ON THE SOURCES OF POLYBROMINATED DIBENZO-*P*-DIOXINS FOUND IN BALTIC PROPER FISH AND SHELLFISH

Peter Haglund[1], Anna Malmvärn[2], Sture Bergek[1], Anders Bignert[3], Lena Kautsky[4], Takeshi Nakano[5], Karin Wiberg[1], Lillemor Asplund[2,6]

[1] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden.
[2] Department of Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden.
[3] Contaminant Research Group, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.
[4] Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden.
[5] Hyogo Prefectural Institute of Public Health and Environmental Science, 654-0037 Kobe, Japan.
[6] Department of Applied Environmental Science, Stockholm University, SE-106 91 Stockholm, Sweden.

Abstract

The spatial distribution of polybrominated dibenzo-*p*-dioxins (PBDDs) in Swedish waters were investigated in order to determine whether the PBDDs, which are found at reasonably high levels in Baltic Proper fish, are of anthropogenic or natural origin. The PBDDs were non-detectable in fish from fresh-water environments, virtually absent in fish from Bothnian Bay/Sea, but present at high levels in samples from Baltic Proper and West Coast. This is strikingly different from their chlorinated analogues, which are present in all fresh-water samples and exhibit an opposite spatial trend. This indicates 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, as compared to pelagic fish, indicate that PBDDs stem from a coastal pollutant source. We hypothesise that they are marine toxins. The temporal variations in the PBDD levels and patterns in perch support the natural formation hypothesis.

Introduction

The Baltic Sea ecosystems are influenced by nutrient enrichment and heavy pollutant loads[1] and there are frequent reports of harmful algal blooms,[2] and mortality and reproduction problems in seabirds[2,3] and fish.[2,4] The salinity of the water decreases with distance from the Atlantic from 30 to 3 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, at least in part, be attributed to increased susceptibility of the organisms due to stress. In the studies underlying this paper a new class of stressors; polybrominated dibenzo-*p*-dioxins (PBDDs), was discovered. These compounds and the closely related polybrominated dibenzofurans (PBDFs) have previously been reported in various samples of anthropogenic origin. Buser reported high levels of PBDD/Fs in pyrolysates of polybrominated diphenyl ethers (PBDEs).[5] Thoma *et al.* also found these in the technical PBDEs, but at lower levels.[6] Furthermore, significant levels were reported in exhaust from cars run on leaded gasoline[7] and in incinerator flue gasses.[8] Thus, it is clear that there has been, and still are, anthropogenic emissions. It is therefore not surprising that Hagberg *et al.* found PBDD/Fs, primarily mono- through hexa-BDFs, in sediments from two Swedish lakes in total concentrations of about 500 pg/g dry weight.[9] However, similar structures have also been found in samples of natural origin. It is well known 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.[10,11,12] MeO-PBDEs, HO-PBDEs and PBDDs have even been found in the Baltic Proper red alga *Ceramium tenuicorne* and cyanobacteria, mussels, and fish.[13,14,15] 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

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 areas. To the best of our knowledge there were no major point-sources in the vicinity. Composite samples were prepared to increase the representativeness and were stored frozen until the time of analysis.

All solvents and chemicals were of high purity and checked for impurities. A mixture of all $^{13}C_{12}$-2,3,7,8-PCDD/Fs except 1,2,3,4,7,8,9-HeptaCDF was used as internal standard (IS), and a mixture of 1,2,3,4-tetraCDD and 1,2,3,4,7,8,9-HeptaCDF was used as recovery standard (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 were synthesized and used for identification.

