

Are the sources of polybrominated dibenzo-*p*-dioxins found in Baltic Proper fish and shellfish anthropogenic or natural?

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Introduction

The Baltic Sea ecosystems are influenced by, for example, nutrient enrichment and heavy pollutant loads (Kautsky & Kautsky, 2000) and there are frequent reports of harmful algal blooms (Kononen, 1992), as well as high mortality and reproduction problems in, e.g. seabirds (Kautsky & Kautsky, 2000) and fish (Kautsky & Kautsky, 2000; Finfo, 2005). The salinity of the Baltic Sea water decreases with distance from the Atlantic from 30 to 3 practical salinity units (psu) due to dilution with freshwater. Thus, all marine and freshwater species that inhabit the sea are under stress due to the difference in salinity between the brackish water and their normal living environments. They are therefore particularly sensitive to toxic compounds. The severity of these effects may, thus, at least in part, be attributed to increased susceptibility of the organisms due to brackish water stress.

In the studies underlying this paper we discovered a new class of stressors; polybrominated dibenzo-*p*-dioxins (PBDDs). These compounds and the closely related polybrominated dibenzofurans (PBDFs) have previously been reported in various samples of anthropogenic origin. Thoma and co-workers have, for instance, reported high levels of PBDD/Fs in pyrolysates of polybrominated diphenyl ethers (PBDEs) (Buser, 1986). They also found these in the technical formulations, but as much lower levels (Thoma, 1986). PBDD/Fs also seem to be formed in incineration processes. Furthermore, significant levels were reported in exhaust from car exhaust for cars run on leaded gasoline (Haglund, 1988) and in flue gasses from incineration processes (Söderström, 2002). Thus, it is clear that there has been, and possibly still are, anthropogenic PBDD/F emissions. It is therefore not surprising that van Bavel co-workers found PBDD/Fs, primarily mono- through hexa-BDFs, in sediments from two Swedish freshwater lakes in total concentrations of about 500 pg/g dry weight (Hagberg, 2005).

However, similar structures have also been found in samples of natural origin. It is well known, for instance, that many organo-bromines are naturally produced, including bromophenols (BPs), hydroxy-polybrominated diphenyl ethers (HO-PBDEs), methoxy-PBDEs (MeO-PBDEs) and HO-PBDDs and MeO-PBDDs (Gribble, 2000; Teuten, 2005; Utkina, 2002). Some of these, MeO-PBDEs and HO-PBDEs, as well as PBDDs, have even been reported to be present in the Baltic Proper red alga *Ceramium tenuicorne* and cyanobacteria, mussels, and fish (Malmvaern, 2005a; Malmvaern, 2005b, Malmvaern, 2005c).

The aim of the present study has been to investigate whether the PBDDs, which are found at reasonably high levels in Baltic Proper fish, are of anthropogenic or natural origin.

Materials and Methods

A large number of samples were collected to study the spatial distribution of the PBDDs and PCDD/Fs. The samples were from the west coast of Sweden, from the three basins of Baltic Sea, the Baltic Proper, The Bothnian Sea and The Bothnian Bay, and from a number of freshwater lakes close to those coastal areas. To the best of our knowledge there were no major point-sources close to the sampling locations. Composite samples were prepared to increase the representativeness of the analysed samples and the composites were stored frozen until the time of analysis. Additional archived mussel tissue lipid extracts were obtained from the collections at ITM, Stockholm University, Sweden.

All solvents and chemicals were of high purity and checked for impurities. A mixture of all $^{13}\text{C}_{12}$ -2,3,7,8-PCDD/Fs except 1,2,3,4,7,8,9-HeptaCDF was prepared from individual standards and was used as internal standard (IS). Two additional congeners, 1,2,3,4-tetraCDD and 1,2,3,4,7,8,9-HeptaCDF were used as recovery standards (RS). Further, a PCDD/F quantification standard was prepared by adding the same amount of IS and RS, as to the samples, to a mixture of all 2,3,7,8-substituted PCDD/Fs. A PBDD quantification mixture, containing 2,7/2,8-DBDD, 2,3,7-TrBDD and 2,3,7,8-TeBDD, was prepared in a similar way. Additional PBDDs, i.e. 1,3-, 1,7-, 1,8-, 1,9-DBDD, 1,3,6-, 1,3,7-, 1,3,8-, 1,3,9-TrBD, 1,3,6,8- and 1,3,7,9-TeBDD were synthesized by Hyogo Prefectural Institute of Public Health and Environmental Sciences and used for congener identification.

