BROMINATED ORGANIC COMPOUNDS IN A HIGH TROPHIC ARCTIC FISH SPECIES, GREENLAND SHARK *(SOMNIOSUS MICROCEPHALUS)*

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Introduction

Polybrominated diphenyl ethers (PBDEs) are chemicals that have been extensively used as flame retardants. PBDEs are highly persistent and bioaccumulative compounds which have been detected in humans and wildlife worldwide [1-3]. Today there is an emerging understanding of other, naturally produced brominated compounds being present in wildlife, particularly from marine environments. For instance, both methoxylated- and hydroxylated-PBDEs (MeO- and OH-PBDEs) are known to be naturally produced in the marine environment [4-7]. Two MeO-PBDEs (6-MeO-BDE47 and 2'-MeO-BDE68), isolated from True's beaked whale have been confirmed as natural products by measurements of the ^{14}C content [8]. OH-PBDEs have also shown to occur as metabolites of the truly anthropogenic PBDEs [9,10].

The Greenland shark (*Somniosus microcephalus*) is a long-lived Arctic fish species that also inhabits the more temperate North Atlantic [11-13]. Adults usually measure 3-5 meters in size, but may reach up to 7 meters [11]. Based on stomach contents the Greenland shark feed on a wide variety of marine species including fish, seals and other marine mammals [14,15]. Long lived high trophic species like the Greenland shark may accumulate high concentrations of contaminants and previous studies on this species have revealed concentrations of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) in the same range as other Arctic top predators, i.e. polar bears [14,16].

In the present study we report concentrations of PBDEs and MeO-PBDEs in ten Greenland sharks collected from the waters around Iceland in the North-East Atlantic. Further, concentrations of two dimethoxylated brominated organic compounds, diMeo-BDE68 and diMeo-BB80, are reported. Both diMeo-BDE68 and diMeo-BB80, previously reported in marine mammals from the Pacific Ocean, are also believed to be of natural origin [17].

Material and Methods

Samples: Since 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. In the present study muscle and liver from ten of these sharks were used. The sharks were collected between 2001 and 2003, all females measuring between 3.55 and 4.8 meters in size.

Chemicals: BDE71 and BDE139, diMeO-BB80, diMeO-BDE68 together with the MeO-PBDEs analysed in the present study, were synthesized in our laboratory [17-19]. BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE138. BDE153, BDE154, BDE183 and BDE190 were purchased from LGC Standards (Wesel, Germany). All solvents used during extraction and cleanup procedures were of highest commercially available purity.

Instruments: Gas chromatography/mass spectrometry (GC/MS) analysis was performed on a Finnigan SSQ 710 coupled with a Varian 3400 gas chromatograph with a split/slitless injector and a CTC A200S autosampler. The capillary column used was a DB5-HT (15 m x 0.25 mm i.d. x 0.1 µm film thickness; J&W Scientific, Folsom, CA, USA) with helium as carrier gas (head pressure 8 psi). The injector was operated in splitless mode (1 min) at a temperature of 280°C. The instrument was operated in the electron capture negative ionisation (ECNI) mode using selected ion monitoring (SIM) with methane as reagent gas, scanning for negative bromide ions, m/z 79 and 81. The ion source and transfer line temperatures were set at 180 and 290°C, respectively.

Extraction and cleanup procedure: Homogenised muscle and liver tissue, in each case 10 g, was extracted according to Jensen et.al. [20] with the exception that c-hexane was used instead of n-hexane. BDE71 (2.5 ng) was added to 1 g of fat from each sample as surrogate standard. Lipids were removed with concentrated sulfuric acid (~1 ml/0.1 g of fat). Two different columns were applied for cleanup. The first one was packed with 0.1 g activated silica (SiO_2) and 0.9 g activated SiO_2 impregnated with concentrated sulfuric acid (2:1, w/w) on top, analytes were eluted with 20 ml of DCM. The second column was packed with 0.7 g activated SiO_2, most of the PCBs were first removed with 15 ml of c-hexane and brominated compounds were then eluted with 15 ml of DCM as mobile phase. Prior to GC/MS analysis BDE139 (60 ng) was added as volumetric standard.

Results and Discussion

PBDEs, MeO-PBDEs and the two dimethoxylated brominated organic compounds, diMeO-BB80 and diMeO-BDE68, were detected and quantified in all samples. Patterns and concentrations were similar between tissues but varied quite a lot between individuals, possibly due to different feeding habits. No correlation between size, a measure of age of this species, and concentrations of the brominated organic compounds was found. This observation is in agreement with previous studies on Greenland sharks where high concentrations of organochlorine contaminants were suggested to reflect accumulation from feeding on high-trophic species like seals rather than being related to size and/or age of the individuals studied [14,16].

Twelve PBDE congeners were analysed (BDE17, BDE28, BDE47, BDE66, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183 and BDE190) and median ∑PBDE concentrations were 49 ng/g (range: 14-210) and 41 ng/g (range: 9.2-160) fat in liver and muscle, respectively. BDE47 was the major PBDE congener in both muscle and liver representing 40-60% of the total PBDE burden followed by BDE99 and BDE100. This pattern is in accordance with other findings in fish and marine mammals from Arctic/North Atlantic waters [21-24]. The median total concentration of PBDEs in this study is higher than found in other fish species and also somewhat higher than in ringed seals from the same regions [22,24]. Concentrations of BDE47 (median 24 ng/g fat in both tissues, figure 1) is in the same range as reported in polar bears and beluga whales [25,26].