Most samples were column extracted. The half-thawed tissues were ground with sodium sulfate (4:1 or more), loaded, spiked with IS, and sequentially extracted with acetone:n-hexane (2.5:1) and n-hexane:diethyl ether (9:1). The perch samples of the time trend study were extracted differently. They were homogenized and extracted with n-hexane:acetone (1:2.5, v:v) and n-hexane:methyl-tert-butyl ether (9:1, v:v). The organic phase was partitioned with a saline: phosphate buffer and re-extracted with the n-hexane: methyl-tert-butyl ether mixture.[16] Finally, the lipid weights were determined gravimetrically. The fat residues of all but the time trend samples were transferred to 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 columns were eluted with n-hexane, and the volume reduced by evaporation. The lipids of the perch for the time trend studies were removed by sulfuric acid and by filtering through 33% H_2SO_4 on silica, with dichloromethane (DCM) as eluent. In the next step, an activated carbon column was used to fractionate the targets according to planarity. Carbon/ Celite mixture was packed in the centre of a glass pipette. The samples were added and eluted with n-hexane, n-hexane/DCM, and 40 ml toluene (back-flush). The dioxins eluted in the last fraction. After evaporation, these fractions were transferred to small multilayer silica columns and were eluted with n-hexane. Tetradecane was added to the samples and to the quantification standard, RS was added to the samples, the volatile solvents were removed by evaporation, and the residues were transferred to GC-vials. The PBDD/Fs were quantified by isotope dilution, using $^{13}C_{12}$-PCDD/Fs as internal standards, by a Micromass Ultima GC-HRMS operating at ≥10,000 resolution, a 60m x 0.25mm x 0.20µm 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 (± 2 mmu) close to the theoretical apex, and were then wider spaced (± 5 mmu). The individual areas were plotted vs. m/z, and a trend line was fitted to the data.

Results

The presence of PBDDs was confirmed by accurate mass determinations and comparisons of retention time data. To find the origin of the PBDDs their geographical distribution (Fig. 1) was scrutinized and a strong spatial trend in their levels was found. 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, PBDDs may be excreted by algae and/or cyanobacteria, assimilated by mussels, and transferred to fish that feed on mussels. The substitution patterns of the major PBDDs in mussels and fish are consistent with formation through condensation of naturally occurring BP congeners; produced through biobromination by bromoperoxidases (BPO) in the presence of bromide.[17]

The temporal variations in the PBDD levels and patterns in perch further support the natural formation hypothesis (Figure 2). The levels fluctuate dramatically between years, much like the variations observed for other marine toxins, e.g. the Paralytic shellfish poisoning (PSP) and Diarrhetic shellfish poisoning toxins. [18] The variation may be associated with changes in the abundance of PBDD producing species in the area, to activity of the PBDD producing biological systems or to the relative proportions of PBDD precursors. The congener

distribution does also support the natural production hypothesis. The shares of metabolically labile tri-BDDs (with vicinal hydrogen substituents that may be epoxidized by the enzyme P450 system) are higher during years with high total PBDD levels (Figure 3), while those of metabolically stable tetra-BDDs (that lack vicinal hydrogen substituents) are higher during years with low total PBDD levels. Thus, at peak exposure the pattern may reflect the amounts assimilated by the organisms; but at low exposure it is skewed towards the more persistent tetra-BDDs, which (partially) persist from the previous exposure peak.

