HALOGENATED ENVIRONMENTAL POLLUTANTS IN GREENLAND SHARK *(SOMNIOSUS MICROCEPHALUS)* FROM THE NORTH-EAST ATLANTIC

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Introduction

The Greenland shark (*Somniosus microcephalus*) is present in Arctic and North Atlantic waters, with adults measuring between 2.4 and 4.3 meters on average. They may reach up to 6.4 meters in size, possibly even larger, and are therefore not only the largest fish found in Arctic waters but also one of the larger shark species in the world [1]. This is a cartilage fish that grows slowly during its whole lifetime and it has been suggested that the growth rate is 0.5-1 cm/year and that their life span might be over 100 years [2]. The Greenland shark is considered an omnivore and have been suggested to feed at the highest trophic level due to stomach contents and concentrations of organochlorine contaminants (OCs) in a similar range as other Arctic predators [3].

A long lived, high trophic fish species like the Greenland shark might easily accumulate OCs and is therefore a sentinel species of great potency for monitoring of such contaminants in the remote Arctic and North Atlantic environments. With a potential limited metabolic capacity, a wide variety of contaminants might also be present in these shark compared to other high trophic species like marine mammals with an efficient metabolism.

Here we summarize what is known so far about concentrations of polychlorinated–*p*-dioxins (PCDDs), polychlorinated dibensofurans (PCDFs), dioxin like polychlorinated biphenyls (DL-PCBs) and also polybrominated diphenyl ethers (PBDEs) in Greenland sharks from the waters around Iceland in the North-East Atlantic [4,5]. Concentrations of six non-dioxin like PCBs (NDL-PCBs), previously not reported, are also included. In addition, we also present new data on concentrations of some perfluorinated compounds (PFCs), which have received a lot of attention the last couple of years. PFCs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been found both in humans and wildlife from different countries all over the world [6-8]. Also in remote areas like the Arctic PFCs have been detected, with higher concentrations higher up in the food chains [9,10]. PFCs analysed in the Greenland sharks were PFOS and a suite of perfluorinated carboxylic acids (PFCAs, C_8-C_{12}). Two metabolic precursors of PFOS in fish[11], N-ethyl- and perfluorooctanesulfonamide (N-EtPFOSA and PFOSA), were also analysed.

Material and Methods

Samples: Since year 2001 Greenland sharks accidentally caught in trawls or entangled in long lines have been collected from the waters around Iceland in the North-East Atlantic. For this project muscle and liver were used from ten of the sharks collected between 2001 and 2003. These ten individuals, all females, were between 3.55 and 4.80 meters in size. Muscle and liver from all ten sharks were analysed for PCDD/Fs and PCBs while the study on PBDEs only included muscle and liver from five sharks. The analysis of PFCs and the two precursors to PFOS was done in muscle and liver tissues from the same ten sharks and also in liver from five other female sharks from the same waters.

Chemicals: Native and ^{13}C-labelled standards used for the analysis of PCDD/Fs, PCBs and PBDEs were purchased from Cambridge Isotopes Laboratories (Andover, USA) or Wellington Laboratories (Guelph, ON, Canada) as described in [4,5]. Labelled $^{13}C_4$- and $^{18}O_2$-PFOS, $^{13}C_4$- and $^{13}C_2$-perfluorooctanoic acid (PFOA), $^{13}C_5$- and $^{13}C_2$-perfluorononanoic acid (PFNA), $^{13}C_2$-perfluorodecanoic acid (PFDA), d_3-N-methylPFOSA and d_5-N-EtPFOSA from Wellington Laboratories (Guelph, ON, Canada). Native PFOS, PFOA, PFNA, PFDA, perfluoroundecanoic acid (PFUA), perfluorododecanoic acid (PFDoDA), PFOSA, and N-EtPFOSA also from Wellington. Optima grade water and methanol were obtained from Caledon Laboratories Ltd. (Georgetown, ON, Canada).

Extraction and Analysis: The extraction and analysis of PCDD/Fs, PCBs and PBDEs have been described elsewhere [4,5,12]. PFCs, N-EtPFOSA and PFOSA were extracted according to Tomy et.al [13]. In short, approximately 0.1g of homogenized Greenland shark muscle and liver was placed in a polypropylene (PP) tube and spiked with a recovery internal standard solution ($^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA and d_3-NMePFOSA). Methanol (2 ml) was added to the samples, vortexed for 1 minute and centrifuged for 5 minutes at 3500 rpm. The methanol extract was transferred to a second test tube and the procedure was repeated twice. The extracts were combined and the solvent was reduced to 0.5 ml, transferred to a micro vial and centrifuged at 13 500 rpm for 10 minutes. The supernatant was carefully transferred to a HPLC vial and spiked with an instrument performance matrix internal standard (IPMIS) solution ($^{18}O_2$-PFOS, $^{13}C_2$-PFOA, $^{13}C_2$-PFNA, and d_5-NEt-PFOSA). Analyses were performed with LC/MS/MS, Agilent 1100 series with a HPLC system from Agilent Technologies (Palo Alto, CA) coupled to a Sciex API 2000 triple quadrupole mass spectrometer (MDS Sciex, ON, Canada) in the negative ion electro spray (ES) mode using multiple reaction monitoring (MRM).

