HYDROXYLATED BROMINATED DIPHENYL ETHERS IN SALMON (*SALMO SALAR*), BLUE MUSSELS (*MYTILUS EDULIS*) AND THE RED ALGAE (*CERAMIUM TENUICORNE*) FROM THE BALTIC SEA – NATURAL PRODUCTION IN BALTIC SEA BIOTA

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Introduction

The presence of methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in biota from northern Europe was recently reported [1, 2, 3, 4]. Hydroxylated polybrominated diphenyl ethers (HO-PBDEs) have also been identified in blood plasma from Baltic salmon [2]. One HO-PBDE congener was identified in human male plasma [5]. It is known that polybrominated diphenyl ethers (PBDE) are metabolized to HO-PBDE in rats, mice and fish [6, 7, 8]. The relatively high amounts of MeO- and HO-PBDEs found in Baltic salmon blood indicated a possible additional source beside PBDE metabolism [2]. To our knowledge, neither HO-PBDE, nor MeO-PBDE, are commercially produced. HO- and MeO-PBDE have been reported to be present in e.g. marine sponges, naturally produced by their symbioticliving cyanobacteria [9]. One MeO-PBDE (2-methoxy- 2',3, 4',5-tetrabromodiphenylether) has also been identified in the green algae, *Cladophora fascicularis*, from Japan [10]. Natural production of brominated phenols (e.g. lanosol) and polybrominated dipheny methanes has earlier been described in several red algae e.g. from the Swedish West cost [11, 12]. These brominated substances were suggested to be secondary metabolites produced by enzymatic bromination in the red algae [13, 14, 15]. The aim of the present study was to investigate potential sources of the HO- and MeO-PBDEs found in Baltic salmon blood and to determine the pattern of these compounds in red algae, blue mussels and salmon.

Methods and Materials

Chemicals: All solvents were of p.a. quality unless otherwise stated. The brominated diphenyl ethers and the halogenated phenoxyphenol standards were synthesized as described elsewhere [16, 17]. Diazomethane was synthesized as described by Fieser and Fieser [18].

Instruments: The gas chromatography mass spectrometry (GC/MS) analysis was performed on a Finnigan MAT SSQ 710, provided with a Varian 3400 gas chromatograph and a fused-silica capillary column (DB-5, 30m, i.d. 0.25μm, 0.25 mm). Helium was used as carrier gas. Electron capture negative ionization (ECNI) was used with methane as reagent gas. The temperature in the ion source was 150 °C. The instrument was scanned from 33 to 1000 *m/z*.

Samples: *Salmon blood:* The blood samples were taken from female sea-run Baltic salmon (*Salmo salar*) as described elsewhere [2].

Mussels: Blue mussels (*Mytilus edulis)* are the biomass-dominating invertebrate species on rocky bottoms in the Baltic Sea and were collected at 3-4 meters depth close to Askö, in the northern Baltic Proper, 70 km south Stockholm.

Red algae: The red algae *Ceramium tenuicorne* were collected at the same site and depth as the blue mussels.

Extraction and clean-up and analysis:

Salmon blood: The salmon blood plasma (5g) were extracted and cleaned-up according to the method earlier described by Asplund et. al., [2]. The phenolic fraction from about thirty plasma samples were pooled and analyzed by GC/MS (ECNI).

Mussels: The samples (7g, fresh weight) were extracted according to Jensen et al. [19] and the lipid amounts were determined gravimetrically, the samples were resolved in hexane and partitioned with KOH (0.5 M KOH in 50% ethanol). The phenolic compounds were extracted after acidification (HCl, 2M) and derivatised with diazomethane. The samples were further cleaned-up on a series of silica/sulfuric acid gel column (1g) eluted with DCM (24 ml), as described in Hovander et al. [20], followed by a silica gel column (0.5g) eluted with DCM (14 ml). Five samples were pooled and analyzed by GC/MS (ECNI).

