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Identification of Polybrominated Dibenzo-*p*-dioxins in Blue Mussels (*Mytilus edulis*) from the Baltic Sea

ANNA MALMVÄRN,*,† YNGVE ZEBÜHR,‡ SÖREN JENSEN,† LENA KAUTSKY,§ ERIK GREYERZ,|| TAKESHI NAKANO,⊥ AND LILLEMOR ASPLUND‡

Department of Environmental Chemistry, Department of Applied Environmental Science (ITM), and Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden, Contaminant Research Group, Swedish Museum of Natural History, SE-104 05 Stockholm, Sweden, and Hyogo Prefectural Institute of Public Health and Environmental Science, 654-0037 Kobe, Japan

Polybrominated dibenzo-*p*-dioxins (PBDDs) are known to be formed as byproducts in connection with the manufacture and combustion of products containing brominated flame retardants. However, to date little is known about the occurrence of PBDDs in biological samples. The aim of the present investigation was to examine the presence of PBDDs in blue mussels (*Mytilus edulis*) from the Baltic Sea employing a procedure adapted for dioxin analysis. Two triBDDs (1,3,7-triBDD and 1,3,8-triBDD) were identified in biota here for the first time. This identification was based on accurate mass determination and comparison of retention times on three gas chromatographic columns of different polarities (PTE 5, SP-2331, and OV1701/heptakis) with synthesized standards, together with comparisons of electron capture negative ionization (ECNI) and electron ionization (EI) mass spectra. In addition, five PBDDs and one polybrominated dibenzofuran (PBDF) were tentatively identified; altogether, one diBDD, three triBDDs, three tetraBDDs, and one triBDF were detected in the blue mussels. To our knowledge this is the first time PBDDs have been identified in biota of the Baltic Sea. The ∑triBDD concentration in the blue mussels was estimated to be 170 ng/g lipids. The origin of these PBDDs remains unclear, but a plausible hypothesis could be biogenic formation in the marine environment.

Introduction

The highly toxic polychlorinated dibenzo-*p*-dioxins (PCDD) have been studied extensively during the past 30 years and found to be worldwide environmental contaminants present in various types of biological samples (*1*). Much less is known about the occurrence of polybrominated dibenzo-*p*-dioxins (PBDDs) in the general environment (*2*). However, the presence of PBDDs in biological samples has been reported, e.g., one tetraBDD in a mixed sample of fish and mussels (*3*), one tetraBDD and one pentaBDD in human adipose tissue (*4*), and hydroxylated and methoxylated PBDDs in Australian marine sponge *Dysidea dendyi* (*5*, *6*). On the other hand, PBDDs have not been detected in salmon, osprey, or human milk from Sweden (*7*) or carp from the United States (*8*).

It is well established that PBDDs are formed as byproducts in connection with the manufacture of brominated flame retardants (BFRs) (*2*), as well as during combustion of products containing such flame retardants (*2*, *9*, *10*). PBDDs are also present in exhaust fumes from motor vehicles (*11*). The knowledge concerning both exposure to and the health effects of these compounds is considerably more limited than in the case of their chlorinated homologues (*12*). The toxicities of different PBDD congeners differ. However, the binding affinity of the aryl hydrocarbon (Ah) receptor for certain triBDD congeners is almost as strong as for 2,3,7,8-tetraCDD (*12*, *13*).

Our earlier, preliminary, study indicated the presence of a triBDD in filter-feeding blue mussels (*Mytilus edulis*) living in the Baltic Sea (*14*). In addition, a putative triBDD, with the same MS fragmentation as a triBDD standard, was discovered in connection with an inventory of PCBs in blue mussels along the Swedish Baltic coast (*15*). The aim of the present investigation was to confirm our preliminary findings and to estimate the concentrations of PBDD in blue mussels, employing an analytical method developed for dioxin analysis and involving gas chromatography−high-resolution mass spectrometry (GC−HRMS).

Materials and Methods

Chemicals. Dichloromethane (DCM), *n*-hexane, and acetone of pesticide grade (Merck, Darmstadt, Germany), DCM and *n*-hexane of HPLC grade (Sigma-Aldrich, Steinheim, Germany), methyl *tert*-butyl ether (MTBE) of HPLC grade, distilled prior to use (Rathburn Chemicals, Walkerburn Scotland, UK), sulfuric acid, phosphoric acid, potassium hydroxide and sodium chloride of *pro analysis* quality, and silica gel (0.063−0.2 mm; Merck) and the ^{13}C-2,2′,3,4,4′,5,5′-heptachloro-biphenyl used as a syringe spike (I. S.) (Cambridge Isotope Laboratories, Andover, MA) were purchased from the sources indicated.

The PBDD/PBDF utilized as standards for identification, i.e., 1,3-/2,7-/2,8-/1,9-diBDD, 1,3,7-/1,3,8-/1,3,6-/1,3,9-/2,3,7-triBDD, 1,3,6,8-/1,3,7,9-/1,3,7,8-tetraBDD, 2,8-/2,7-diBDF, and 2,3,8-triBDF, were synthesized as described elsewhere (*16*). The congeners detected were quantified in relationship to 1,3,8-triBDF, 2,3,7-triBDD, 2,3,7,8-tetraBDF, 1,2,3,4-tetraBDD, and 2,3,7,8-tetraBDD, all of which were kind gifts from Prof. Stephen Safe (Texas A & M University, College Station, TX).

