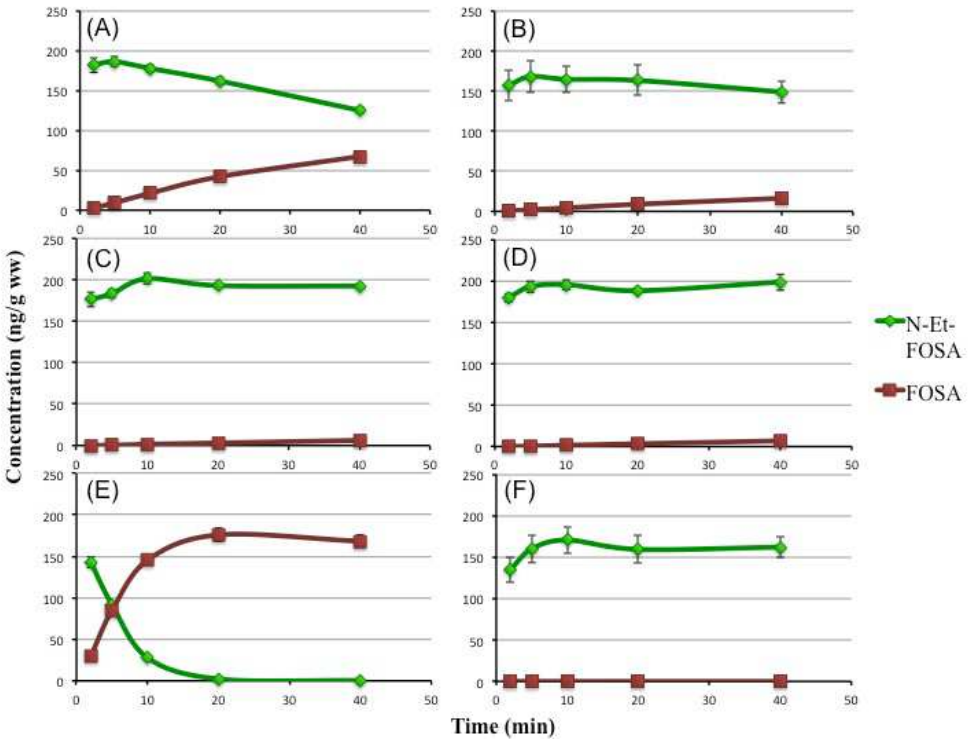

Figure 5.2 Time course representation microsome biotransformation assay of N-Et-FOSA to FOSA by (A) High polar bear (PB), (B) Low PB, (C) High ringed seal (RS), (D) Low RS, (E) Wistar-Han Rat and (F) heat inactivated rat microsomes. Each time point is a duplicate inter day (n=3 per assay). The starting N-Et-FOSA concentration was 300 nM.

PFOS was monitored in all pools, however, its detected concentrations did not exceed the blank concentration and were then considered as not quantifiable (data not shown). As mentioned above, the FOSA to PFOS reaction has been seen previously in rat. However the reaction was only observed in complete liver slices, indicating that the reaction requires enzymes or cofactors, which are not contained in extracted microsomes but are present in liver tissues (Xu et al.,2004). It is known that CYP families can be

induced by the presence of contaminants, increasing their hepatic levels (Bandiera 1997). It is possible that the CYP necessary to initiate the FOSA degradation and the PFOS formation has to be induced by the presence of contaminants, a biological response that cannot be achieved in extracted microsomes as the cell machinery (i.e. the nucleus and the rough and smooth endoplasmic reticulum). Another hypothesis as to why PFOS formation cannot be achieved in detectable amounts, is that the detoxification machinery in the hepatocyte is already induced by the presence of high amounts of PFAS (Table 4.1) and released in the cytoplasm. Thus, through the microsome extraction process, said machinery is actually separated from the microsome fraction, used in this experiment.

