POLYBROMINATED DIOXINS AND DIBENZOFURANS: A GLOBAL CONCERN?

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Abstract
The PBDD/Fs were analyzed in mollusc and crustacean samples from the Baltic Sea, NE Atlantic, NW Atlantic, Mediterranean Sea, S Atlantic, N Pacific, S Pacific and Indian Ocean and were found in all samples. Thus, they are ubiquitous in the marine environments. There was an enormous spread in concentrations, which spread four orders of magnitudes between all of the samples and three orders of magnitude between samples from the same water (North East Atlantic). The highest levels were detected in the Baltic Sea (about 20,000 pg/g dry weight). Similar concentrations were found in opposite corners of the world, i.e. North East Atlantic and South Pacific. For the remaining bodies of water the number of samples was to low to allow any meaningful comparisons of concentrations, but these all fall within the range of the North East Atlantic and South Pacific samples. The ubiquity of the pollutants, high peak concentrations, and high variability indicate that the PBDD/Fs have to be considered in environmental and human risk assessments.

Introduction
Natural organohalogen compounds are very common in nature. As of year 2003, more than 3800 organohalogen compounds, mainly containing chlorine (ca 2200) or bromine (ca 1950) but a few with iodine (95) and fluorine (100), had been reported to be produced by living organisms or formed during natural abiogenic processes, such as volcanoes, forest fires, and other geothermal processes[1]. The oceans are the single largest source of biogenic organohalogens, which are biosynthesized by myriad seaweeds, sponges, corals, tunicates, bacteria, and other marine life. Many of the organohalogen compounds are produced through the action of haloperoxidases, which catalyze the oxidation of halides. They are named according to the most electronegative halide that they can oxidize; chloroperoxidases can catalyze the oxidation of chloride, as well as bromide and iodide, bromoperoxidases (BPOs) react with bromide and iodide, whereas iodoperoxidases are specific to iodide.

Many of the natural organohalogen compounds share structural features with anthropogenic persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs), polychlorinated phenols (PCPs), polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), and polychlorinated phenoxyphenols (pre-dioxins)[2]. Polybrominated phenols (PCP analogues) and polybrominated anisols have long been known to be naturally produced in significant quantities[3]. Methoxy- and hydroxy-PBDEs (MeO-PBDEs and HO-PBDEs, respectively) have been frequently reported in marine organisms, and it have been unequivocally proven that two of the MeO-PBDEs are of natural origin[4,5,6]. Recently, polybrominated dioxins and polybrominated dibenzofurans (PBDD/Fs) have been found in samples of fish[7,8], bivalves[8,9], macroalgae[10], cyanobacteria[10] and marine sponge[11] from the Baltic Sea and the North Sea.

Since the MeO-PBDEs and HO-PBDEs have been detected in samples from all over the world and the structurally closely related dihydroxy-PBDDs have been detected in marine sponge from the waters outside Australia (close to the anti-pole of the Baltic) and BPOs are present in many of the species that have been found to contain high concentrations of PBDDs and are ubiquitous in the marine environments world wide[12]; we hypothesized that the PBDD/Fs are also ubiquitous in the marine environments.

Since the PBDD/Fs have been detected at high levels and some of the detected PBDDs are potent aryl hydrocarbon (Ah) receptor agonists it was also concluded that the PBDD/Fs may be of environmental and health concern. The aim of the current study was therefore to investigate the global occurrence and levels of the PBDDs.

Experimental

Samples. The samples were bought from supermarkets, obtained as certified reference materials, or collected within environmental monitoring programs (Sweden and New Zealand). More information on the samples are listed in Table 1.

Table 1. Sample description, including sample number, species and sampling location.