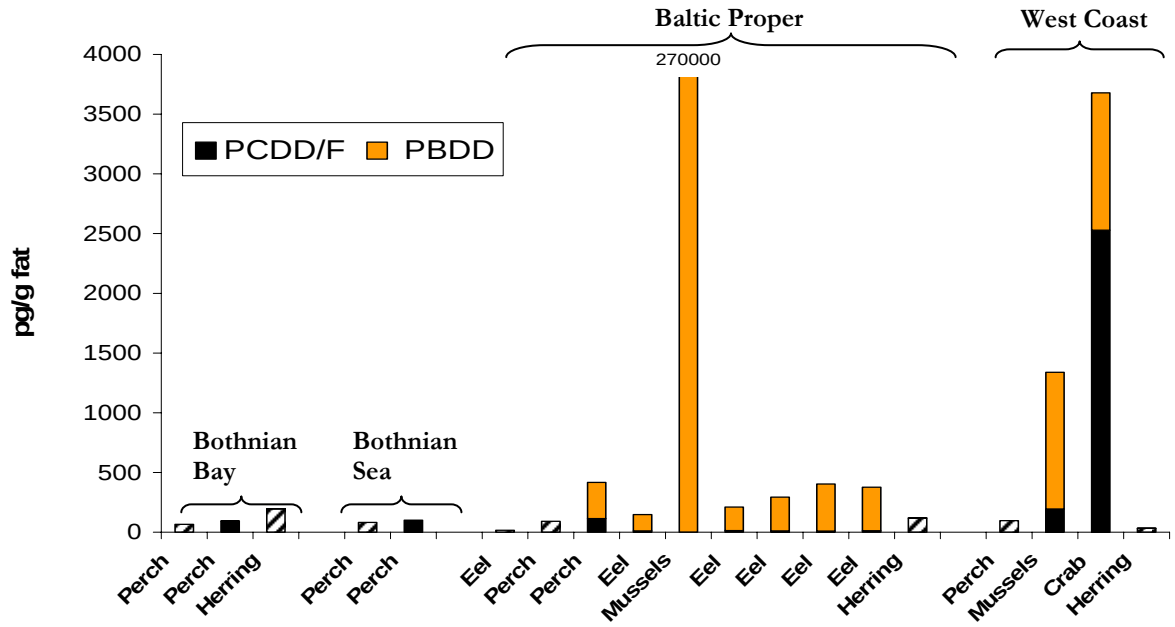

Figure 1. Levels of PBDDs (upper, orange) and PCDD/Fs (lower, black) in fish and shellfish from Swedish waters. Fish from inland lakes (perch, eel) and open waters (herring) are indicated with hatched bars; samples from the costal (littoral) zone with solid bars. The bar of Kvädöfjärden mussels is truncated.

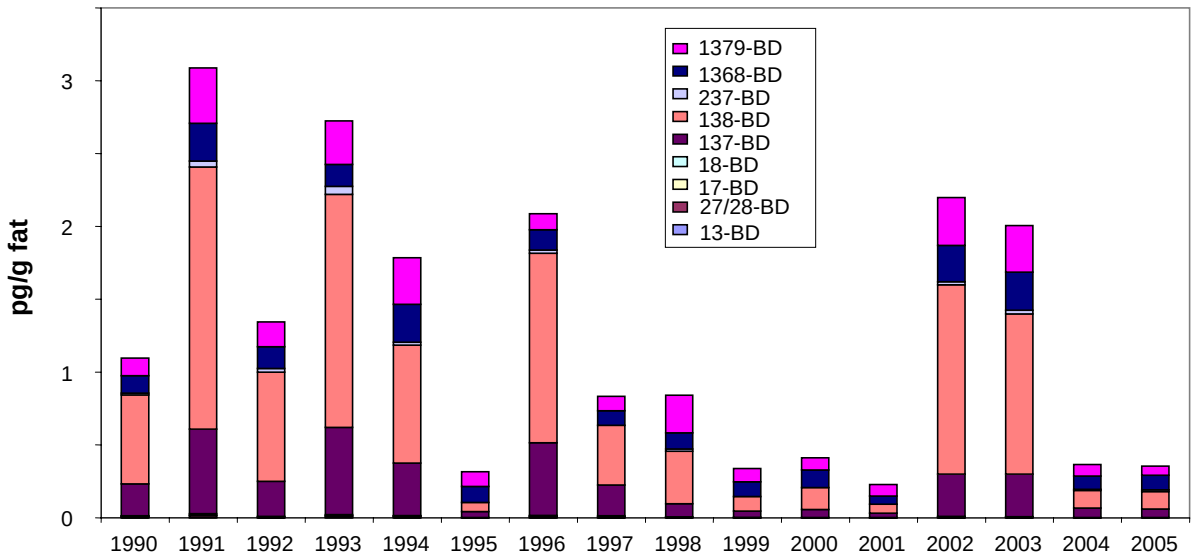

Figure 2. Levels (pg/g wet weight) and patterns of PBDDs in perch collected from Kvädöfjärden (a background site with high PBDD levels) during the period 1990 to 2005.