Instruments. Fractionation was performed initially using a semipreparative μBondapak aminopropylsilica column (300 × 7.8 mm, 10-μm particles; Waters, Milford, MA), a Hitachi L-620 intelligent pump fitted with a valve injector (Rheodyne 7125), equipped with a 180-μL injector loop, (Rheodyne 7067-005), automatic valve station and switching valves; and a UV−Vis detector (Hitachi L 4200) operating at 254 nm. The second fractionation was carried out with a 2-(1-pyrenyl)-ethyldimethylsilylated silica column (PYE, 150 × 4.6 mm, 5-μm particles; Cosmosil, Nacalai Tesque, Kyoto, Japan). A Peltier column oven (model 7955; Jones Chromatography,

* Corresponding author phone: +46-8-163781; fax: +46-8-163979; e-mail: anna.malmvarn@mk.su.se.
† Department of Environmental Chemistry, Stockholm University.
‡ Department of Applied Environmental Science (ITM), Stockholm University.
§ Department of Botany, Stockholm University.
|| Swedish Museum of Natural History.
⊥ Hyogo Prefectural Institute of Public Health and Environmental Science.

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Lakewood, CO) was employed to control the temperature of the columns (*17*, *18*).

GC−High-Resolution MS. GC−HRMS analyses were performed utilizing an HP 6890 GC (Hewlett-Packard, Avondale, PA) coupled to an Autospec Ultima magnetic sector mass spectrometer (Micromass, Ultricham, UK). Electron ionization (EI) was achieved at 32 eV and detection was carried out in the selected ion monitoring (SIM) mode. The instrument was set to a resolution of 10 000 and an acceleration voltage of 8000 V. The samples were on-column-injected to the nonpolar PTE 5 capillary column (15 m × 0.25 mm i.d., with 0.25-μm film; Supelco, Bellefonte, PA) with a siltek deactivated silica column (2 m × 0.53 mm; Restek, Bellefonte, PA) as the retention gap.

The samples were injected at 100 °C and the GC oven temperature was increased at a rate of 10 °C min^{-1} until 310 °C was reached and maintained for 5 min. The temperatures of the ion source and transfer line were kept at 250 and 290 °C, respectively. High-boiling perfluorokerosene (PFK) was used for calibration.

Other analyses were also performed on this instrument, but employing a SP-2331 column (30 m × 0.25 mm i.d. and 0.25-μm film thickness; Supelco) instead. In this case the column run was programmed as follows: 100 °C (2 min); increased at 15 °C min^{-1} until 200 °C was reached; and increased 4 °C min^{-1} to 265 °C (6.5 min). The instrument parameters were the same as those described above, with the exception that temperature of the transfer line was set to 265 °C.

GC−Low-Resolution MS. GC-LRMS analyses were performed on a single quadrupole, Finnigan MAT SSQ 710, connected to a Varian 3400 gas chromatograph and a split/splitless injector operated in the splitless mode. The GC capillary column (10 m, 0.25 mm i.d.) was coated with a mixture of OV1701 and heptakis (2,3-di-*O*-methyl-6-*O*-*t*-hexyl)-β-cyclodextrin (1:1) (*19*) and helium was used as the carrier gas (head pressure of 6 psi). The split was kept closed for 1 min and the GC oven temperature was programmed as follows: 80 °C (2 min); 20 °C min^{-1} until 170 °C was reached, 1 °C min^{-1} up to 200 °C (36 min); and increase of 2 °C min^{-1} to 220 °C. The mass spectrometer was run in the electron capture negative ionization (ECNI) mode with an electron energy of 70 eV, and the spectra scanned from 50 to 500 m/z. The temperatures of the ion source and transfer line were set to 120 and 250 °C, respectively.

Samples. Blue mussels (*Mytilus edulis*) were collected in September 1999 at a depth of 1−2.5 m along the Swedish coast close to Oxlesund (N 57° 33′, 62, E 16° 42′,00) in the Baltic Proper. The mussels, 25−40 mm in diameter, were hung in a string bag overnight to allow discharge of excrement, and the total mussel tissue (40 g fresh weight, without shells) was stored at −20 °C until analysis in September 2003.

Extraction and Cleanup Procedures. Extraction and cleanup procedures are illustrated in Figure 1.

Extraction and Sulfuric Acid Treatment. The extraction of the frozen mussel tissue was based on a method described by Jensen et al. (*20*, *21*) with the modification that diethyl ether was replaced with MTBE. The sample (40 g) was divided into five equal parts which were extracted separately. The lipid content of the extracts thus obtained was determined gravimetrically to be 1.4%. To remove lipids, each sample, dissolved in *n*-hexane (4 mL), was treated with concentrated sulfuric acid (2 mL), following which the sulfuric acid phase was re-extracted with *n*-hexane (3 mL). This entire procedure was repeated once and, finally, all of the hexane extracts were pooled.