Red algae: Five sampled were pooled (85 g, fresh weight), extracted and cleaned-up essentially in the same way as the mussel sample described above. The samples were analyzed by GC/MS (ECNI). Solvent blanks followed the samples during extraction and clean-up.

Result and Discussion

In a previous study we reported the presence of HO-PBDEs and MeO-PBDE in Baltic Salmon blood [2]. Two of the HO-PBDEs present in the salmon blood were identified [21]. At this symposium, Marsh and coworkers present the identification of an additional four HO- PBDE congeners in the salmon plasma by comparison with authentic synthesized standards [16].

Mass chromatograms (ECNI) of the phenolic fraction of blood plasma from salmon (*Salmo salar*), blue mussel tissue (*Mytilus edulis*) and the red algae (*Ceramium tenuicorne*) from the Baltic Sea are shown in Figure 1. The chromatograms show the bromine ion *m/z* 79 of the diazomethane derivatized samples. The structures of the identified HO-PBDEs, marked Br_{4a}, Br_{4b}, Br_{4c}, Br_{5a}, Br_{5b}, Br_{5c}, Br_{5d} and Br_6, are given in the GC/MS chromatograms. The identification was made by compression of ECNI mass spectra and comparison of GC-retention times of sample peaks and the synthesized reference standards. In addition, the sample contains at least two monochlorotetrabromo-HO-PBDEs, one of them identified as shown in the chromatogram Br_4/Cl_a, and one with unknown structure Br_4/Cl_b. All the identified HO-PBDEs have the hydroxy group in the *ortho* position relative to the diphenyl ether linkage and have 2,4-dibromo-substitution in the other ring. The HO-PBDE congener patterns of the salmon samples differ from the patterns of the blue mussels and *Ceramium* samples. Three additional OH-PBDEs (Br_{5c}, Br_{5d} and Br_6) were detected in the algae and mussel samples and the structures are given in Figure 1c. The red algae samples are dominated by one pentaBDE (Br_{5d}) and one hexaBDE (Br_6) congeners. These OH-PBDEs are also present in the blue mussels though to a less extent than in the red algae. These two species are commonly found mixed on rocky bottoms, thus suggesting that these compounds may easily be taken up by the blue mussels living in dense stands of red algae. In the salmon blood sample these HO-PBDEs (Br_{5d} , Br_6) are present at only low levels. The difference in the chromatographic pattern between the samples may indicate differences in bioavailability, uptake and/or elimination of the different HO-PBDEs to the blue mussel and salmon. Another possibility is differences in the blue mussel and salmon metabolism or a selective retention of the HO-PBDEs in the salmon blood.