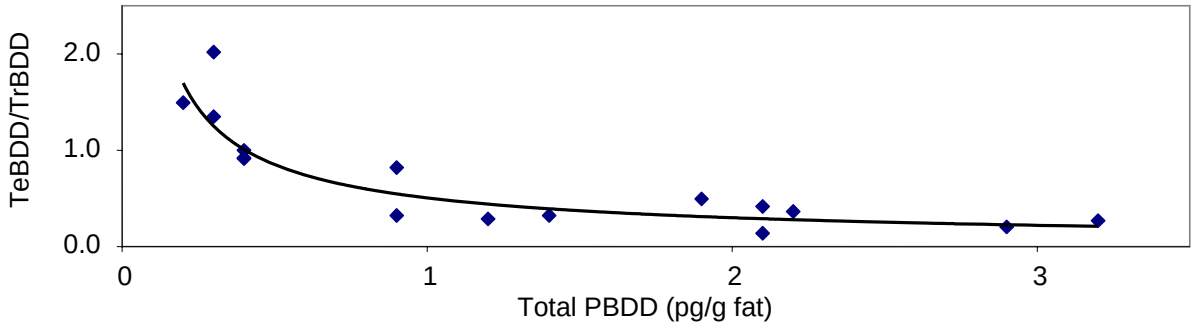

Figure 3. Correlation between the TeBDD/TrBDD ratio and the total PBDD level in Kvädöfjärden perch.

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.[19,20] The latter has also been shown to cause early life stage mortality in rainbow trout, with a REP of 0.017.[21] 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 to 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.

Acknowledgements
Financial supports were obtained from the Swedish EPA through their POP screening program and through a FORMAS grant. Technicians at the Contaminant Research Group, Swedish Museum of Natural History and at The National Food Administration is acknowledged for their assistance in sample collection and preparation.

References

1. Kautsky L, Kautsky N. In: *Seas at the Millennium: An Environmental Evaluation*, Sheppard C (ed), Elsevier Science, Amsterdam, 2000: 121.
2. Kononen K. *Finn Mar Res* 1992; 261: 3.
3. Hario M, Hirvi J, Hollmén T, Rudbäck E. *Environ Pollu*t 2004; 127:411.
4. Swedish Board of Fisheries, Report Finfo 2005:05, www.fiskeriverket.se/publikationer/finfo/finfo.htm.
5. Buser HR. Environ Sci Technol 1986; 20:404.
6. Thoma H, Rist S, Hauschulz G, Hutzinger 0. *Chemosphere* 1986; 15:2111.
7. Haglund P, Egebäck K-E, Jansson, B. *Chemosphere* 1988; 17:2129.
8. Söderström G, Marklund S. *ES&T* 2002; 36:1959.
9. Hagberg J, Grahn E, van Bavel B, Lindström G. *Organohalogen comp* 2005; 67:2030.
10. Gribble GW. *Environ Sci & Pollut Res* 2000; 7:37.
11. Teuten ETL, Xu L, Reddy CM. *Science* 2005; 307:917.
12. Utkina NK, Denisenko VA, Virovaya MV, Scholokova OV, Prokof'eva NGJ. *Nat Prod* 2002; 65:1213.
13. Malmvärn A, Marsh G, Kautsky L, Athanasiadou M, Bergman A, Asplund L. *ES&T* 2005; 39:2990.
14. Malmvärn A, Eriksson U, Kautsky L, Asplund L. *Oranohalogen comp* 2005; 67:1380.
15. Malmvarn A, Zebuhr Y, Jensen S, Kautsky L, Greyerz E, Nakano T, Asplund L. *ES&T* 2005; 39:8235.
16. Jensen S, Reutergårdh L Jansson B. *Fish.Tech..Pap*. 1983; 212: 21-33
17. van Pée K-H, Unversucht S. *Chemosphere* 2003; 52:299.
18. Cambella A, Ibarra D, Diogene J, Dahl E. *Oceanography* 2005; 18:158.
19. Mason G, Farrell K, Keys B, Piskorska-Pliszczynska J, Safe L, Safe S. *Toxicology* 1986; 41:121.
20. Mason G, Zacharewski T, Denomme M.A, Safe L, Safe S. *Toxicology* 1987; 44:245.
21. Hornung MW, Zabel EW, Peterson RE. *Toxicol Appl Pharmacol* 1996; 140:227.