HYDROXYLATED- AND METHOXYLATED- POLYBROMINATED DIPHENYL ETHERS AND POLYBROMINATED DIBENZO-*P*-DIOXINS IN RED ALGA FROM THE BALTIC SEA

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Introduction

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been identified in blood plasma from Baltic salmon as well as in blue mussels from the Baltic Sea [1-3]. The origins of OH-PBDEs are likely both anthropogenic and biogenic. Brominated flame retardants (BFRs), e.g. polybrominated diphenyl ethers (PBDEs), are known to be metabolized to OH-PBDEs in rats, mice and fish [4-8]. However, OH-PBDEs have also been reported to be naturally produced in the marine environment e.g. in sponges [9,10] and were recently identified in red algae from the Baltic Sea [3].

The methylated analogs, methoxylated polybrominated diphenyl ethers (MeO-PBDEs) have also been found in the alga *Ceramium tenuicorne*, in fish, seals, and birds from the Baltic Sea [1-3,11-13]. Two of the MeO-PBDEs identified in the red alga *Ceramium tenuicorne* (MeO-BDE68 and MeO-BDE47), were recently isolated from whale and have been confirmed to be of natural origin by the measurement of the ^{14}C content [14].

In the present study, OH-PBDEs and MeO-PBDEs in the red algae from the Baltic Sea were quantified. This alga was also investigated for its contents of polybrominated dibenzo-*p*-dioxins (PBDD), which recently was identified in blue mussels (*Mytilus edulis*) from the same area [15].

Materials and Methods

Chemicals. All solvents were of *p.a*. quality unless otherwise stated. Diazomethane was prepared in house from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) [16], Sigma-Aldrich (Steinheim, Germany). The methoxylated halogenated diphenyl ether standards were synthesized as described elsewhere [17]. The PBDD standards were synthesized as described elsewhere [18].

Instruments. The GC-HRMS analyses of the PBDDs were performed on a Micromass Autospec Ultima magnetic sector mass spectrometer. Electron ionization (EI) was achieved at 32 eV and the detection carried out in selected ion-monitoring (SIM) mode. The instrument was set to a resolution of 10 000 and an acceleration voltage of 8000 V. The samples were injected on column to a non-polar PTE 5 capillary column (15 m × 0.25 mm i.d., with 0.25 µm film thickness) with a siltek deactivated silica column (2 m × 0.53 mm) as the retention gap. The analyses of the MeO- and OH-PBDEs were performed on a Varian 3400 GC, equipped with an electron capture detector (ECD), and a split/splitless injector operated in splittless mode. A DB-5 column (30 m x 0.25 mm i.d. and 0.25 µm film thickness) was used.

Sample. The red macroalgae, *Ceramium tenuicorne* (Kütz.) Waern, were collected at 3-4 meters depth close to Askö, an island in the Stockholm archipelago, which adjoins the northern Baltic Proper.

Extraction and cleanup. The isolation procedure of MeO- and OH-PBDEs in red algae are shown in Figure 1. Briefly, each algal sample (total six replicates and around 10 g f.w. in each) were homogenized and extracted with a method originally developed by Jensen and coworkers [19]. After gravimetric determination of the extractable material, the surrogate standards (S.S.) 4'-MeO-BDE121 (1.5 ng) and 4'-OH-BDE121 (43.3 ng) were added. Phenolic compounds were then isolated from neutral compounds by partitioning with potassium hydroxide (0.5 M in 50% EtOH) as described earlier [20]. The fractions containing phenolic compounds were derivatized with diazomethane [20]. Further cleanup, of the phenolic and neutral fractions, was done by the use of two different columns; the first contained silica (activated 300° C over night) impregnated with sulfuric acid (2:1, w/w 1 g), while the second was packed with silica (activated; 1 g). Finally, the volumetric standard (V.S) BDE-138 was added prior to GC analysis.