Fractionation on a Silica Gel Column. The solvent volume was reduced under nitrogen to approximately 100 μL and then transferred to a Pasteur pipet that contained silylated glass wool and silica gel (1 g; activated 300 °C overnight), pre-washed with *n*-hexane (10 mL). This column was then eluted successively with 5 mL of *n*-hexane (fraction 1), 13 mL of hexane (fraction 2 containing PBDDs), and 12 mL of DCM (fraction 3 containing MeO−PBDEs).

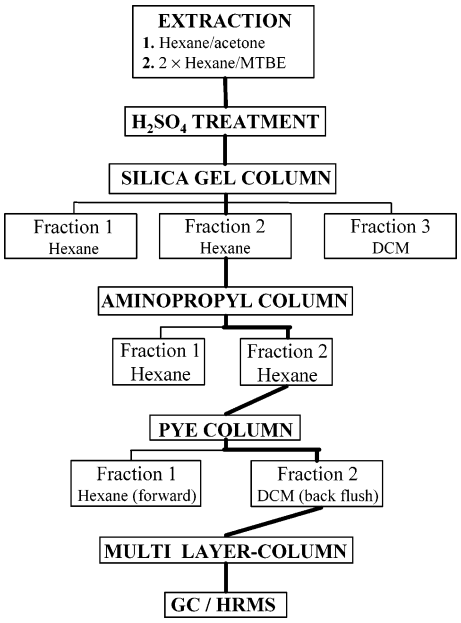


FIGURE 1. Procedure for the extraction and cleanup of the analytes from blue mussel tissue.

Fractionation on an Aminopropyl Column. Fraction 2 from the silica gel column was subsequently further fractionated at room temperature on an aminopropyl silica column, employing elution with *n*-hexane in the straight-phase mode. The first fraction thus obtained contained primarily aliphatic and one-ring aromatic compounds and was not analyzed further. The second fraction, obtained after 3−12 min at a flow rate of 3 mL/min, contained compounds with two aromatic rings, including polyhalogenated-*p*-dibenzodioxins (PXDD, X = Cl, Br), dibenzofurans (PXDF), and polyhalogenated biphenyls (PXB) (*22*), and was collected for further cleanup.

Fractionation on a PYE Column. The diaromatic fraction collected from the aminopropyl column was reduced under nitrogen to a volume of 20 μL and thereafter further fractionated on a 2-(1-pyrenyl)-ethyldimethylsilylated silica (PYE) column to separate the coplanar compounds (e.g., PXDD and PXDF) from less planar compounds (e.g., mono- to tetra-ortho-substituted PCB). In the first step, the mono- to tetra-ortho-substituted PCBs were eluted with *n*-hexane, (10.5 min with a flow rate of 0.7 mL/min at −0.5 °C) in the forward direction; following which the target compounds, PXDD and PXDF, were back-flushed out from the column with DCM (10 min with a flow rate of 1.5 mL/min at 25 °C).

Afterward, the coplanar fraction was eluted (with *n*-hexane) through a short open Pasteur pipet column containing three layers of modified silica (0.3 g). From the top to the bottom: silica treated with concentrated sulfuric acid (40% w/w), potassium silicate (silica allowed to react with potassium hydroxide in methanol), and silica deactivated with 10% water. This final cleanup step was designed to remove contaminants that "bled" from the PYE column. The fraction thus obtained was reduced in volume to 15 μL prior to GC−MS analysis.

The method was validated utilizing PBDD standards and blanks were run in parallel with the samples through the entire procedure. Both the extraction and cleanup were performed in the dark or with protection by aluminum foil to prevent debromination of the analytes.