Other POPs such as PBDEs are known to also undergo hepatic biotransformation in polar bears (Krieger et al., 2016). The 2,2', 4,4'-tetrabrominated diphenyl ether (BDE-47) and 2,2',4,4', 5-pentabrominated diphenyl ether (BDE-99) both produce eleven individual hydroxylated metabolites when incubated in presence of polar bear microsomes (Krieger et al., 2016). Similarly to the N-Et-FOSA dealkylation, the oxidation of BDE-47 and 99 is mediated by CYP, specifically CYP2B in the case of BDEs (Krieger et al., 2016).

5.2.3 *In vitro* N-Et-FOSA Results and Observed Concentration Patterns

Previously, we observed that there was a major difference in PFOS : FOSA ratio between polar bear and ringed seal (Chapter 4). As enzymatic reactions are mediated in part by substrate availability and polar bears metabolically convert N-Et-FOSA to FOSA more rapidly than ringed seals, our results further suggest that PFOS precursor biotransformation contributes to tissue levels and thus affects the FOSA : N-Et-FOSA

and PFOS : FOSA concentration ratios (Figures 5.1 and 5.2). The high variability in EROD activities across polar bears from different regions (i.e. EG and Iceland) and different years (2008-2011) (Table 1) showed an impact on the biotransformation of N-Et-FOSA (Figures 5.1 and 5.2) (Letcher et al., 2014). In chapter 3, a major difference in PFOS : FOSA concentration ratio was also observed, which could be linked to a biotransformation speed difference between the two subpopulations as suggested by our results (i.e. one population above the biotransformation cut-off the other below).

5.3 Conclusion

N-Et-FOSA depletion was significant ($p > 0.01$) over a 40-minute incubation period for only the High PB polar bear microsomal pool. For all four pools, there was significant formation of FOSA and especially for the High PB pool. However, overall the rates of N-Et-FOSA depletion and FOSA formation were slow. As was discussed, the results suggest that there is likely a minimum level of enzyme activity that must exist to observe a measurable rate of N-Et-FOSA depletion and FOSA formation for both polar bears and ringed seals. The metabolic rates reported here are consistent with the results in Chapter 3 and 4, where precursors are more abundant when PFOS has lesser concentrations. Overall, the results presented in this chapter further suggest the implication of PFOS precursor metabolism in the levels of N-Et-FOSA, FOSA and PFOS.

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Chapter 6

Conclusions and Future Directions

6.1 Conclusions

This study examined the behavior of PFASs in the liver and adipose tissues of three "hotspot" subpopulations of polar bears and in the EG ringed seal. This was the first study in a decade to have compared PFAS levels and patterns in the livers of polar bears from Hudson Bay and East Greenland. To our knowledge, it is also the first study to report the presence of PFEtCHxS in any Arctic wildlife species. Also, this is the first report of PFBA and PFHxA in ringed seals and in polar bears. Finally, this study suggests that there is a minimum level of enzyme activity that is necessary for extracted liver microsomes to be able to observe significant in vitro degradation of e.g. N-Et-FOSA and FOSA precursors.

This study outlined the importance of understanding the fate of PFASs in the Arctic marine food web, notably in polar bears and the dearth of information available. In Chapter 3, we underlined major differences between three subpopulations of polar bears, SHB, WHB and EG. Although the subpopulations are affected by the same global emission of PFASs, they are affected in different ways. Be it because of the differences in atmospheric circulation, providing the EG subpopulation with greater amounts of PFOS and PFOS related products and increasing the proportion of volatile precursors in the SHB and WHB subpopulations or because of diet differences between the three subpopulations, increasing the biomagnification of PFOS in the EG bears, the fate of PFASs differ across the three subpopulations.

In Chapter 4, we examined great differences both in PFAS levels and in percent composition between polar bears and ringed seals, suggesting that biomagnification does not occur in a simple manner. Again, underlining the importance of understanding the fate of PFASs, we suggested that biomagnification should consider the eating habits of polar bears, revealing large BMFs for PFCAs, the total absence of PFOS and the presence of FOSA in non-quantifiable range in ringed seal blubber. This raised multiple interrogations regarding the diet of polar bears and the influence of biotransformation on PFAS exposure.