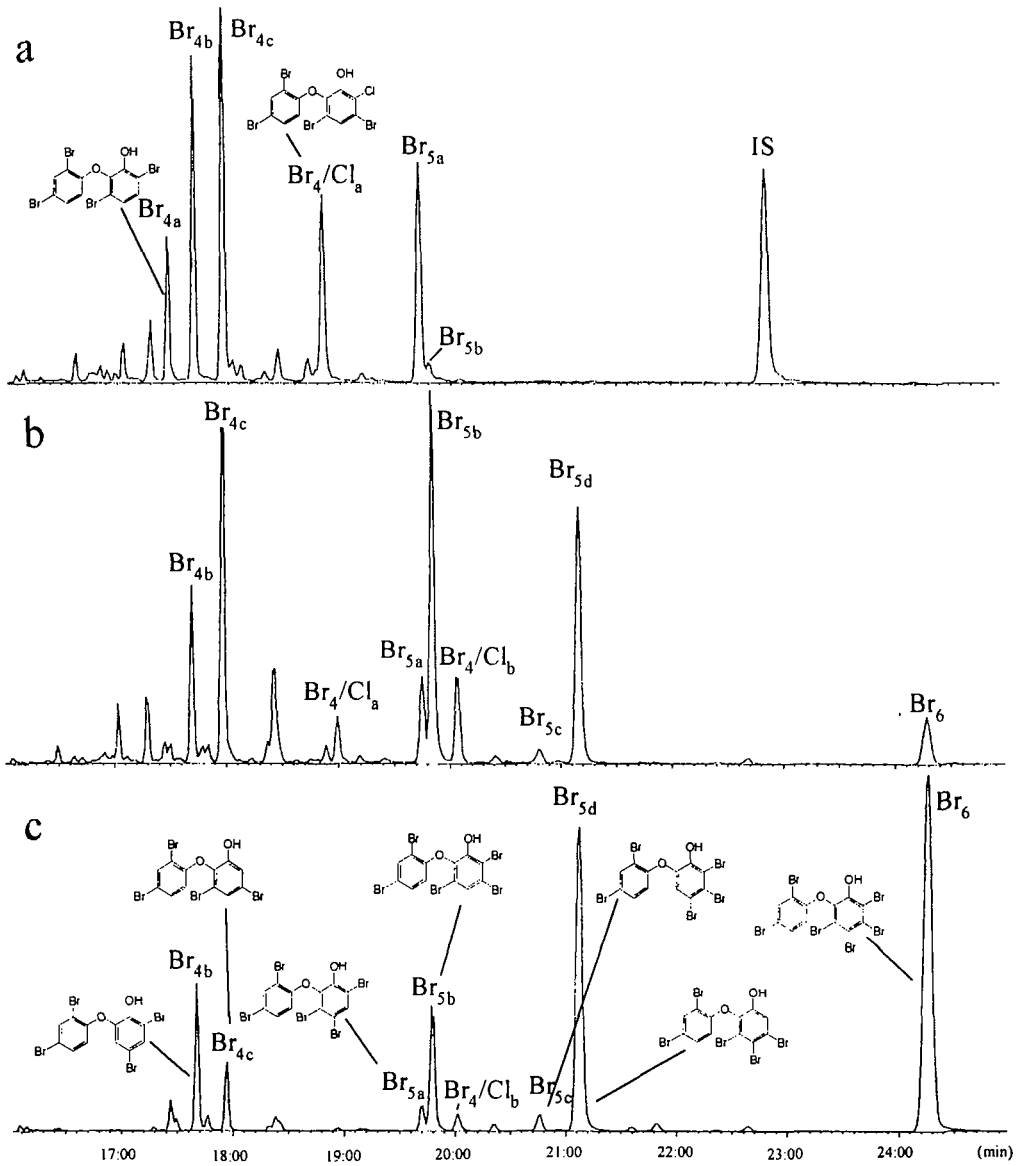

Figure 1 GC/MS (ECNI) chromatograms of the methylated phenolic fraction of the samples: a) Blood plasma from Baltic salmon (*Salmo salar*) b) Blue mussel (*Mytilus edulis*) c) Red algae (*Ceramium tenuicorne*). IS (BDE-138).

The investigated salmon feed in the southern Baltic and could have a different exposure compared to the blue mussel and red algae sample collected in the northern Baltic.

The neutral fraction of the algae samples was also analyzed by GC/MS. This fraction contained MeO-BDEs but the levels were considerably lower (about 100 times lower) than the corresponding HO-PBDEs in the phenolic fraction.

The substitution pattern of HO-PBDEs in the salmon, mussel and algae samples have similarities with the pattern reported for the naturally produced HO-PBDEs, originating from marine sponges/cyanobacteria and algae [22, 23, 24, 25, 9, 14, 10]. This indicates that the HO-PBDEs present in the Baltic salmon plasma most likely are naturally produced. To our knowledge this is the first time HO-PBDEs have been identified in red algae species from the Baltic Sea. However, further studies have to be carried out to prove whether the OH-PBDEs are produced by the red algae. Further, other sources of OH-PBDEs, for examples, the production via cyanobacteria present in the pelagic and benthic ecosystem of Baltic Sea, will need to be investigated.

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