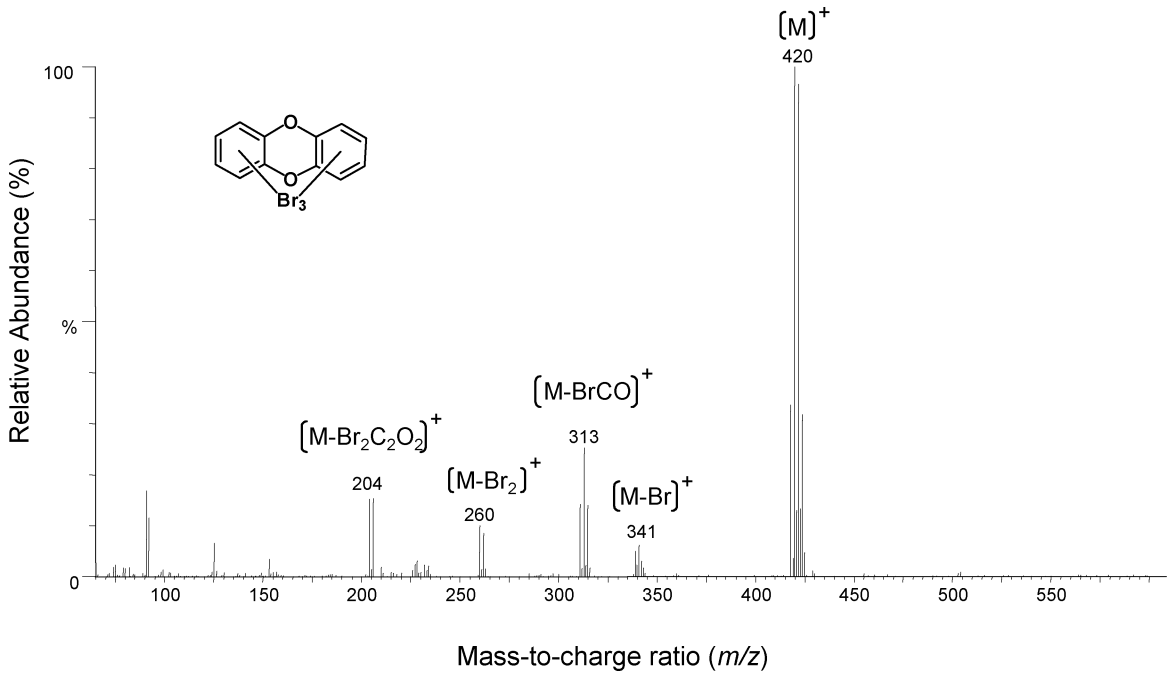


FIGURE 2. Full-scan MS (EI) spectrum of the triBDD (C) in the mussel sample.

Accurate Mass Determination. Accurate determination of the mass of triBDD was first carried out with a magnetic scan over the range of 50−500 daltons (Da) at a resolution of 10 000. Secondary mass calibration was performed employing high-boiling PFK as the standard. In addition, a voltage scan over the mass range from 410 to 433 Da was also performed, encompassing both the triBDD M^+-cluster and two ions from PFK. In a third attempt, based on the method suggested by Grange et al. (*23*), an intensity profile for the response was constructed with an SIM descriptor using small increments (0.002 Da) around the theoretical mass of triBDD (22 discrete masses), as well as over the mass peaks of PFK ions (5 discrete masses). This experiment was performed at resolutions of 10 000 and at 20 000.

Quantification. The sample was analyzed in SIM mode, monitoring two ions, one quantification ion, and one qualifier ion (the two most intense ions of the molecular ion isotope distribution clusters) for every substance. For analysis of PBDD, the following ions were monitored: 341.8715 and 339.8735 (diBDD); 405.7850 and 403.7870 (triBDF); 419.7819 and 421.7800 (triBDD); 499.6904 and 497.6925 (tetraBDD), and for tetraBDF only the 481.6975 ion. As lockmass and lockmass check, the 430.9728 ion originating from PFK was used. Quantification was achieved by comparison with a standard mixture containing known amounts of 1,3,8-triBDF, 2,3,7-triBDD, 2,3,7,8-tetraBDF, and 2,3,7,8-/1,2,3,4-tetraBDD. ^{13}C-2,2′,3,4,4′,5,5′-heptachlorobiphenyl (405.8427) was employed as a syringe spike and was used in the response factor calculations. It was added to the sample following cleanup, since this compound is otherwise separated from the analytes on the PYE column.

Results

Identification. The full-scan GC−MS (EI) spectrum of the triBDD present in the dioxin fraction from the PYE column (Figure 2) revealed a molecular ion ($m/z = 418$) and a tribromine isotope cluster, as well as, an intense ion at $m/z = 311$, a characteristic fragment ion $[M - BrCO]^+$, produced by EI ionization of PBDD (*24*). The identity of the triBDD ($C_{12}H_5O_2Br_3$) was then further confirmed by accurate mass determination. A magnetic scan at a resolution of 10 000 indicated a mass of 417.788, which is 4 millidalton (mDa) more than the correct value of 417.7840. Furthermore, a second determination employing voltage scan produced a similar result. In a third attempt based on the method suggested by Grange et al. (*23*), the mean value of three runs at a resolution of 20 000 was 417.7840, which is no more than 0.04 mDa greater than the theoretical value of 417.78396. This latter approach is both more sensitive and known to be more accurate than other more commonly used methods (*23*−*27*).

Gas Chromatographic Separation. The dioxin fraction prepared from the blue mussels was analyzed for di- to tetraBDD/BDF by GC−HRMS, SIM. The GC−HRMS chromatogram (sum of the SIM ions) from the PTE 5 column is shown in Figure 3. The retention time of the triBDD present was identical to the retention time for the three triBDD congeners employed as authentic reference standards, namely, 1,3,6-, 1,3,7-, and 1,3,8-triBDDs (C), which all coelute on the PTE 5 column. Additionally, PBDDs were also found in the sample and tentatively identified as 2,3,7-triBDD (D), 2,3,7,8-tetraBDD, and/or 1,2,3,4-tetraBDD (G). Furthermore, there were indications that the sample also contained two other tetraBDDs (E and F), one diBDD (A), and one triBDF (B), but due to the lack of standards and the possibility of coelution, these identifications are only preliminary. The results are summarized in Table 1: altogether, one diBDD, three triBDDs, three tetraBDDs, and one triBDF were detected in the blue mussels.