In Chapter 5, the metabolic pathway of N-Et-FOSA -> FOSA -> PFOS was observed in EG ringed seals and polar bears in order to better understand the effect of such metabolism on the exposure and levels of PFOS and its precursors. Looking at the EROD activities across all samples, it was hypothesized that a minimal CYP monooxygenase activity is necessary in order to degrade N-Et-FOSA significantly, offering a possible explanation for the differences in ratios of PFOS to FOSA and N-Et-FOSA between polar bear subpopulations and between ringed seals and polar bears. Although the FOSA -> PFOS reaction remains to be elucidated, Chapter 5 helped better understand parts of the behavior of PFASs in the Arctic marine food web.

Parts of this study highlighted the importance of precursors and short-chained PFASs in the total PFAS exposure and the necessity to consider these chemicals when establishing PFAS levels. In Chapters 3 and 4, short-chained PFASs, considered as replacement chemicals for PFOS due to their shorter carbon chain, were detected in both ringed seals and polar bears from EG. Arguably, these chemicals (i.e. PFBS, PFEtCHxS, PFBA, PFHxA and FBSA) were detected in lesser concentrations than PFOS or PFOA,

but they have been introduced in recent years, thus having less time to bioaccumulate. This raises the concern regarding replacement chemicals for PFOS, hinting towards their ability to bioaccumulate and biomagnify through the Arctic marine food web.

As mentioned before, Chapter 5 focused on the biotransformation of N-Et-FOSA. We observed the ability of polar bears certain ringed seals to form FOSA from N-Et-FOSA. This metabolic pathway, which is suspected of leading to PFOS, revealed an effect of precursors on apparent levels of PFASs. Indeed, even if polar bears and ringed seals are exposed to N-Et-FOSA, only trace levels are detectable due to their ability to degrade the precursor. This shows that liver levels are not always an accurate depiction of the exposure of the animal if the metabolic capabilities are not considered as well.

6.2 Future Directions

Even with fresh and enzymatically active samples, this study was unable to observe the FOSA degradation into PFOS. As this seems to be a major pathway that greatly affects polar bears, it would be important to better understand this reaction. Since this reaction has previously been seen in complete rat liver slices, this should be the starting point of future studies (Xu et al., 2004). Due to harvesting quotas, it is difficult to obtain large amounts of polar bear liver tissues. Therefore, a preliminary study aiming to recreate the FOSA degradation and PFOS formation in rat, polar bear and ringed seal liver slices should be performed. As the reaction could not be observed in extracted liver microsomes, an experiment should be realized to locate the reaction. Using cell-cultured hepatocytes as a starting point, the experiment would consist in using subcellular fractioning to identify the location of the FOSA degradation starting from the complete

cell, followed by the cytosol, the nucleus and the microsomes. As the CYP responsible the dealkylation of N-Et-FOSA has been revealed, using the optimal reaction, in order to better understand the FOSA degradation, CYP specific inhibitors could be used to identify the exact CYP family responsible for the mediation of the reaction (Benskin et al., 2009; Xu et al., 2004). Finally, it would be possible to verify if PFOS, FOSA and N-Et-FOSA levels have an effect on the responsible CYP amounts in polar bear liver tissues (Bandiera et al., 1997). A correlation would indicate the induction of the specific CYP to counterbalance the presence of the contaminants.

Chapter 3 revealed major differences in PFAS levels and percent composition in the livers of polar bears from EG and HB. Limited information is currently known on the differences between these polar bear subpopulations. Information on the diet differences between these subpopulations is available, hinting that the differences noticed in this study could be associated with the integration of freshwater/terrestrial related food in the EG food web, increasing the trophic levels, therefore increasing the exposure to PFOS (McKinney et al., 2010). However, little is known on the differences of metabolic capabilities between these polar bear subpopulations. It would be interesting to compare the abilities of both polar bears and their prey from these distinct regions to degrade N-Et-FOSA using extracted liver microsomes (Letcher et al., 2014). As a compliment, a study comparing other contaminants like PBDEs or PCBs in EG, SHB and WHB polar bears could be realized to examine if the same type of differences occur for other contaminants (Krieger et al., 2016). To further extend the polar bear subpopulation comparison, a new circumpolar study comparing metabolic capabilities to PFAS levels in EG, SHB, WHB, High Arctic, Alaska and Svalbard could be realized.

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