Brominated Dibenzo-*p*-Dioxins – A New Class of Marine Toxins?

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Abstract

The levels of polybrominated dibenzo-*p*-dioxins (PBDDs) were measured in marine fish, mussels and shellfish. PBDDs were non-detectable in samples from the freshwater environments, and their levels were successively higher in samples from the marine environments of the Bothnian Bay and Bothnian Sea, the West Coast of Sweden and the Baltic Proper. The levels in Baltic Proper littoral fish generally exceeded those of their chlorinated analogues (PCDDs). This is alarming as some Baltic fish species already are contaminated by chlorinated dioxins to such an extent that they cannot be sold on the European market. By comparing spatial trends in PBDD and PCDD distributions, and PBDD patterns in fish, mussels and algae; we show that the PBDDs are probably produced naturally, and propose a route for their biosynthesis. We further show that the levels of PBDDs are high (ng/g fresh weight) in mussels, and that the levels increase over time. Finally, we discuss the possibility that the PBDDs have adverse biological effects, and that the levels are increasing as a result of global warming and eutrophication.

Introduction

The marine environments that are generally both the most productive and most heavily affected by human activities are situated in shallow coastal areas. Consequently, many coastal ecosystems are severely disturbed, including those in the Baltic Sea; a semi-enclosed sea connected to the Atlantic Ocean via the narrow, shallow Danish Straits. Its ecosystems are influenced by nutrient enrichment and heavy pollutant loads (1) and there are frequent reports of harmful algal blooms (2), as well as high mortality and reproduction

problems in marine mammals (3,4), seabirds (3,5) and fish (3,6). The severity of these effects may be attributed, at least in part, to increased susceptibility of the organisms due to brackish water stress (1). The salinity of the Baltic Sea decreases with distance from the Atlantic from 30 to 3 practical salinity units (psu) due to dilution with freshwater (Fig. 1). 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, and are therefore particularly sensitive to toxic compounds.

Here we report the occurrence of a new class of stressors; polybrominated dibenzo-*p*-dioxins (PBDDs). Our investigations suggest that these are naturally produced in contrast to the ubiquitous and highly toxic chlorinated analogues, the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), which primarily stem from anthropogenic activities (7). This hypothesis is supported by data showing spatial trends in the distribution of PBDDs and PCDD/Fs in fish, similarities in relative PBDD abundances in fish, mussels (*Mytilus edulis*) and PBDD-containing algae, and biosynthesis considerations. The report also includes a discussion concerning the potential current and future effects of these compounds on the ecosystem and human health.

Materials and Methods

Samples. Fish, mussels and shellfish were collected 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 costal areas. These were used to study the spatial distribution of the PBDDs and PCDD/Fs. The samples were either collected from background areas for environmental monitoring purposes or from commercial fishing-

vessels or fish-markets for food control purposes. These are denoted 'monitoring' and 'food control', respectively, in Supporting Information Table S1. The collection was coordinated by the Swedish Environmental Protection Agency, the National Food Inspectorate and the Swedish Museum of Natural History. Composite samples were prepared to increase the representativeness of the analysed samples (see Tables 1 and S1). The preparation was made by personnel at the National Food Inspectorate or the Swedish Museum of Natural History and the composites were stored frozen until the time of analysis. Additional archived mussel tissue lipid extracts were retrieved from the collections at the Department of Applied Environmental Research at Stockholm University and were used to study the temporal trends in PBDD levels over the period 1995-2003.

Chemicals. All solvents were of high purity and checked for impurities. Celite® 545 was obtained from Fluka, AX-21 carbon from Anderson Development (MI, USA) and silica (Kieselgel 60, 0.063–0.200 mm) of chromatography grade, and anhydrous sodium sulfate, sulfuric acid and potassium hydroxide of *pro analysis* grade from Merck. The silica was washed with methanol and dichloromethane and activated and stored at 120˚C prior to use. Similarly, sodium sulfate was activated at 550˚C for 48h. AX-21 carbon (Anderson Development, MI, USA) was mixed with Celite in the proportions 7.9/92.1, and the mixture was cleaned by Soxhlet extraction in toluene for 12 h and dried at 130◦C overnight.