The samples were mixed with sodium sulfate (4:1 or more) and were then loaded into glass columns, spiked with IS, and were sequentially extracted with acetone: *n*-hexane (2.5:1) and *n*-hexane: diethyl ether (9:1). The extracts were collected in round bottom flasks. Finally, 99.5% ethanol was added to each flask, and the lipid weights were determined gravimetrically after complete solvent removal by rotary evaporation.

The fat residues were quantitatively transferred to pre-washed multilayer silica columns containing (from the bottom): glass wool, 35% KOH/silica, silica, 40% H_2SO_4 on silica, 20% H_2SO_4 on silica, silica and Na_2SO_4 . The column was eluted with *n*-hexane, and the volume was reduced by rotary evaporation. In the next step, an activated carbon column was used to fractionate the target compounds according to planarity. Carbon/ Celite mixture was packed in the centre of a glass pipette with glass wool on either side. The sample extract was transferred to the column *n*-hexane and eluted with *n*-hexane followed by *n*-hexane/DCM, and finally 40 ml toluene (back-flush). The PCDD/Fs and PBDD/Fs elute in the last fraction. After evaporation, the samples were transferred to miniaturized multilayer silica columns and were eluted with *n*-hexane. Prior to GC-HRMS analysis, tetradecane keeper was added to the samples and to the quantification standard, RS was added to the samples, the volatile solvents were removed by rotary evaporation, and the residues were transferred to GC-vials with 150 μl inserts.

The PBDD/Fs were quantified by isotope dilution, using $^{13}\text{C}_{12}$ -PCDD/Fs as internal standards, by a Micromass Ultima GC-HRMS operating at $\geq 10,000$ resolution, a 60m x 0.25mm x 0.20 μm Supelco SP-2331 GC column, helium at 1.0 ml/min, and a GC oven temperature program of 190°C for 2 min, raise at 3°C/min to 280°C, hold for 10 min.

Accurate mass determinations were performed on selected samples using multiple (symmetrically distributed) SIR channels over a molecular ion distribution cluster ion. The SIR ions were closely spaced ($\pm 2\text{mmu}$) close to the theoretical apex, and were then wider spaced ($\pm 5\text{ppm}$). The areas of the individual peaks were plotted vs. their m/z values, a trend line was fitted to the data, and the experimental molecular weight was obtained through the curve apex.

Results

The presence of PBDDs was confirmed by accurate mass determinations and comparisons of retention time data. To find the origin of the PBDDs we scrutinized their geographical distribution (Fig. 1) and found a strong spatial trend in their levels. The PBDDs were non-detectable in fish from the fresh-water environments, virtually absent in fish from the Bothnian Bay/Sea, but present at high to very high levels in samples from Baltic Proper and West Coast. This is strikingly different from their chlorinated analogues (PCDD/Fs), which are present in all fresh-water samples and exhibit an opposite spatial trend. These findings indicate that the two classes of pollutants originate from different sources; and absence of PBDDs in freshwater samples rules out long-range air transport as a substantial contributor. Instead, the elevated levels of PBDDs in Baltic Proper littoral fish (perch and eel), as compared to pelagic fish (herring) from the same area, indicate that PBDDs stem from a pollutant source in the coastal (littoral) zone. We hypothesized that they are marine toxins.

Since the relative abundances of the various isomers, as manifested in GC-MS profiles, are almost identical in these species, it is likely that they share a common source and food-web pathway. Thus, the PBDDs may be excreted by algae and/or cyanobacteria, assimilated by mussels, and transferred to fish that feed on mussels/ mussel larvae. The substitution patterns of the major PBDDs in mussels and fish are consistent with formation through condensation of naturally occurring BP congeners, which are produced through biobromination by bromoperoxidases (BPO) in the presence of bromide (van Pée, 2003).