No.	Species	Species, latin	Country	Location
Baltic Sea				
1	Blue mussels	*Mytilus edilus*	Sweden	Kvädöfjärden
2	Blue mussels	*Mytilus edilus*	Sweden	Kvädöfjärden
3	Blue mussels	*Mytilus edilus*	Sweden	Kvädöfjärden
4	Blue mussels	*Mytilus edilus*	Sweden	Kvädöfjärden
N. Atlantic Ocean				
5	Blue mussels	*Mytilus edilus*	Sweden	Havstensfjorden
6	Blue mussels	*Mytilus edilus*	Sweden	Sannäsfjorden
7	Common cockle	*Cerastoderma edule*	Sweden	Kalvöfjorden
8	European oysters	*Ostrea edilus*	Sweden	Sannäsfjorden
9	Edible crab, meat	*Cancer pagurus*	Sweden	Skagerack
10	Edible crab, pancreas	*Cancer pagurus*	Sweden	Skagerack
11	Blue mussels	*Mytilus edilus*	Norway	Åfjorden
12	Blue mussels	*Mytilus edilus*	Denmark	Limfjorden
13	Blue mussels	*Mytilus edilus*	Denmark	Limfjorden
14	Blue mussels	*Mytilus edilus*	Ireland	W. Irland*
15	Edible crab, meat	*Cancer pagurus*	Ireland	W. Irland*
16	Edible crab, roe	*Cancer pagurus*	Ireland	W. Irland*
17	Edible crab, pancreas	*Cancer pagurus*	Ireland	W. Irland*
18	Blue mussels	*Mytilus edilus*	Netherlands	Wadden Sea
19	Blue mussels	*Mytilus edilus*	Netherlands	Wadden Sea
20	Blue mussels	*Mytilus edilus*	Netherlands	Oesterschelde
21	Razor clams	*Ensis directus*	Netherlands	Zeeland*
22	Oysters	*Crassostrea gigas*	Netherlands	Zeeland*
23	Oysters	*Crassostrea gigas*	France	Atlantic coast*
24	Blue mussels	*Mytilus edilus*	Spain	Galicia*
25	Lobster, meat	*Homarus americanus*	Canada	Prince Edward Island
26	Giant scallop	*Placopecten magellanicus*	USA	NE U.S. coast*
Mediterranian Sea				
27	Blue mussels	*Mytilus galloprincialis*	France	Ethang de Thau
28	Blue mussels	*Mytilus galloprincialis*	Italy	La Specia Gulf
29	Carpet shell clams	*Venerupis Pullastra*	Italy	Adriatic coast*
S. Atlanic Ocean				
30	Edible brown mussel	*Perna perna*	Brasil	Guanabara Bay
N. Pacific Ocean				
31	Blue mussels	*Mytilus edilus*	S. Korea	Busan
32	Blue mussels	*Mytilus edilus*	S. Korea	Busan
33	Blue mussels	*Mytilus edilus*	Thailand	?
34	"Mud crab"	?	Thailand	Songkhla
S. Pacific Ocean				
35	Green lip mussels	*Perna canaliculus*	New Zeeland	Marlborough Sounds
36	Blue mussels	*Mytilus galloprovincialis*	New Zeeland	Wellington harbour
37	Green lip mussels	*Perna canaliculus*	New Zeeland	Auckland region
38	Green lip Mussels	*Perna canaliculus*	New Zeeland	Auckland region
39	Oyster	*Saccostrea glomerata*	New Zeeland	Auckland region
40	Diloma	*Diloma subrostrata*	New Zeeland	Auckland region
41	Diloma	*Diloma subrostrata*	New Zeeland	Auckland region
42	Blue mussels	*Mytilus edilus*	Chile	Puerto Montt
43	Blue mussels	*Mytilus edilus*	Chile	Puerto Montt
44	Blue mussels	*Mytilus edilus*	Chile	Puerto Montt
45	Blacklip Abalone	*Haliotis rubra*	Australia	South East Coast

* Exact location unknown.

Extraction. The samples were mixed with an excess of sodium sulfate (4:1 or more) in a high-speed blender, allowed to equilibrate for at least 30 min, and mixed a second time. The samples were then loaded into glass columns (40 mm internal diameter), spiked with IS, and were sequentially extracted with 300 mL acetone/n-hexane (2.5:1) and 300 mL n-hexane/diethyl ether (9:1). The extracts were collected in round-bottom flasks. Finally, 50 mL of 99.5% ethanol was added to each flask, and the lipid weights were determined gravimetrically after complete solvent removal by rotary evaporation. All windows and lights in the laboratory used for extraction and cleanup were equipped with UV-absorbing plastic foil to prevent debromination of PBDD/Fs.