Among the naturally produced compounds analysed, four MeO-PBDEs (2'-MeO-BDE68, 6-MeO-BDE47, 6-MeO-BDE85 and 6-MeO-BDE137), diMeO-BB80 and diMeO-BDE68 were quantified in all samples. 6-MeO-BDE47 with median concentrations of 74 and 79 ng/g fat in muscle and liver, respectively, was in fact the major brominated organic compound found followed by BDE47 and 2'-MeO-BDE68 (Figure 1).

Up to three different tetrabrominated methoxylated diphenyl ethers have been quantified in fish and marine mammals from Arctic/North Atlantic waters [22,26,27]. No MeO-PBDE congener information was reported in previously mentioned studies, making accurate comparisons impossible. However, in this study we report concentrations of 6-MeO-BDE47 that are higher compared to the fish and similar or higher compared to the marine mammals. 6-MeO-BDE47 has also been reported in adipose tissue of polar bears, but at lower concentrations [28].

Higher concentrations of contaminants in this study compared to other Arctic fish are most likely due to the longer lifespan and higher trophic level of the Greenland shark than other fish species. Other factors, like for instance the ability to degrade and excrete contaminants, might also influence the levels of the contaminants compared to marine mammals.

Concentrations of diMeO-BB80 and diMeO-BDE68 were similar in muscle compared to liver, as were the other brominated compounds analysed. Median concentrations of diMeO-BB80 were 6.7 and 7.0 ng/g fat in muscle and liver, respectively and for diMeO-BDE68 6.5 and 4.7 ng/g fat, respectively. Previously diMeO-BB80 and diMeO-BDE168 have been reported in marine mammals from Japanese coastal waters [17,29]. Compared to these two studies concentrations in Greenland shark is generally lower for diMeO-BB80 and more similar for diMeO-BDE68. When comparing concentrations of 6-MeO-BDE47 and 2'-MeO-BDE68, similar concentrations are present in fresh blubber of striped dolphin and minke whales compared to the Greenland shark [17]. High concentrations of 6-MeO-BDE47 have also been reported in various marine mammals from Australia (46-1900

ng/g fat) and in killer whales from Japanese waters (420-880 ng/g fat) [29,30], exceeding those presented in this study. Although concentrations and patterns of the two dimethoxylated compounds together with MeO-PBDEs in this high trophic Arctic species differs from species found in more temperate waters, the results of this study show that the sources for these compounds are globally distributed.

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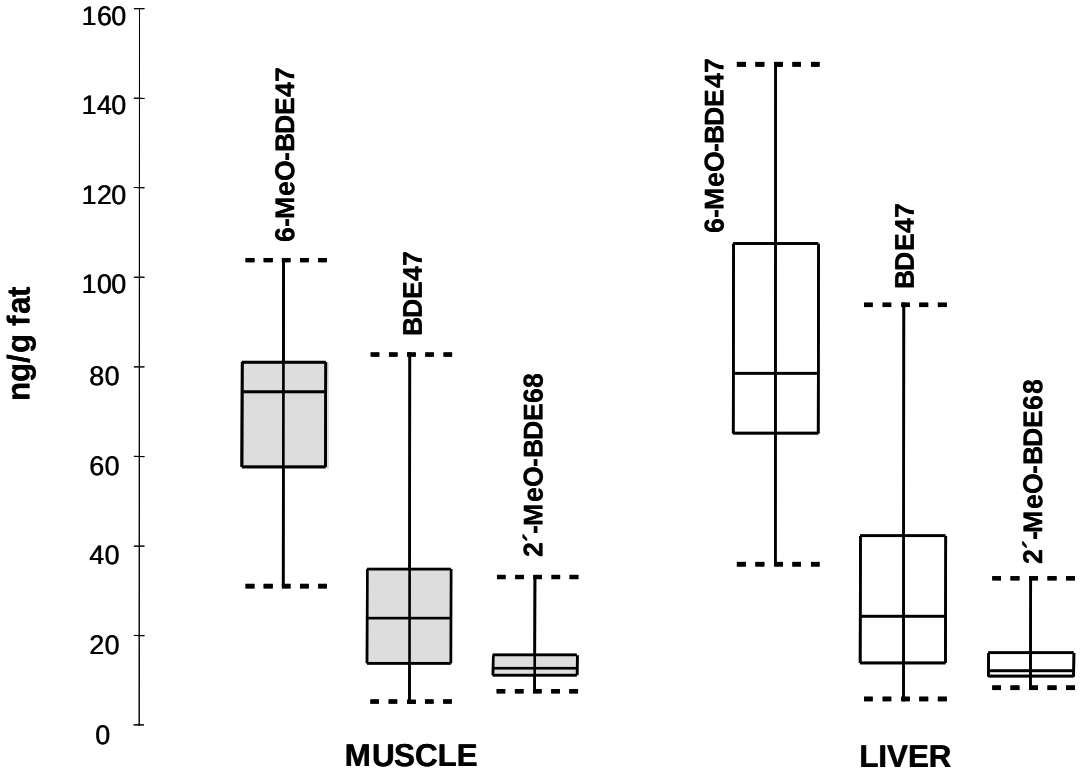

Figure 1: Box-and-whisker-plots of the three major brominated organic compounds in muscle (grey) and liver (white) of the ten Greenland sharks analysed. Median, 25th and 75th percentile together with min and max concentrations are shown.