Results and discussion

Concentrations of PCDD/Fs, DL-PCBs, NDL-PCBs and PBDEs present in muscle and liver of the Greenland sharks analysed are summarised in Table 1. As previously stated some of these concentrations are in the same range or higher than reported from fish and marine mammals from the Arctic environment [4,5]. For instance, the mean concentration of PCDDs in Greenland shark liver is 71 pg/g fat, which is slightly lower compared to what has been reported in polar bear liver from East Greenland but for PCDFs the concentrations are higher in the Greenland shark (460 pg/g fat) [14]. For PBDEs the major congener, among the 20 congeners detected, was BDE-47 in both muscle and liver with mean concentrations of 45 and 50 ng/g fat, respectively. The mean concentration of BDE-47 in Greenland shark is higher or in the same range as in polar bears from Svalbard (41 ng/g fat) and East Greenland (51 ng/g fat) [15].

Among the six NDL-PCBs analysed, CB-153 followed by CB-138 were the major congeners. The mean concentration of CB-153 was 1200 and 1300 ng/g fat for muscle and liver, respectively. A previous study on Greenland sharks from the Canadian Arctic reported a mean concentration of around 500 ng/g fat, about two times lower than in this study [3]. The mean summed concentration of the six NDL-PCBs including CB-105, CB-118 and CB-156 in muscle and liver was 4000 and 4300 ng/g fat, respectively. These concentrations are about four times higher than in ringed seal and 10 to 20 times higher than in fish from East Greenland, and again in the same range as in polar bears from the Arctic [16,17].

Table 1: Mean concentrations and ranges (min-max) of PCDD/Fs (pg/g fat), PCBs (ng/g fat) and PBDEs (ng/g fat) present in Greenland sharks from the North-East Atlantic[4,5,18].

		Muscle		**Liver**	
	n	**mean**	**min-max**	**mean**	**min-max**
PCDD/Fs	10	13	1.6-30	530	47-1600
DL-PCBs:					
non-*ortho* PCBs	10	0.36	0.04-0.99	7.8	0.97-23
mono-*ortho* PCBs	10	670	190-1600	760	170-1600
NDL-PCBs [a]	10	3400	790-8200	3600	770-8900
PBDEs [b]	5	75	7.7-200	94	11-280

Congeners included in sum concentrations for [a] NDL-PCBs: CB-28, -52, -101, -138, -153, -180 and [b] for PBDEs: BDE-17, -28, -47, -49, -66, -75, -99, -100, -119, -153, -154, -155.

Even if previous mentioned contaminants in these sharks are present at elevated levels the preliminary data for PFCs presented here shows that their burden of PFCs are low. PFOS which is generally the major PFC detected in biota, was below the method detection limit (MDL), 1.9 ng/g in all samples analysed. This was also the case for the PFCAs with high MDLs for PFOA (53 ng/g) and PFNA (55 ng/g) but lower for PFDA, PFUA and PFDoDA (3.0-9.6 ng/g w.w.). The only fluorinated compound actually quantified among the eight analysed in these samples was PFOSA that was present above the MDL (0.4 ng/g) in eight of the liver samples. The concentrations ranged between 0.40 and 2.6 ng/g w.w. with a mean concentration of 1.2 ng/g w.w.. PFOSA and

related compounds have shown to be metabolic precursors to PFOS in fish [11]. The fact that PFOSA and not PFOS were present in these sharks might be an indication of a limited metabolic capacity for the Greenland shark as earlier suggested [3]. The PFOSA concentration was similar to what has been reported in liver of ringed seal from East Greenland [19]. However, in these seals PFOS was present at concentration ranging up to 130 ng/g w.w.. This is most likely due to different metabolic capacity by the ringed seal and the Greenland shark.

Several studies have shown that higher concentrations of PFCs are present at higher trophic levels of the food chain [9,10]. PFOS has for instance been shown to be one of the most prominent contaminants in polar bears [10,20]. Since the Greenland shark also is a high trophic species in the marine ecosystem their low burden of PFCs is possibly somewhat unexpected, especially in the liver since PFOS and other PFCs are said to preferentially bind to proteins in liver rather than to lipids [21]. Also, due to the fact that PFCs, such as PFOS have been detected in various Arctic fish species from the Canadian Arctic and also from East Greenland and Icelandic waters, it is to expect them in these sharks as well [9,10,22,23]. As seen in Figure 1 PFOSA, the only fluorinated compound detected in these Greenland sharks, is present at almost three orders of magnitude lower than for instance CB-153 and also lower than the DL CB-118 and BDE-47.

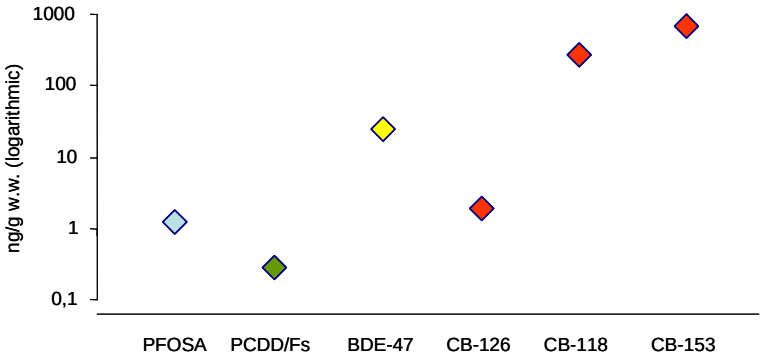

Figure 1: Mean logarithmic concentrations (ng/g w.w.) of PFOSA, PCDD/Fs, BDE-47, CB-153 and the DL CB-126 and CB-118 in Greenland shark liver from the North-East Atlantic.

In conclusion, even if elevated levels of more traditional contaminants are present in these Greenland sharks, their burden of PFCs, especially PFOS, are low not only compared to marine mammals but also to other Arctic fish species.

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