Cleanup. The fat was dissolved in n-hexane and transferred to a pre-washed (100 mL n-hexane) multilayer silica column (35 mm diameter) containing (from the bottom): glass wool, 6 g of 35% KOH/silica (w/w), 3 g of silica, 17 g of 40% H2SO4 on silica (w/w), 7 g of 20% H2SO4 on silica (w/w), 3 g of silica, and 7 g of Na2SO4. The column was eluted with 200 mL of n-hexane, and the volume was reduced to approximately 1 mL by rotary evaporation. In the next step, an activated carbon column was used to fractionate the target compounds according to planarity. Half a gram of carbon/ Celite mixture was packed in the center of a glass pipet. mL, cut at both ends) with glass wool on either side. Before use, the column was washed with 4 mL of dichloromethane (DCM)/methanol/toluene 15/4/1 (v/v/v), 1mL of DCM, and 5mL of n-hexane. The sample extract was transferred to the column with 3 _ 1 mL n-hexane and eluted with 30 mL of n-hexane followed by 40 mL of n-hexane/DCM, 1/1 (v/v), and then 40 mL of toluene. Before the elution of fraction 3, the column was turned upside down. This elution scheme resulted in three fractions. Fraction 1 contained the bulk of PCBs, fraction 2 contained the mono-ortho CBs, and fraction 3 contained non-ortho CBs, PCDD/Fs, and PBDD/Fs. After rotary evaporation into 40 íL of tetradecane (keeper), the sample was transferred to a pre-washed miniaturized multilayer silica column (5mmdiameter) containing KOH/silica, silica, 40% H2SO4 on silica, and Na2SO4, and was eluted with 8 mL of n-hexane. Prior to gas chromatography-highresolution mass spectrometry (GC-HRMS) analysis, RS was added to each sample and to the quantification standard, and the volatile solvents were removed by rotary evaporation in small pear-shaped flasks. The residues were transferred to 2 mL vials with 150 µL inserts.

GC-HRMS Analysis. The PBDD/Fs were quantified by isotope-dilution GC-HRMS using a Micromass Ultima system (Waters Corp., Milford, MA) operating in EI mode (34 eV) at 10,000 resolution. The two most intense ions of each molecular ion isotope distribution cluster were monitored; and the selected ion recording (SIR) descriptor was divided in time segments, during which only one homologue group was monitored, to enhance the sensitivity. A 60 m x 0.25 mm x 0.20 µm Supelco SP-2331 column was used for the GC separation (Bellefonte, Pennsylvania) with a constant flow of helium carrier at 1.0 mL/min, and a GC oven temperature program as follows: 190 °C for 2 min, raise at 3 °C/min to 280 °C, hold for 10 min. The $^{13}C_{12}$-TCDD was used as internal standard.

Results and Discussion

The PBDD/Fs were found in all samples and, thus, are ubiquitous in the marine environments. The results of the analyses of bivalve samples are shown in Figure 1. There was an enormous spread in concentrations, which spread four orders of magnitudes between all of the samples and three orders of magnitude between samples from the same water (North East Atlantic). The highest levels were detected in the Baltic Sea. Similar concentrations were found in opposite corners of the world, i.e. North East Atlantic and South Pacific. For the remaining bodies of water the number of samples was to low to allow any meaningful comparisons of concentrations, but these all fall within the range of the North East Atlantic and South Pacific samples.

In contrast, the homologue patterns of the samples were quite similar (Figure 2) with the exception for the Baltic Sea samples, which was richer in PBDDs, in particular TrBDDs. This may indicate that there is a common natural source, or sources, of PBDD/Fs in the major Seas of the world, and that other species dominate in the brackish Baltic Sea environment. It is also well known that the organisms of the Baltic Sea are under stress due to a difference in the salinity between the surrounding water and their normal living environment (that may be either fresh or marine water). Potentially, such stress can affect the production of natural halogenated compounds.