The PTE 5 column was used for tentative identification of the PBDD congeners present in the mussel sample, the PBDD standard employed was a mixture of several triBDD congeners of which 1,3,7-, 1,3,8-, and 1,3,6-triBDD, as mentioned above, coelute on this column. Therefore, two additional GC columns were also tested in an attempt to separate these triBDD congeners. First, an SP-2331 column, on which the retention order for the triBDDs is known (*16*), produced a slightly better separation of these congeners than did the PTE 5 column (Figure 4). This figure shows the presence of two triBDD congeners, i.e., 1,3,7-triBDD and 1,3,8-triBDD, in the sample, and four congeners in the standard mixture.

Even better separation was achieved employing a column normally used for the separation of chiral compounds (Figure

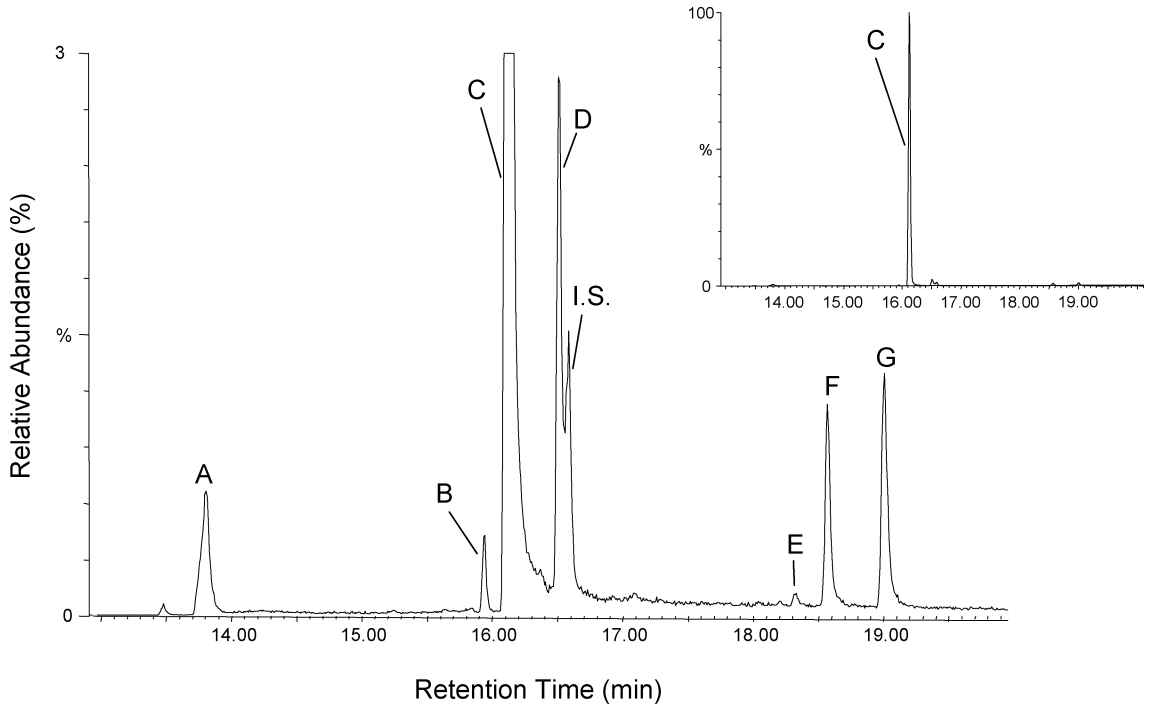
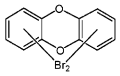
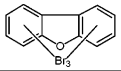
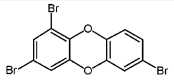
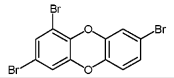
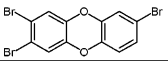
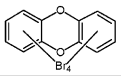
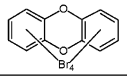
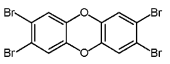
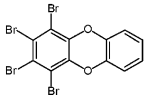


FIGURE 3. GC−HRMS (EI) chromatogram (sum of the SIM ions) of the di- to tetraBDDs/BDFs, detected in the PYE fraction isolated from the blue mussels, on the nonpolar PTE 5 column. The ^{13}C-2,2′,3,4,4′,5,5′-heptachloro-biphenyl was used as a syringe spike (I. S.). The structures and names of the peaks marked in the chromatogram are shown in Table 1.