A mixture of all $^{13}C_{12}$-2,3,7,8-PCDD/Fs except 1,2,3,4,7,8,9-HeptaCDF was prepared from individual standards obtained from Cambridge Isotope Laboratories (Andover, MA, USA) and was used as internal standard (IS; 250 pg/component/sample). Two additional congeners, 1,2,3,4-tetraCDD and 1,2,3,4,7,8,9-HeptaCDF, were obtained from the same

source and was used as recovery standards (RS; 250 pg/component/sample). 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 (EPA-1613CSS; Wellington Laboratories, Guelph, ONT, Canada) (100-1000 pg per component). A PBDD quantification mixture, containing 2,7/2,8-DBDD, 2,3,7-TrBDD and 2,3,7,8-TeBDD (140-500 pg per component), 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 prepared by Hyogo Prefectural Institute of Public Health and Environmental Sciences to be used for PBDD congener identification. All standards were made up in toluene.

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.

An alternative extraction method had been used to obtain the archived mussel sample extracts. It was based on liquid-liquid extraction using similar organic solvent mixtures, as was used in the column extraction procedure above, and is described in detail elsewhere (8).

All windows and lights in the laboratory used for extraction and clean-up were equipped with UV-absorbing plastic foil to prevent debromination of PBDD/Fs.

Clean-up. 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 35% KOH/silica (w/w), 3 g silica, 17 g 40% H_2SO_4 on silica (w/w), 7 g 20% H_2SO_4 on silica (w/w), 3 g silica and 7 g Na_2SO_4. The column was eluted with 200 ml *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 centre of a glass pipette (10 ml, cut at both ends) with glass wool on either side. Before use, the column was washed with 4 ml dichloromethane (DCM)/methanol/toluene 15/4/1 (v/v/v), 1 ml DCM and 5 ml *n*-hexane. The sample extract was transferred to the column with 3×1 ml *n*-hexane and eluted with 30 ml *n*-hexane followed by 40 ml *n*-hexane/DCM, 1/1 (v/v) and then 40 ml toluene. Before the elution of fraction 3, the column was turned upside down. This elution scheme resulted in three fractions. Fraction 1 contains the bulk of PCBs, fraction 2 mono-*ortho* CBs, and fraction 3 non-*ortho* CBs, PCDD/Fs and PBDD/Fs. After rotary evaporation to approximately 1 ml, the sample was transferred to a pre-washed miniaturized multilayer silica column (5 mm diameter) containing KOH/silica, silica, 40% H_2SO_4-silica and Na_2SO_4, and was eluted with 8 ml *n*-hexane. Prior to gas chromatography – high resolution mass spectrometry (GC-HRMS) analysis, 40 µl of tetradecane (keeper) was added to each sample and to the quantification standard, RS was added to the samples, 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 samples were analyzed for PCDD/Fs by isotope-dilution GC-HRMS using a VG 70/250S (Waters Corp., Milford, MA, USA) operated in EI mode (34 eV) at 8,000 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 0.32 mm x 0.32 µm J&W DB5MS column (Agilent, Palo Alto, CA, USA) was used for the GC separation with helium carrier gas at 18 psi head pressure. Each analysis was initiated by injecting a 3-µl aliquot of the sample in split-less mode, thereafter the GC oven was temperature programmed as follows: 200˚C for 2 min, raise at 3˚C/min to 300˚C, hold for 4 min. The performance of the analyses fulfilled the requirements laid down in the European commission directive 2002/69/EC.

The PBDD/Fs were quantified in a similar way, but using a Micromass Ultima GC-HRMS (Waters Corp., Milford, MA, USA) operating at ≥10,000 resolution, a 60m x 0.25mm x 0.20µm Supelco SP-2331 GC column (Bellefonte, Pennsylvania, USA), a constant flow of helium carrier at 1.0 ml/min, and a slightly different GC oven temperature program: 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.