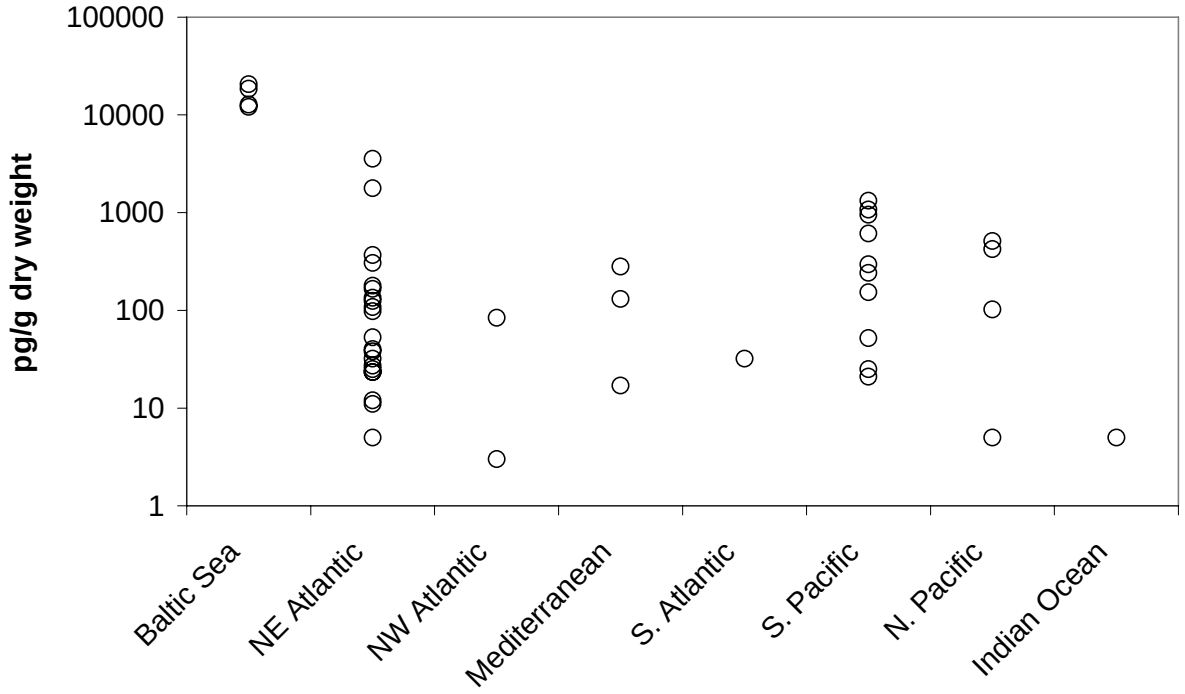

Figure 1. Concentrations of PBDDs in mussel samples from all over the world.

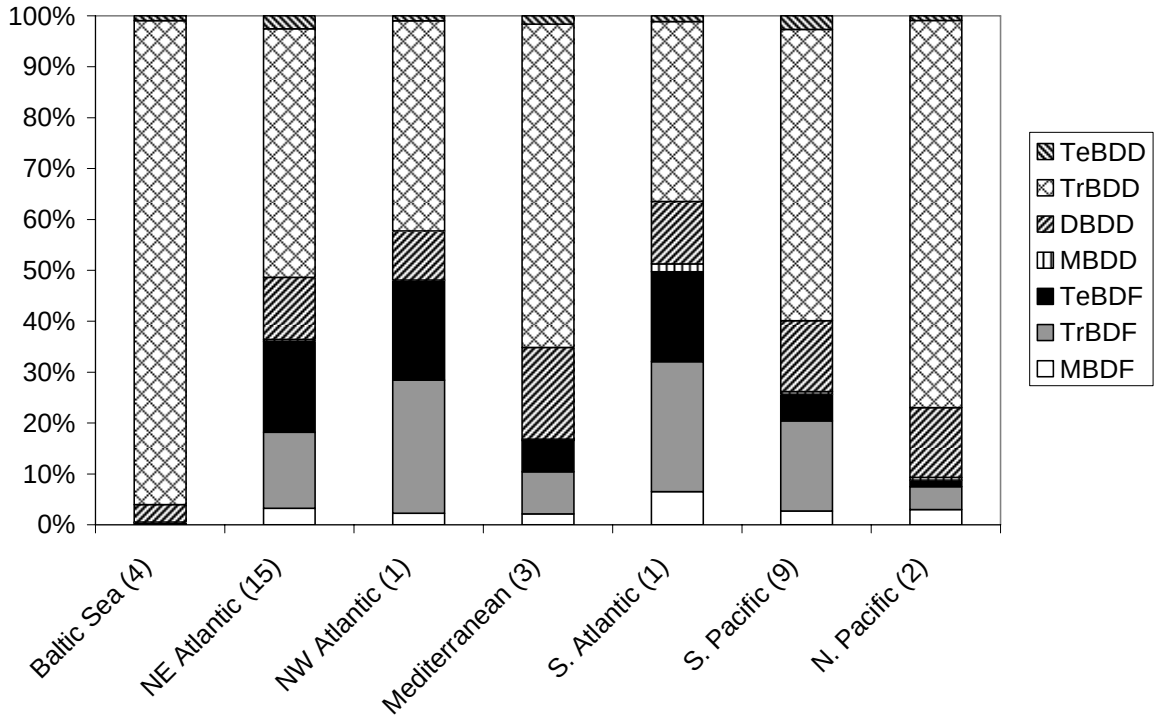

Figure 2. Average homologue contributions for mussel samples collected in seven bodies of water.

Comparisons of the concentrations of PBDD/Fs in samples from the North East Atlantic reveal that oysters generally seems to have higher concentrations than mussels and that the concentrations in crustaceans such as crabs and lobster can be of similar magnitude as those of mussels (Figure 3). The observation of higher concentrations in oysters than in mussels concurs with the results of a Scottish study[13].

