6-HYDROXYLATED- 2,2′,4,4′-TETRABROMODIPHENYL ETHER IN HERRING (*Clupea harengus)* PLASMA FROM THE BALTIC SEA

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Introduction
Organic halogenated contaminants (OHCs) in the Baltic Sea have been monitored since the 1970s. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been a part of the National Swedish Environmental Monitoring Program for a long time[1]. Lately more research focus has been directed towards OHC metabolites and natural products such as hydroxylated polychlorinated biphenyls (OH-PCBs)[2,3,4], hydroxylated polybrominated diphenyl ethers (OH-PBDEs)[2,5-7] and methoxylated polybrominated diphenyl ethers (MeO-PBDEs)[5-8]. Phenolic compounds are of emerging interest since several adverse effects have been linked to them[9-11]. For example, OH-PBDE (6-OH-BDE47) has recently been shown to act as an inhibitor of oxidative phosphorylation (OXPHOS) in Zebrafish[10].

The Baltic herring is a pelagic species with high impact on the ecosystem; it is one of the dominant pelagic fish species and is the prey of several wildlife species feeding on Baltic Sea biota. The herring has shown to suffer from adverse effects such as a decrease in biomass[12] and fat content[1]. We hypothesize that herring blood can indicate a general exposure for phenolic compounds in pelagic species. Hence this study aims to quantify 6-OH-BDE47 in Baltic herring **(***Clupea harengus)* plasma and compare to other, well known OHCs, such as CB-153, BDE-47 but also to the naturally produced 6-MeO-BDE47[13-15].

Materials and Methods
Samples: Blood samples from 20 herrings *(Clupea harengus)* were collected during May 2007 from Askö marine research station (N58°49', E17°37') in the Baltic Sea (location marked in Figure 1). Samples were stored frozen (-20°C) until start of chemical analysis.

Chemicals: 2-propanol, *n*-hexane and dicloromethane (DCM) were of pesticide grade (Merck, Darmstadt, Germany), methyl *tert*-butyl ether of HPLC grade, distilled before use (Rathburn Chemicals, Walkerburn, Scotland,UK), water of HPLC grade (Scharlau Chemie, Barcelona, Spain), potassium hydroxide (Merck, Darmstadt, Germany), sulfuric acid (BDH, WWR International Ltd, Pool, England), phosphoric acid (Merck, Darmstadt, Germany), hydrochloric acid (Merck, Darmstadt, Germany), sodium chloride (Merck, Darmstadt, Germany) all of *pro analysis* quality and silica gel (0.063-0.2 mm, Merck, Darmstadt, Germany) were purchased from indicated sources. Diazomethane was prepared in house from *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide (Diazald)[16] obtained from Sigma-Aldrich (Steinheim, Germany). The OH- and MeO-PBDE reference compounds were synthesised in house[17,18] and the PBDE reference compounds were purchased from LGC Promochem (Wesel, Germany).

Analytical method: All samples were analysed individually, about 0.5 g (fresh weight) were extracted according to Hovander *et al*. (2000) with 2-propanol, methyl-*tert*-butyl ether and *n*-hexane[19]. Lipids were determined gravimetrically. Separation of neutral and phenolic compounds was carried out with potassium hydroxide partitioning (0.5 M in 50% ethanol). The halogenated phenolic compounds (HPCs) were derivatized with diazomethane. Both fractions were cleaned-up with concentrated sulfuric acid and sulfuric acid/silica gel (33% w/w) columns (1 g) applying DCM (28 ml) as the mobile phase. Analysis of brominated compounds was carried out by gas chromatography-mass spectrometry (GC-MS) while analysis of PCBs was performed with GC-ECD (electron capture detection).

Instrumental analysis: GC-MS analysis was performed on a Finnigan MAT SSQ710 (ThermoFinnigan, Bremen, Germany), coupled to a Varian 3400 GC (Varian Inc., CA, USA) equipped with a spilt/splitless injector (held at 260°C) running in splitless mode for 0.7 min. Two capillary columns were used; a Thermo TR5 SQC capillary

column (30 m x 0.25 mm i.d., 0.25 µm particle size; Thermo Electron Corporation, Bellefonte, DA, USA) for the neutral fraction and a HT8 SGE capillary column (25 m x 0.22 mm i.d., 0.25 µm particle size; SGE SGE, Zulte, Belgium) for the fraction containing the methylated HPCs using helium as carrier gas (head pressure 12 psi). The GC-oven temperature program was: 80°C (1 min), 15°C min^{-1} to 310°C (12 min). The MS instrument operated in electron capture negative ionization (ECNI) mode using selected ion monitoring (SIM), with methane as regent gas, scanning for m/z 79 and 81. The ion source and the transfer line temperatures were set at 180°C and 280°C respectively.