Accurate mass determinations were performed on selected samples using multiple (symmetrically distributed) SIR channels over a molecular ion distribution cluster ion (normally $[M+2]^+$). The channels were spaced by 2 milli mass units (mmu) closest to the theoretical peak apex and by 5 mmu further away. The areas of the individual peaks were determined and plotted vs. their m/z values, a "smoothed" trend line was fitted to the data, and the experimental molecular weight (g/mol) was obtained through the curve apex.

Results and Discussion

Perch from Swedish waters were initially screened for mono- though hepta-BDD/Fs and monobromotrichloro- through monobromopentachlorodibenzo-*p*-dioxins, but only, di- through tetraBDDs was detected. Subsequent studies were therefore focused on these. The tentative identification of PBDDs was confirmed by separating the individual congeners chromatographically and determining their molecular weights by gas chromatography – high-resolution mass spectrometry (GC-HRMS) (9). The molecular weights of all the identified PBDDs were within ±0.002 mass units of the expected weights. Furthermore, their relative GC retention times corresponded, on two complementary columns (one polar and the other non-polar), with those of authentic reference standards.

In an attempt to identify the origin of these PBDDs we scrutinized their geographical distribution (Fig. 1) and found a strong spatial trend in their levels. The PBDDs were non-detectable in samples from the freshwater environments, and their levels were successively higher in samples from the marine environments of the Bothnian Bay and Bothnian Sea, the West Coast of Sweden and the Baltic Proper. This is strikingly different from the PCDD/Fs, which are present in all freshwater samples and, further, exhibit an almost opposite spatial trend in the marine environment, with the highest levels in samples from the Bothnian Bay and Bothnian Sea, and successively lower levels in the Baltic Proper and the West Coast (Table 1). These findings indicate that the two classes of pollutants originate from different sources. The absence of PBDDs in freshwater samples suggests that long-range air transport from distant sources is not a substantial contributor. Instead, the elevated levels of

PBDDs in Baltic Proper littoral fish (perch, *Perca fluviatilis*; and eel, *Anguilla anguilla*), as compared to pelagic fish (herring, *Clupea harengus*) from the same area, with the exception of perch and herring from the Bothnian Bay, in which TrBDDs occur at similar (low) levels; indicate that PBDDs stem from a pollutant source in the coastal zone (Table 1). Since levels of PBDDs are similar in all mussel samples collected along the south-eastern shoreline (10), as are those of all eel samples from this shoreline, the source is probably of diffuse character; which is consistent with the natural production hypothesis.

Further, it is well documented that many classes of organobromine compounds are naturally produced, including *inter alia* bromophenols (PBPs), bromoanisols, hydroxy- and methoxy-polybrominated diphenyl ethers (HO-PBDEs and MeO-PBDEs), and hydroxy- and methoxy-PBDDs (11,12,13). Two of these classes, MeO-PBDEs and HO-PBDEs, as well as PBDDs, have been shown to be present in the Baltic Sea red alga *Ceramium tenuicorne* (Kütz.), cyanobacteria samples, blue mussels, and fish (10,14,15 and Table 1). Since the relative abundances of the various isomers, as manifested in GC-MS profiles (10,14,15, and Fig. 1B), are similar in these species, it is likely that they share a common source and food-web transfer pathway. Thus, the PBDDs may be excreted by algae and/or cyanobacteria, assimilated by filter feeders like mussels, and then transferred to fish, e.g. perch, which consume considerable amounts of mussels and mussel larvae (16).