The comparisons also revealed differences in tissue distribution of crabs, which have much higher concentrations in their roe and hepatopancreas than in their meat.

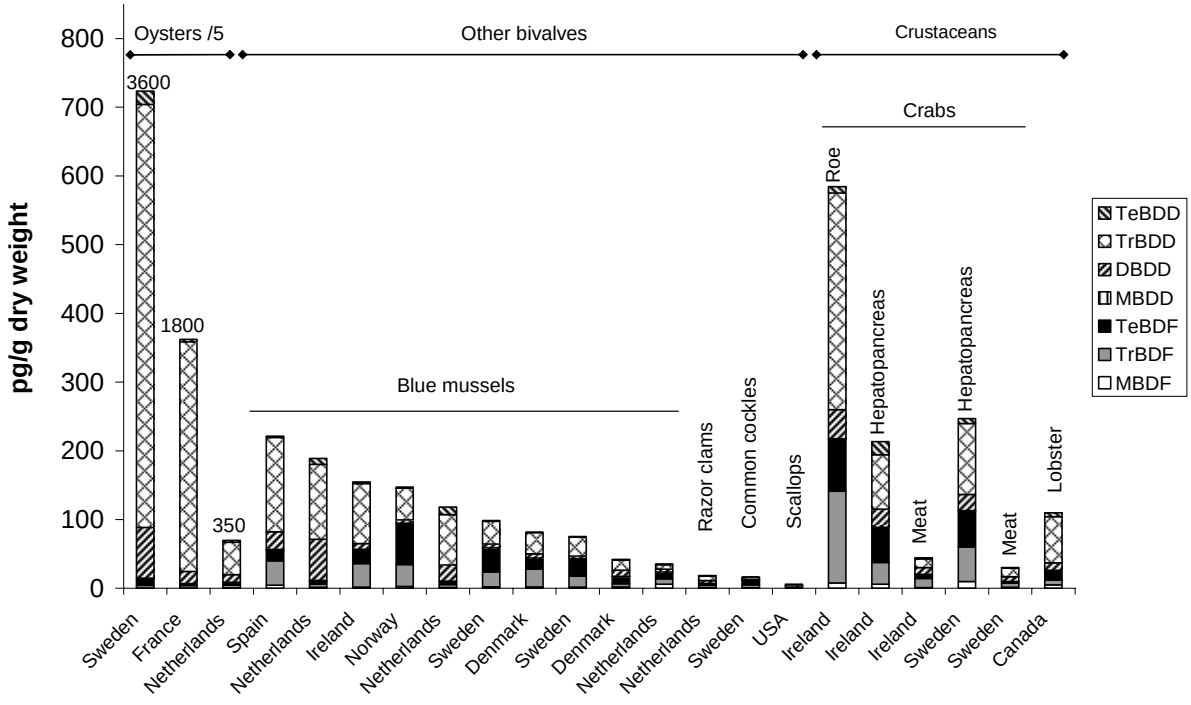

Figure 3. Concentrations of PBDD/Fs in bivales and crustaceans from the North East Atlantic.

The toxicity of PBDDs closely resembles that of the PCDD/Fs as they share a common mechanism of action, mediated through binding to the cytosolic aryl hydrocarbon (Ah)-receptor, causing effects such as lethality, wasting, teratogenesis, reproductive impairment, chloracne, immunotoxicity, and enzyme induction[14]. The Ah-binding affinities are structure-dependent and, interestingly, the PBDDs are generally equally as or even more potent than their corresponding chlorinated analogues[15-17].

Thus, our detection of high levels of PBDDs in some seafood samples is alarming from a human food safety point of view, especially since the levels of PCDD/Fs in such food already are close to or even exceed cost recommendations. Fatty fish from the Baltic Sea do, for instance frequently exceed European Commission's maximum residue limits (MRLs) of 4 pg TCDD equivalents (TEQ)/g fish muscle[18].

In addition, the potential ecotoxicological effects of the PBDDs must also be considered, as PBDDs have been shown to cause early life stage mortality in rainbow trout (*Oncorynchus mykiss*)[19] and mussels are key components of the littoral foodwebs. Thus, it is likely that many species are exposed to, and potentially affected by, PBDDs.

In conclusion, the ubiquity of the pollutants, high peak concentrations, and high variability in concentrations indicate that the PBDD/Fs have to be considered in future environmental and human risk assessments. To support such efforts, additional data on the toxic potency of major PBDD/Fs are needed.

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