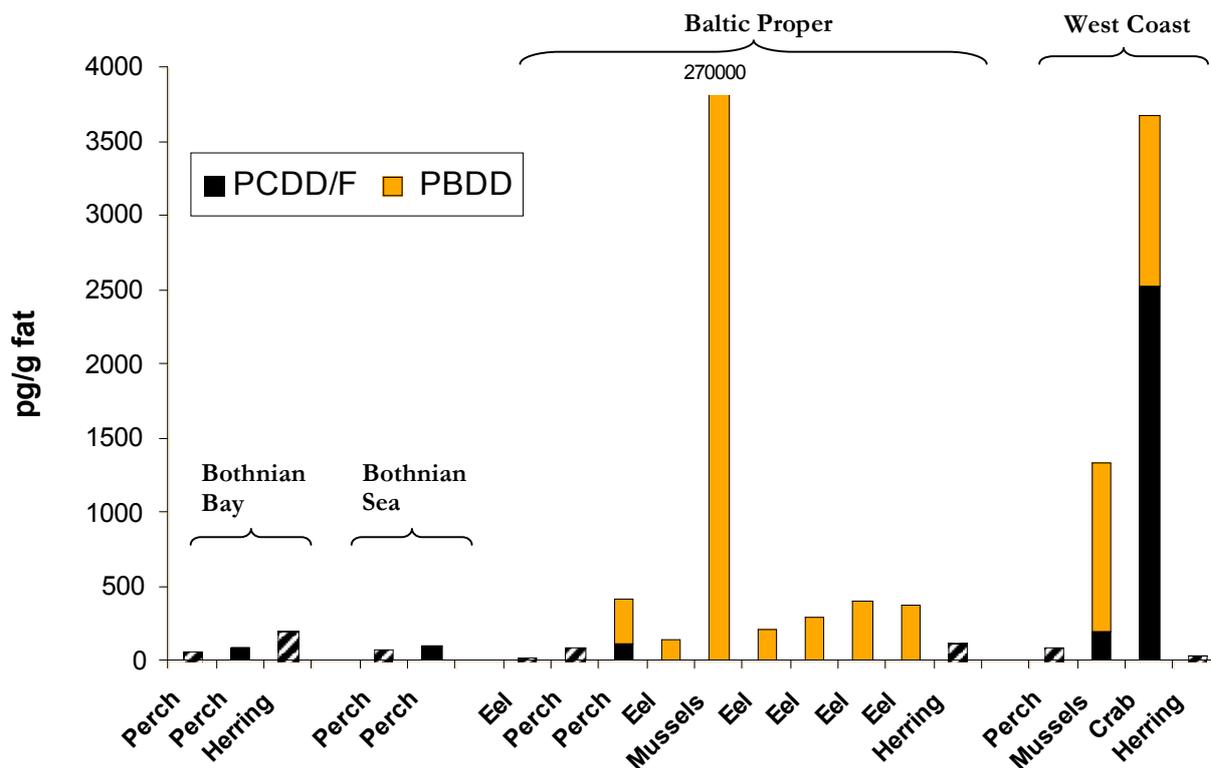


Figure 1. Levels of PBDDs (upper, orange) and PCDD/Fs (lower, black) in fish and shellfish from Swedish waters. Fish from inland lakes (perch, eel) and open waters (herring) are indicated with hatched bars; samples from the costal (littoral) zone with solid bars. The bar of Kvädöfjärden mussels is truncated.

The PBDDs are potent dioxin-like compounds. The relative equivalency potencies (REPs) vs TCDD of 2,7/2,8-DBDD and 2,3,7-TrBDD in the Ah-binding assay are 0.65 and 0.85, respectively (Mason, 1986; Mason, 1987). The latter has also been shown to cause early life stage mortality in rainbow trout, with a REP of 0.017 (Hornung, 1996). If we use these REPs to make a very rough assessment of the dioxin toxic equivalencies (TEQs) we find Ah-TEQs and trout mortality-TEQs of crab and eels close to the EU maximum residue limits (MRLs) for food, and Ah-TEQs of the Kvädöfjärden mussels exceed the MRL 100-fold.

It is, therefore, critical to obtain more information on the toxicological properties of not only 2,7/2,8-DBDD and 2,3,7-TrBDD, but also other abundant PBDDs, especially 1,3,7- and 1,3,8-TrBDD, and three of TeBDDs with unknown substitution. It is also necessary to obtain a better understanding of the environmental occurrence and fate of these emerging pollutants.

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