Moreover, the substitution patterns of the major PBDDs in mussels and fish (Fig. 1B and Table 1) are consistent with formation through condensation of naturally occurring PBP congeners, which are produced through biobromination by bromoperoxidases (BPOs) in the presence of bromide (17,18). Due to the electronic properties of the hydroxy group the *ortho* and *para* positions are preferentially brominated (19) and, thus, 2-BP, 4-BP, 2,4-

dibromophenol (DBP), 2,6-DBP, and 2,4,6-tribromophenol (TrBP) are expected to be major products. The condensation of BPs to PBDDs is presumably also catalyzed by BPO, as the horseradish chloroperoxidase is known to catalyze the dimerization of chlorophenols to PCDDs (20).

Our suggested biosynthesis routes for the PBDDs are outlined in Fig. 2. Direct condensation of BPs explains the occurrence of 1,3-, 1,8-, and 2,7-dibromodibenzo-*p*-dioxin (DBDD), 1,3,8- and 2,3,7-tribromodibenzo-p-dioxin (TrBDD), and 1,3,6,8- and 2,3,7,8-tetrabromodibenzo-p-dioxin (TeBDD). Additional congeners may be explained by Smiles rearrangement via an intermediate anion, i.e. 1,7- and 2,8-DBDD, 1,3,7-TrBDD and 1,3,7,9-TeBDD (21). The high relative abundance of the congeners formed from naturally abundant BP precursors is consistent with such a formation mechanism. Taking all these findings and established facts into consideration, it is most likely that the PBDDs found in this study are of natural origin.

Although biogenic, the PBDDs may still be toxic and, thus, pose a threat to the health of humans consuming PBDD-containing food. The toxicity of PBDDs closely resembles that of the PCDD/Fs as they share a common mechanism of action. Several PBDDs bind to the cytosolic aryl hydrocarbon (Ah)-receptor, which plays a central role in mediating 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like effects, such as: lethality, wasting, teratogenesis, reproductive impairment, chloracne, immunotoxicity, and enzyme induction (22). The Ah-binding affinities are structure-dependent and, interestingly, the PBDDs are generally equally or even more potent as their corresponding chlorinated analogues (23,24,25). Thus, our detection of high levels of PBDDs in Baltic Proper fish is alarming (Table 1), especially since the levels of PCDD/Fs in many catches of fatty Baltic fish

already exceed the European Commission's maximum residue limits (MRLs) of 4 pg TCDD equivalents (TEQ)/g fish muscle (26).

TEQs are used to facilitate risk assessments of samples with different proportions of the various PCDD/Fs, and correspond to the sum of the products of the concentrations of the individual toxicant congeners and their toxic equivalency factors (TEFs). Originally, the legislation applied to the seventeen 2,3,7,8-substituted PCDD/Fs (27), but it was recently revised to include 12 dioxin-like PCBs (26). In the 2005 WHO re-evaluation of TEFs it was stated that "If the mixed halogenated (bromine- and chlorine-substituted) dioxins and dibenzofurans are indeed detected in humans and their food, these should definitely be considered for inclusion in the TEF scheme" (28). It is therefore conceivable that the EU legislation will be expanded further in the future to accommodate PBDD/Fs.

The potency of dioxin-like compounds is assessed using, *inter alia* Ah-receptor binding, Aryl Hydrocarbon Hydroxylase (AHH) and Ethoxy Resorufin-*O*-Deethylase (EROD) enzyme induction measurements, and fish reproduction (rainbow trout) tests. Such data are available for two of the PBDD components: 2,7/2,8-DBDD and 2,3,7-TrBDD. The relative equivalency potencies (REPs), as compared to TCDD, of these compounds in the EROD, AHH, and Ah-binding assays are 0.0018 and 0.023, 0.017 and 0.018, and 0.65 and 0.85, respectively (23), and the 2,3,7-TrBDD REP in the rainbow trout early life stage mortality assay is 0.017 (29). We used these REPs to assess the TEQs of selected samples, and found that the Ah-receptor-PBDD-TEQ of the crab and the eels, and the rainbow trout-PBDD-TEQ, and EROD- and AHH-PBDD-TEQs of the Kvädöfjärden mussels, are close to the PCDD/F-MRL, while the Ah-receptor-PBDD-TEQs of the Kvädöfjärden mussels exceed the MRL by almost two orders of magnitude. Although these calculations were not

performed using internationally accepted TEFs, they indicate that the PBDDs occur at levels that may cause concern. It is, therefore, important 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.