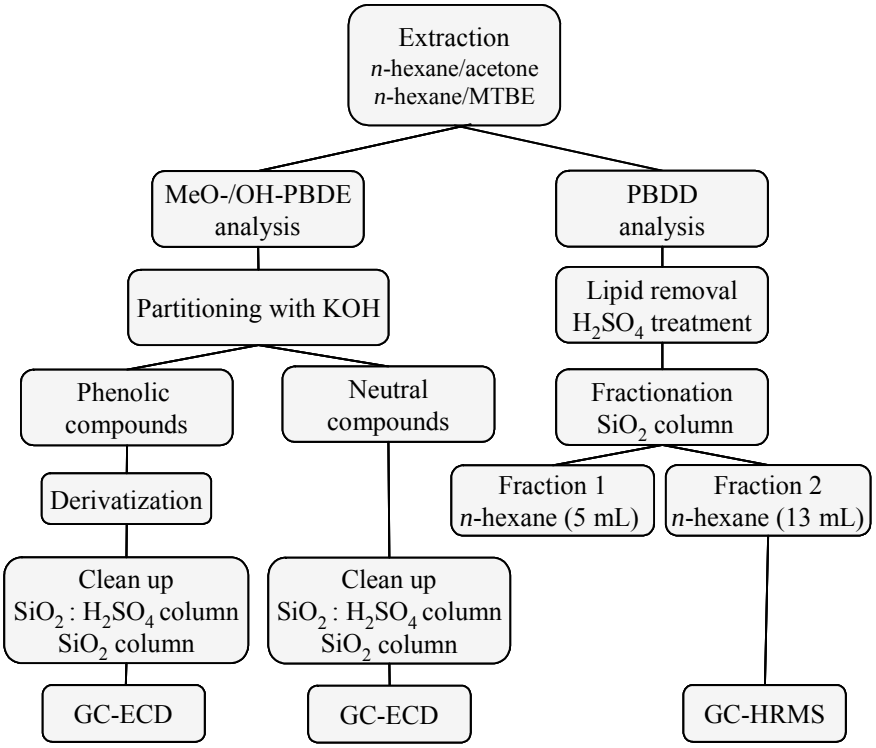

Figure 1. Procedure for the extraction and clean up of the red algal samples for analyses of OH-PBDEs, MeO-PBDEs and PBDDs.

PBDDs were isolated from a separate red algae sample (total 85 g f.w.), from the same batch. To remove lipids the samples was treated with conc. sulfuric acid. As a last clean up step, the extract was fractionated on an activated silica gel column (1 g) [15]. The solvent volume of fraction 2 was reduced prior to GC-HRMS analysis (Figure 1).

Quantification. The MeO-PBDE and methylated OH-PBDE congeners in the red algae were quantified by GC-ECD. The quantification was performed using single point external authentic reference standards in the concentration range of the linear relationship of the GC system. The linearity of the GC-ECD instrument was investigated by running standards at multiple concentration levels in parallel with the samples.

Result and Discussion

Four MeO-PBDEs and seven OH-PBDEs, recently identified [3], were quantified in red algae from the Baltic Sea. Chromatograms of the phenolic and neutral fractions of one of the algal replicates are shown in Figure 2 and the quantified congeners in each fraction are marked with the abbreviation name.

The mean values ($n=6$), in ng/g lipid weight, for the methylated OH-PBDEs in the phenolic fraction were 1600 for 2'-OH-BDE-68, 3300 for 6-OH-BDE-47, 1100 for 6-OH-BDE-90, 4400 for 6-OH-BDE-99, 500 for 2-OH-BDE-123, 3400 for 6-OH-BDE-85, and 3700 for 6-OH-BDE-137. The levels of the quantitated MeO-PBDEs in the neutral fraction were considerably lower; 160 for 2'-MeO-BDE-68, 310 for 6-MeO-BDE-47, 60 for 6-MeO-BDE-85, and 60 for 6-MeO-BDE-137. The reason for this is not clear, one explanation could be that the OH-PBDEs are the main product formed by the algae and the MeO-PBDEs are a secondary product, formed by methylation of OH-PBDEs in the environment.