GC analysis was performed on a Varian CP-3800 GC (Varian Inc., CA, USA) equipped with a split/splitless injector (held at 260°C), running in splitless mode for 2 min, and a EC detector (held at 360°C). Separation was achieved on a CP-Sil8 capillary column (25 m x 0.15 mm i.d., 0.12 µm particle size, Varian Inc., CA, USA) with hydrogen as the carrier gas (head pressure 50 psi). The GC oven temperature program was: 80°C (2 min), 10°C min^{-1} to 300°C (5 min).

Results and discussion

The herring plasma concentration of CB-153 is about 10 times higher than the most abundant PBDE congener, i.e. BDE-47 (Figure 2). The 6-OH-BDE47 concentration is higher, i.e. four times higher compared to CB-153 (Figure 2) and in the same range as ΣDDTs (Figure 3). It is also significantly higher than 6-MeO-BDE47 in the plasma (Figure 2). Comparison to other studies is difficult due to the lack of recent data of OHCs in blood from the Baltic region, however, muscle concentrations in Baltic herring are measured yearly and are similar to the levels on fresh weight (f.w.) basis in the herring blood for both CB-153 and BDE-47[1]. The concentrations of 6-MeO-BDE47 (Figure 2) in blood are however somewhat lower than what is found in muscle tissue reported from environmental monitoring of Baltic herring muscle tissue[20]. Also, it is notable that the levels 6-MeO-BDE47 and BDE-47 in the blood are similar. In herring muscle tissue the levels of 6-MeO-BDE47 is about four times higher than BDE-47[20]. Kierkegaard *et al*. (2004) have shown that 6-MeO-BDE47 in Baltic herring muscle tissue varies along the Swedish coast line [21].

The high concentrations of 6-OH-BDE47 presented herein (Figure 2) is, to our knowledge, not comparable to any previous studies on wildlife blood. OH-PBDEs have been found and quantified in concentrations ranging from 1.5 pg g^{-1} f.w. for 6-OH-BDE47 in white bass and bowfin blood from the Detroit River[22] to concentrations of 3.5 ng g^{-1} f.w. for ΣOH-PBDEs in glaucous gull blood from Svalbard[23]. The level of 6-OH-BDE47 in the Baltic herring plasma is three times as high as ΣOH-PBDEs in glaucous gull. Also, Asplund *et al.* (1999) have estimated the concentration of 6-OH-BDE47 in salmon blood to 30 ng g^{-1} lipid weight (l.w.) (2 % lipids)[7], to compare with the mean concentration in herring blood herein, 1200 ng g^{-1} l.w. (mean 0.95 % lipids).

Several OH- and MeO-PBDEs have been isolated as natural products[13-15] OH-PBDEs have also been reported as metabolites of PBDEs[24-26]. Natural production of 6-OH-BDE47 has been reported[27-29], however, 6-OH-BDE47 can also be formed through metabolism of BDE-47[24,30]. The much higher concentrations of 6-OH-BDE47 than BDE47 presented here contradicts metabolism and gives a clear indication of that 6-OH-BDE47 herein is of natural origin.

The Baltic herring shows a range of adverse effects, like population decrease, both in number, biomass[12] and lipid content[1] as well as egg mortality[31,32]. As suggested by others, herring egg mortality is closely linked to filamentous red and brown algae toxicity[31,32] and as algae contain a range of phenolic compound[5,33], the HPCs may play a role in the manifestation of adverse effects. 6-OH-BDE47 has been shown to act as a strong inhibitor of the oxidative phosphorylation in Zebrafish[10]. The lowest observed adverse effect level (LOEL) given in the exposure study by van Boxtel *et al*. (2008) was of a water concentration of 12.5 nM corresponding to a calculated internal no observed adverse effect level (NOAEL) of 150 nM (lipid basis)[10]. The concentration herein is only a factor 40 lower (4 nmol g^{-1} l.w.), but a comparison is difficult since little is known about the correlation between the external and the internal dose. However, considering additive effects of uncouplers of mitochondrial oxidative phosphorylation the margin of safety may be small. Also, the Baltic herring has a central role in the Baltic food chain and thus the predator exposure may be substantial.