The potential ecotoxicological effects of the PBDDs must also be considered. The most abundant PBDDs in fish, DBDDs and TrBDDs, have many vicinal hydrogen's and are expected to be easily metabolized and excreted (30). It is, therefore, likely that the fish exposure is high. Furthermore, there are currently severe problems with the recruitment of perch, pike (*Esox lucius*) and salmon (*Salmo salar*) along the Baltic south-eastern coast (3,6). The fish spawn and the eggs hatch, but few fry survive, possibly due to exposure to marine biotoxins. If this is the case, PBDDs are plausible causal agents, as they have been shown (see above) to cause early life stage mortality in rainbow trout (*Oncorynchus mykiss*) (29). Furthermore, the occurrence of high levels of PBDDs (ng/g w.w.) in blue mussels is alarming, as these are key components of the littoral food web (accounting for >90% of the animal biomass) (31). Thus, it is likely that many species apart from those studied here are exposed to, and potentially affected by, PBDDs.

The discovery of increasing PBDD levels in mussels from Kvädöfjärden (Fig. 3), which corresponds to an average annual increase of 11%, further increases our concerns. This temporal trend can, in part, be attributed to an increase in lipid content (5% annual increase). The remaining increase may be explained by changes in the macroalgal community structure due to eutrophication and global warming. More specifically, the abundance of filamentous green and red algae, which are favored by high nutrient concentrations and high water temperatures, has been increasing, leading to the shading and

decline of perennial brown algae such as *Fucus vesiculosus* (1). One of the red algae that has been increasing in abundance is the PBDD-containing *Ceramium tenuicorne* (10). Another possibility is that PBDD-containing cyanobacteria, which are increasing due to the relatively high levels of available phosphorous in the water during summer (32), are responsible for, or contributing to, the increase in PBDD levels. The large year-to-year variations in PBDD levels observed, c.f. 1995 and 1996, and 2000 and 2001 in Fig. 3, are consistent with such a biological formation process. The abundance of cyanobacteria varies greatly between years. Further studies are however needed to clarify whether there is a casual relationship or not.

Finally, since BPO-containing and organobromine-producing organisms are ubiquitous (11); PBDD biotoxins are potentially a global concern. The extent to which the PBDDs affect, and will affect, the marine ecosystems and human health remains to be seen. The primary factors that will determine the severity of such effects are the occurrence and distribution of PBDD-producing species, and the levels, relative abundance, and toxicity of the biotoxins formed. However, the findings reported and cited here suggest that nutrient enrichment and global warming influence these variables.

Acknowledgements

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Supporting Information Available

Tables containing detailed sample descriptions and PBDD levels for all individual congeners are provided in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Table 1. Levels of selected PBDDs and PCDD/Fs in composite biota samples, expressed as pg/g wet weight. The number of composites analyzed is denoted n. Geometric mean values are given for samples with n>3, and maximum values are given, in *italics*, for the congeners that did not occur in all samples. 2,3,7,8-TeBDD was only found in perch from location 15 at 0.011 pg/g wet weight. The levels of the individual samples and of additional PBDDs (3U1, 4U1, 4U2, and 4U3) are given as Supporting Information (Table S2). ND: Not detected; NA: Not analyzed.