TABLE 1. Structures and Levels (per g lipid) of the Compounds Detected in Blue Mussels from the Baltic Sea

	Abbreviation	Structure	id. no.[a]	ng/g
Dibromo-dibenzo-*p*-dioxin	DiBDD[b]		A **	n.q.
Tribromo-dibenzo-*p*-furan	TriBDF		B **	1
1,3,7-Tribromo-dibenzo-*p*-dioxin	1,3,7-TriBDD		C *	160
1,3,8-Tribromo-dibenzo-*p*-dioxin	1,3,8-TriBDD			
2,3,7-Tribromo-dibenzo-*p*-dioxin	2,3,7-TriBDD		D **	7
Tetrabromo-dibenzo-*p*-dioxin	TetraBDD[b]		E **	0.1
Tetrabromo-dibenzo-*p*-dioxin	TetraBDD		F **	n.q.
2,3,7,8-Tetrabromo-dibenzo-*p*-dioxin	2,3,7,8-TetraBDD		G **	2
1,2,3,4-Tetrabromo-dibenzo-*p*-dioxin	1,2,3,4-TetraBDD			

[a] The identifying letters assigned to the PBDD/PBDFs here are the same as those in Figure 3. *1,3,7-triBDD and 1,3,8-triBDD were identified by comparison of their retention times on three GC columns with those of standards and by accurate mass determination. **Additional PBDDs/PBDF present in the sample were only tentatively identified using a single GC column. [b] The diBDD (A) and the tetraBDD (F) were not quantified (n.q.).

5), revealing the presence of two triBDDs in the sample and four in the standard mixture. The four triBDD congeners in the standard mixture could be completely separated on this chiral column, however, it was not possible to establish the identity of the individual triBDDs, since the order of their elution was unknown. The peak C (Figure 4) in the blue

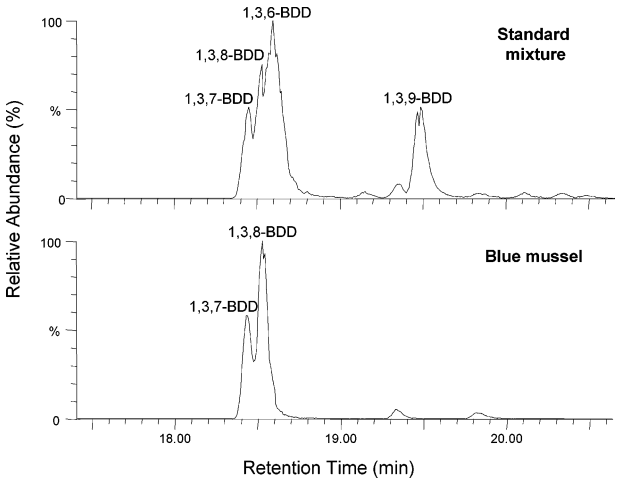


FIGURE 4. GC−HRMS (EI, SIM) chromatograms of the standard solution (containing 1,3,7-triBDD, 1,3,8-triBDD, 1,3,6-triBDD, and 1,3,9-triBDD; upper chromatogram) and the blue mussel sample (lower chromatogram), obtained employing a polar SP-2331 column.

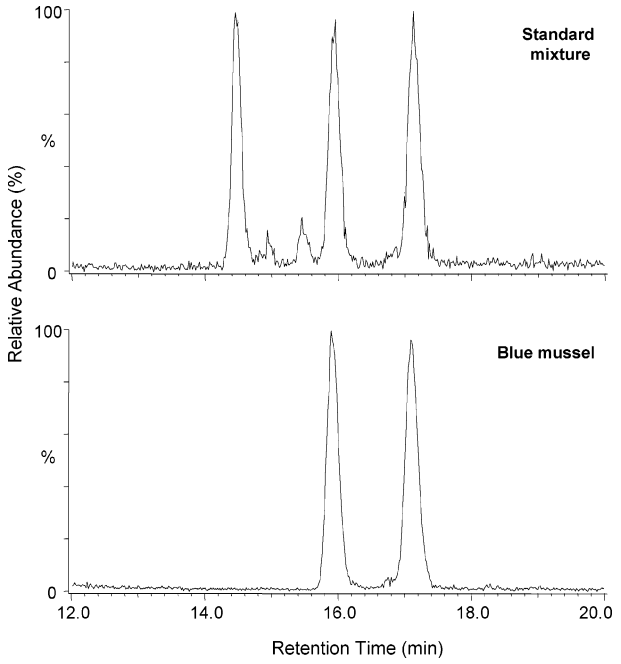


FIGURE 5. GC−MS (ECNI) chromatograms of the molecular ion (*m/z* 418, 420) of the triBDD analyzed on a chiral column. The upper chromatogram depicts the standard mixture (see Figure 4 caption) and the lower chromatogram depicts the mussel sample.

mussel sample represented two triBDD congeners, i.e., 1,3,7-triBDD and 1,3,8-triBDD.

Quantification. The quantification was performed on the PTE 5 column and concentrations in the mussel sample were calculated by comparison with the responses of the authentic PBDD standards analyzed in parallel. In this manner the concentrations of the PBDDs in blue mussel tissue were estimated to be 160 ng/g (l.w.) for 1,3,7-triBDD + 1,3,8-triBDD, 7 ng/g for the 2,3,7-triBDD, and 2 ng/g for the 2,3,7,8-tetraBDD + 1,2,3,4-tetraBDD (Table 1).