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Figure 1: Askö sampling site for Baltic herring plasma.

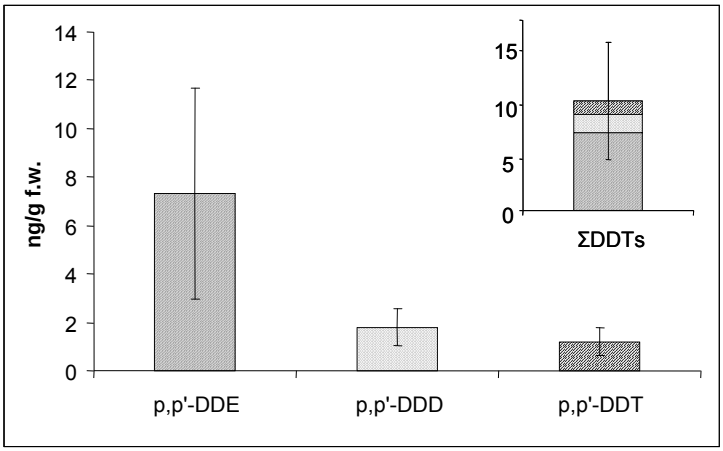

Figure 3: sDDTs in Baltic herring plasma, mean concentrations in ng g^{-1} f.w. (± standard error).

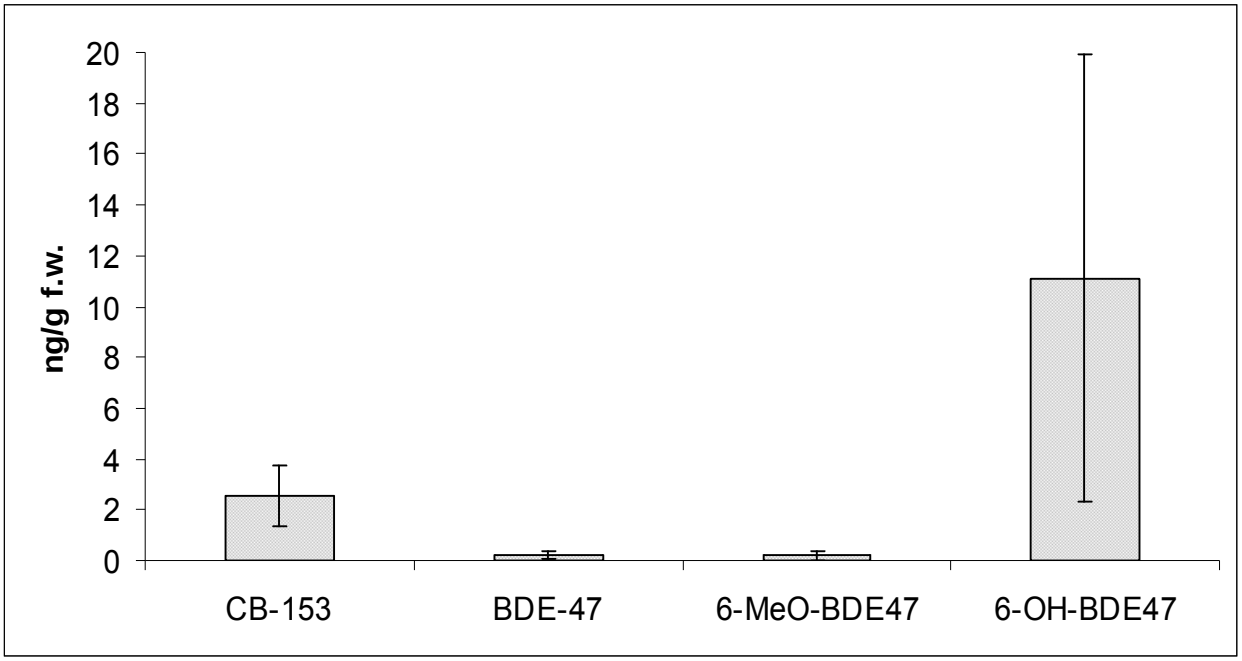

Figure 2: CB-153, BDE-47, 6-MeO-BDE47 and 6-OH-BDE47 in Baltic herring plasma, mean concentrations in ng g^{-1} f.w. (± standard error). The mean lipid content in the herring plasma was 0.95 % ± 0.42 % (standard error).