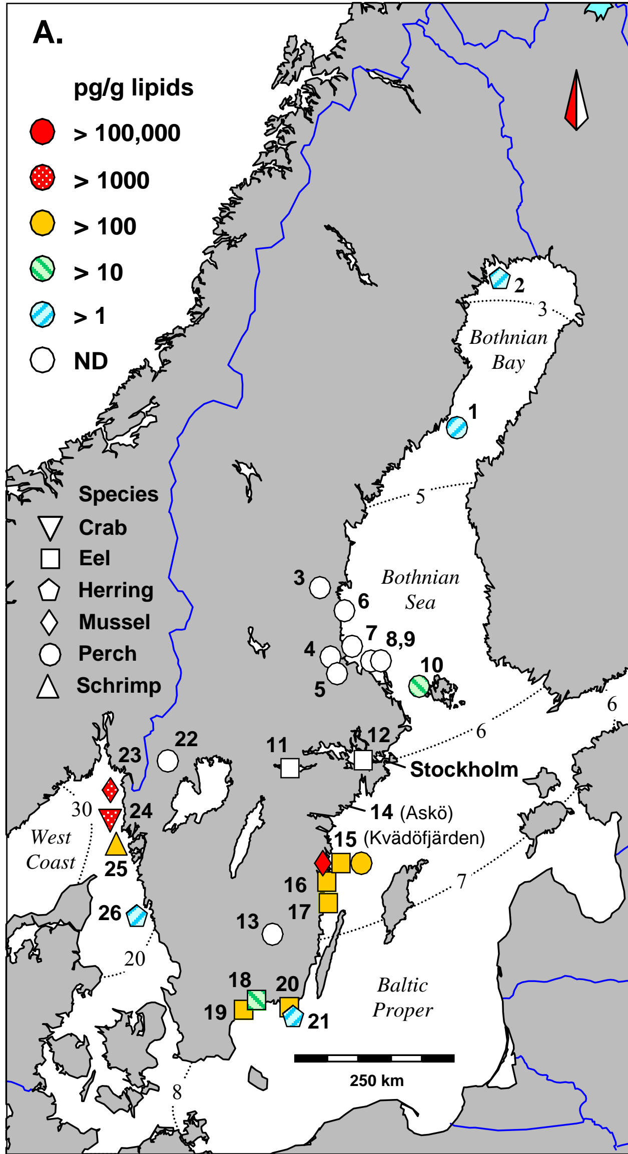
Location #, type,		Lipids	DBDDs				TrBDDs			TeBDDs		PBDD	PCDD/F	
species (n)		(%)	13	27/28	17	18	137	138	237	1368	1379	Total	Total	TEQ
Bothnian Bay														
1 Coast	Perch (8)	0.76	ND	ND	ND	ND	*0.002*	*0.008*	ND	ND	ND	*0.010*	0.71	0.24
2 Sea	Herring (16)	2.2	ND	ND	ND	ND	*0.009*	*0.023*	ND	ND	ND	*0.032*	4.3	1.0
Bothnian Sea														
3 Lake	Perch (8)	0.53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.35	0.10
4 Lake	Perch (1)	0.69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.56	0.13
5 Lake	Perch (1)	0.69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.87	0.13
6-9 Coast	Perch (4)	0.66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.67	0.23
10 Sea	Perch (1)	0.64	ND	ND	ND	ND	0.025	0.081	ND	ND	ND	0.11	0.78	0.27
Baltic Proper														
11 Lake	Eel (1)	21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.6	0.90
12 Lake	Eel (1)	21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.4	0.84
13 Lake	Perch (8)	0.55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.50	0.14
15 Coast	Perch (8)	0.66	0.15	0.49	0.072	0.27	0.27	0.66	0.016	0.013	*0.008*	2.0	0.75	0.092
	Eel (1)	14	0.62	2.4	0.25	0.92	2.5	11	ND	0.10	0.020	19	1.6	0.67
	Mussels (1)	1.5	32	250	26	44	1400	2100	110	0.36	0.53	4100	NA	NA
16 Coast	Eel (1)	25	2.8	9.1	1.0	5.5	6.2	23	0.14	ND	ND	49	3.6	1.5
17 Coast	Eel (1)	17	1.2	4.6	0.41	1.6	9.6	28	ND	0.27	ND	48	1.9	0.74
18 Coast	Eel (1)	19	1.5	7.1	0.45	3.7	12	48	0.18	0.15	ND	75	1.8	0.64
19 Coast	Eel (1)	21	0.16	0.49	0.056	0.23	2.6	9	ND	ND	ND	13	2.6	0.97
20 Coast	Eel (1)	14	1.0	4.5	0.50	2.1	9.5	32	0.070	ND	ND	51	1.7	0.70
21 Sea	Herring (16)	2.2	ND	ND	ND	ND	*0.010*	*0.015*	ND	ND	ND	*0.025*	2.6	0.88
West Coast														
22 Lake	Perch (8)	0.63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.61	0.18
23 Coast	Mussels (1)	2.1	0.33	2.2	0.18	1.1	3.4	15	0.09	0.28	0.04	24	4.1	1.1
24 Sea	Crab (1)	0.87	ND	2.4	ND	ND	4.7	1.3	0.52	0.19	ND	10	22	0.85
25 Sea	Shrimps (1)	1.5	ND	0.24	0.010	ND	1.5	0.14	ND	0.007	0.021	1.9	2.5	0.44
26 Sea	Herring (16)	5.3	ND	ND	ND	ND	*0.009*	*0.031*	ND	*0.026*	ND	*0.063*	1.7	0.45