Discussion

In an earlier study we reported the presence of an unknown brominated compound in blue mussels from the Baltic Sea, which was tentatively identified as a triBDD (*14*). However, since ortho substituted OH-PBDEs were present in the phenolic fraction of the mussel sample, in relatively high concentrations (*28*), artificial formation of PBDD during the cleanup and analysis had to be addressed. For this reason, a new mussel sample was analyzed here employing a method adapted for dioxin analysis, including GC−HRMS (Figure 1) (*17*).

As a first step, a silica gel column was used to separate dioxins from brominated phenols and OH−PBDEs. Thereafter a PYE column was employed to separate planar compounds, such as dioxins, from less planar substances. The putative PBDDs were recovered in the second hexane fraction eluted from the silica gel column and in the coplanar fraction obtained from the PYE column. Thus, the putative PBDDs behave in the same manner as the PBDD standards tested in these chromatographic systems.

Independent of the previous study by Malmvärn et al. (*14*), there were indications for the presence of a triBDD in blue mussels collected along the southern Swedish Baltic coast in connection with a PCB inventory study (*15*). A GC/ECD chromatogram of one of the blue mussel samples from this inventory study is shown in Figure 6. The first part of the chromatogram shows organohalogen substances (OHS) such as DDE and PCB in low levels. In the later part of this chromatogram, three peaks dominate: the putative triBDD itself, followed by two methoxylated brominated diphenyl ethers (MeO−BDE68 and MeO−BDE47). These three peaks appeared in all samples except for those collected in the southernmost part of the Baltic Sea (Table 2).

At present, little information concerning occurrence and transport of PBDDs is available. In most investigations concerning PBDDs, the samples are collected in areas directly exposed to automobile exhaust fumes and/or other products of combustion (*2*, *11*). Among the few studies on the presence of PBDDs in biological material, 2,3,7,8-tetraBDD has been detected in the adipose tissue of Japanese subjects at levels of 0.1−4.2 pg/g lipids (*4*) and one tetraBDD, of unknown structure was found, but not quantified in a mixed sample of fish and mussels (*3*).

In the present study the concentration of the two coeluting triBDD congeners was estimated to be as high as 160 ng/g lipids; whereas the concentrations of the other PBDD/PBDF congeners were 20−1600-fold lower (Table 1). The ∑PCDD concentrations in blue mussels from the Baltic Sea has been reported to be 310 pg/g dry weight (*29*) approximately i.e., 70-fold lower than the ∑PBDD concentrations in the blue mussels analyzed here.

The knowledge concerning the toxicity of brominated dioxins has been summarized in a recent review (*12*). In general, all of the toxic effects observed with PCDD/PCDF have also been observed in the limited number of studies on PBDD/PBDF (*2*). With respect to binding to the Ah receptor, 2,3,7,8-tetraBDD exhibits a relative binding affinity of 0.66 compared to the corresponding chlorinated homologue (2,3,7,8-tetraCDD), which is considered to be the most toxic of the chlorinated homologues and whose binding affinity has been assigned a value of 1. The corresponding value for 2,3,7-triBDD is 0.8, which is substantially greater than for the chlorinated homologue of this compound. On the other hand, 2,3,7-triBDD has been reported to be less potent than 2,3,7,8-tetraBDD as an inducer of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities (*12*). With regard to toxicity in fish, all PBDD congeners investigated cause mortality at an early stage of life, with 2,3,7,8-tetraBDD being suggested to be even more potent than 2,3,7,8-tetraCDD in this respect (*30*).

The triBDD congeners (1,3,7- and 1,3,8-triBDD) shown here to be present in blue mussels from the Baltic Sea are substituted with bromine in two of the 2,3,7,8-positions, a substitution pattern which results in a high degree of toxicity in the case of the chlorinated dioxin homologues. To our knowledge, the toxicity of these triBDD congeners has not