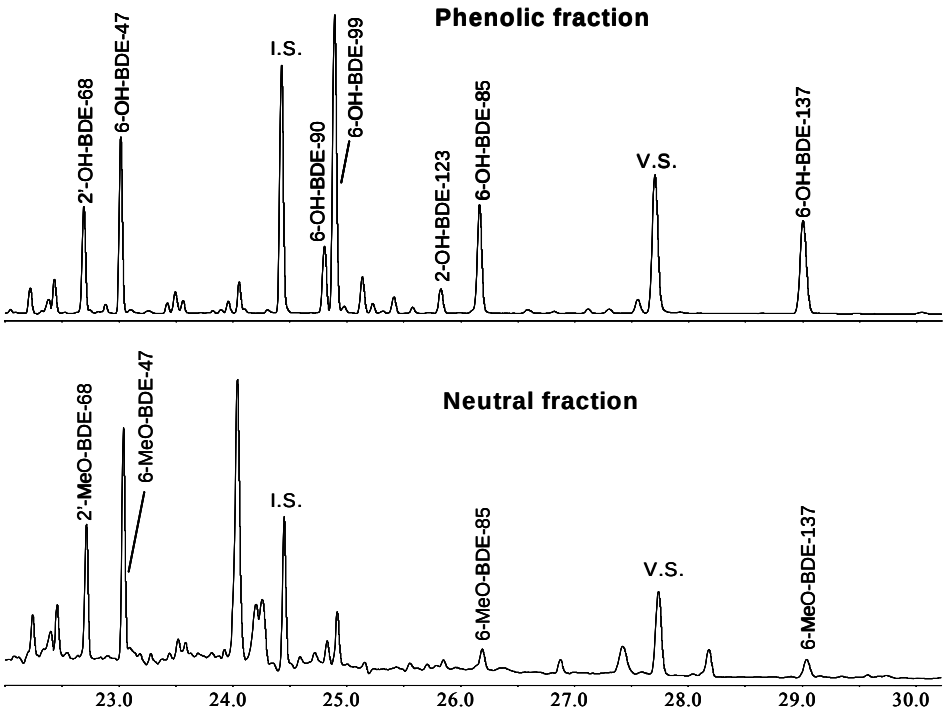

Figure 2. The upper GC chromatogram shows the methylated phenolic fraction (diluted 30 times compared to the neutral fraction) and the lower GC-chromatogram shows the neutral fraction of the red alga.
4'-OH-BDE-121 and 4'-MeO-BDE-121 was used as surrogate standards (S.S.) and BDE-138 as volumetric standard (V.S).

In a previous study, a triBDD was detected in the neutral fraction of the red alga *Ceramium tenuicorne* collected in the Baltic Sea [3].To confirm these results, the present study was designed, and a new algal sample, of the same species, was extracted and analyzed (Figure 1).
A full-scan GC-HRMS (EI) spectrum of the triBDD present in the algae revealed a molecular ion (*m/z*=418) and a tri-bromine isotope cluster, as well as, an abundant ion at *m/z*=311 with a di-bromine isotope cluster, a characteristic fragment ion $[M\text{-}BrCO]^+$, produced by EI ionization of PBDD [11]. The identity of the triBDD ($C_{12}H_5O_2Br_3$) was confirmed by accurate mass measurement with voltage scan at 10 000 resolution.
The corresponding peak was detected in blue mussels and recently identified as two triBDDs (1,3,7-triBDD and 1,3,8-triBDD), which coelute on the non-polar column applied [15]. At this stage no effort has been performed to investigate if the triBDD, in the algal sample, also consist of more than one triBDD congener. The present study stresses the fact that naturally produced dioxins are part of the Baltic Sea environment.

Acknowledgements
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