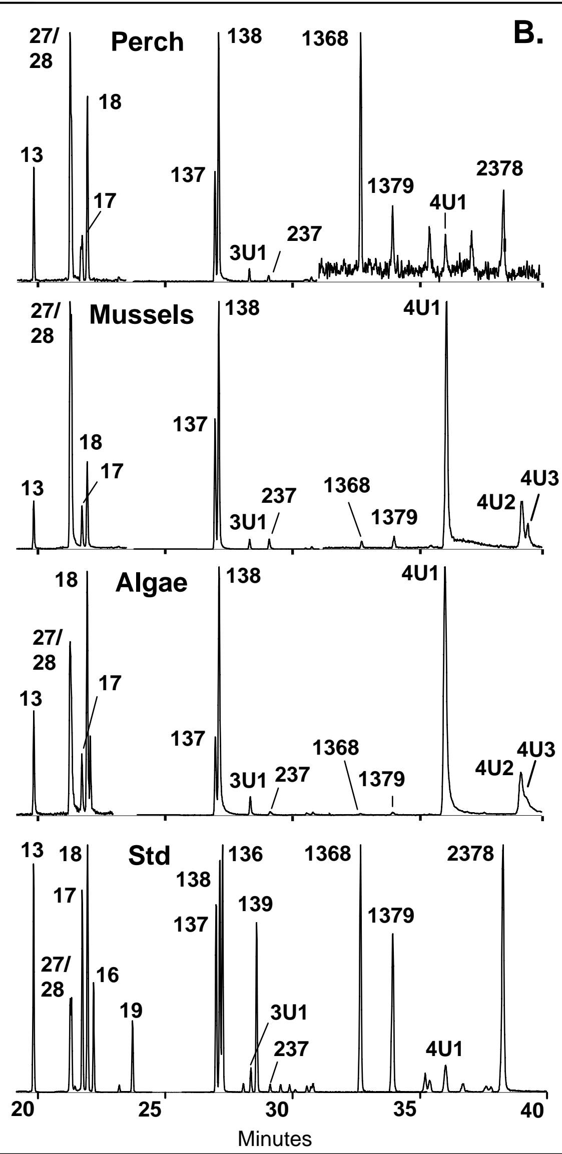
Figure legends.