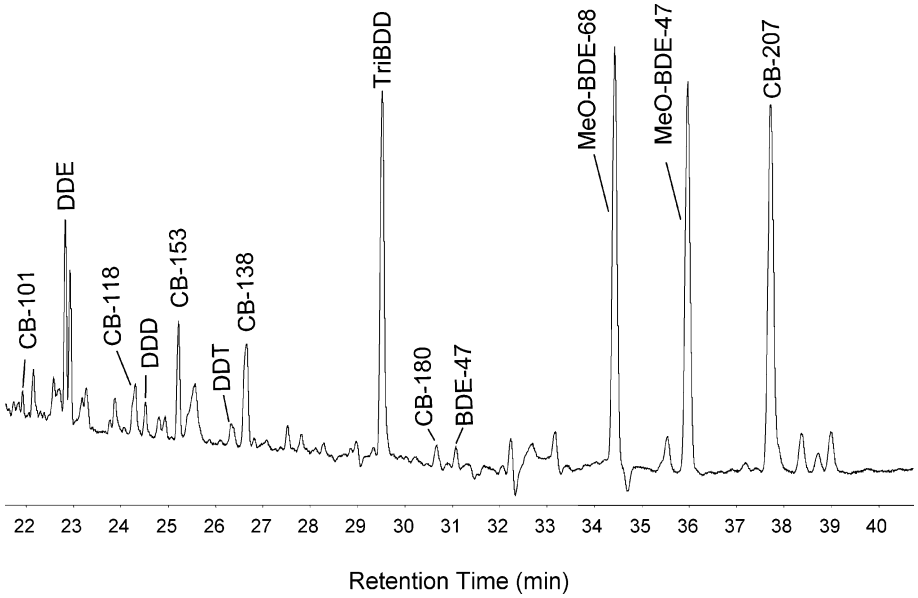


FIGURE 6. GC chromatogram of an extract from blue mussels reported from the PCB inventory study (*15*). The GC was fitted with a nonpolar column (CP-Sil 8CB; 60 m × 0.25 mm i.d., with 0.25-μm film). The mussel sample was extracted according to Jensen et al. (*21*) and the lipids were removed by treatment with sulfuric acid prior to injection onto the Varian 3400 GC, equipped with an electron capture detector (ECD). The 2,2′,3,3′,4,4′,5,6,6′-nonaclorobiphenyl (CB-207) was used as an internal standard (I. S.). The BDE-47 concentration in this mussel sample was estimated to be around 10 ng/g lipids.

TABLE 2. Concentrations, in ng/g Lipid Weight, of Compounds Analyzed in Blue Mussels from the Baltic Sea in Connection with the PCB Inventory Study (*15*)[a]

location	∑ DDT[b,c]	∑ PCB[c,d]	CB-153	TriBDD[e]	MeO−BDE68[f]	MeO−BDE47[f]	lipid %
(1) Archipelago of Stockholm	52	380	42	30	120	190	1.2
(2) Södermanland	58	380	65	50	110	180	0.9
(3) Östergötland	42	210	40	60	150	220	1.0
(4) Småland	51	260	49	160	300	350	1.2
(5) Gotland	60	190	37	220	250	570	0.9
(6) Blekinge	44	220	43	70	300	350	1.0
(7) Skåne	104	180	33	n.d.	n.d.	n.d.	0.9

[a] The sampling sites are shown on the map and the lipid content of the mussels is given in % of fresh weight. [b] ∑DDT is the sum of DDE, DDD, and DDT. [c] ∑DDT and ∑PCB concentrations are mean values of five replicates of homogenized mussels from each location. [d] ∑PCB was calculated from the major PCB, CB-138, since the PCB pattern in mussels from the studied area is known to be rather constant. The quantification of the DDT and PCB congeners was performed in relation to standard curves. [e] The concentrations of triBDD were estimated on GC-ECD by comparison with the response factor of the quantified triBDD in the mussel sample from Oxlesund (Materials and Methods section). [f] The quantification of the MeO−PBDEs was achieved by comparison with the corresponding individual authentic external standards.

yet been tested. The introduction of nonlateral bromine substituents tends to decrease the affinity of binding to the Ah receptor (*13*); but even if the triBDDs identified are only moderately toxic, their high concentrations (160 ng/g) in blue mussels may make them of ecotoxicological relevance and significance for human exposure.

The origin of the PBDDs isolated from blue mussels in the Baltic Sea remains unknown. They may originate from anthropogenic activities, but biogenic formation is also a plausible explanation for the presence of triBDD in the marine environment. Such biogenesis could possibly be direct and/or involve the dimerization/biotransformation of precursor molecules such as brominated phenols and/or OH−PBDE. In this context, OH−PBDEs have been found in relatively high concentrations in both blue mussels and red algae (*28*) and, in addition, are known to be natural products formed, for instance, in marine sponges (*31*−*34*).

Since all of the OH−PBDEs identified to date in blue mussels from the Baltic Sea (*28*) are substituted with a hydroxyl- group in the position ortho to the diphenyl ether oxygen and a bromine substituent in the same position in the other phenyl ring, they may give rise to PBDD via ring closure. If this is the case, the possible precursors of 1,3,7-triBDD and 1,3,8-triBDD would be OH−BDE47 and OH−BDE68, respectively, both of which are present in blue mussels (*28*). Although the remaining PBDDs present in these mussels

appear to have no potential OH−PBDE precursors, the identities of these congeners is uncertain, since certain PBDD congeners coelute on the nonpolar GC-column and, in addition, many standards are yet not available. Furthermore, the concentrations of such congeners were considerably lower, which may indicate that they arise from anthropogenic sources. One possible approach to determining whether the origin of PBDDs is antopgenic or natural could be to investigate the ^{14}C content of these compounds (*35*, *36*). This was preformed recently with two MeO−PBDEs (MeO−BDE68 and MeO−BDE47) isolated from whale, in which case the high content of ^{14}C confirmed the natural origin of these substances (*37*).

To our knowledge, PBDDs of natural origin have never been identified, even though both hydroxylated and methoxylated PBDDs, as well as nonhalogenated dioxins are known natural products (*5*, *6*, *38*). The triBDDs are present at high levels in blue mussels, and moreover, the PCB inventory study indicates that these compounds are widely spread in the Baltic environment (Table 2). Both these observations support the hypothesis that the PBDDs in blue mussels are of natural origin. Further investigation is required in order to evaluate the ecotoxicological and toxicological significance of these PBDDs found in the Baltic Sea.

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