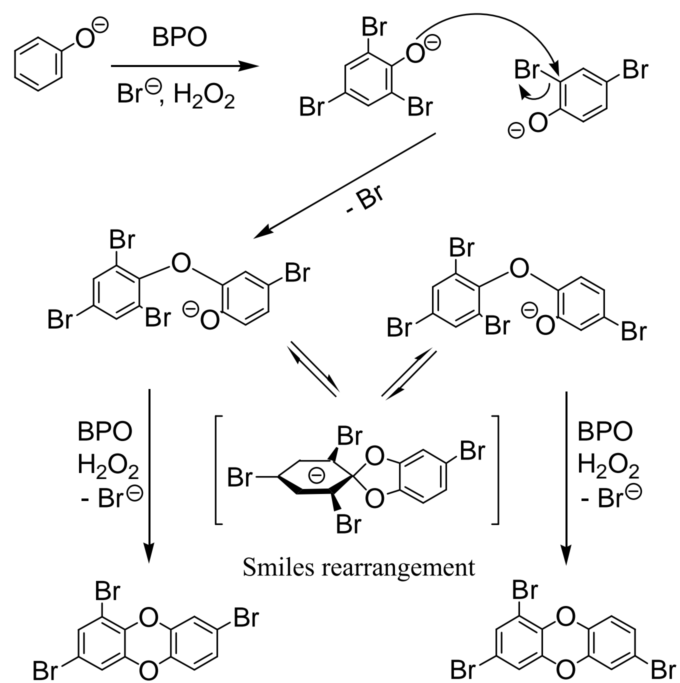
Fig. 1. Occurrence of PBDDs in biota. **(A)** Geographic distribution of PBDDs. The numbers in bold font refer to sample locations and standard font to salinities (psu). ND: not detected. Detailed sample descriptions are given as Supporting Information (Table S1). **(B)** GC-MS profiles of DBDD, TrBDD and TeBDD in perch and mussels from Kvädöfjärden, *Ceramium tenuicorne* algae from Askö, and a standard (Std). The numbers refer to the bromine substituent positions. One unknown TrBDD (3U1), and three TeBDDs (4U1, 4U2, and 4U3), are also included.

Fig. 2. Proposed biosynthesis routes for PBDDs. **(A)** Biosynthesis scheme for the bromoperoxidase (BPO)-mediated conversion of 2,4-di- and 2,4,6-tribromophenols to 1,3,7-TrBDD and 1,3,8-TrBDD. **(B)** Bromophenol precursors for the PBDDs found in this study. Abundant bromophenols and PBDDs are indicated in bold. S: Smiles rearrangement products.

Fig. 3. Temporal trend of total PBDDs (ng/g fresh weight) in mussels from Kvädöfjärden.

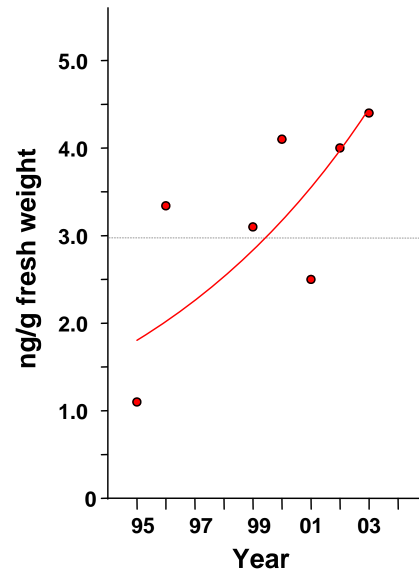

A.
pg/g lipids
> 100,000
> 1000
> 100
> 10
> 1
ND
Species
Crab
Eel
Herring
Mussel
Perch
Schrimp
Bothnian Bay
Bothnian Sea
Stockholm
14 (Askö)
15 (Kvädöfjärden)
West Coast
Baltic Proper
250 km


B.
Perch
Mussels
Algae
Std
Minutes



B.

Bromophenols				PBDDs
2,4,6	+	**2**	→	1,3
2,4	+	**2,4**	→	**2,7** 2,8 S
2,4	+	**2,6**	→	**1,8** 1,7 S
2,4,6	+	**2,4**	→	**1,3,8** 1,3,7 S
2,4	+	2,4,5	→	2,3,7
2,4,6	+	**2,4,6**	→	**1,3,6,8** 1,3,7,9 S
2,4,5	+	2,4,5	→	2,3,7,8


ng/g fresh weight
5.0
4.0
3.0
2.0
1.0
0
95
97
99
01
03
Year

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