

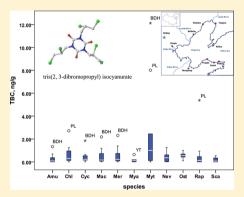


Tris(2,3-dibromopropyl) Isocyanurate, Hexabromocyclododecanes, and Polybrominated Diphenyl Ethers in Mollusks from Chinese Bohai Sea

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Supporting Information

ABSTRACT: A novel brominated flame retardant (BFR), tris(2,3-dibromopropyl) isocyanurate (TBC), as well as hexabromocyclododecanes (HBCDs) and polybrominated diphenyl ethers (PBDEs), were analyzed in 11 species of mollusks collected from nine coastal cities around the Chinese Bohai Sea in 2009 and 2010. The detection frequencies were 100%, 99%, and 77% for PBDEs, HBCDs, and TBC, respectively. Concentrations of Σ HBCDs ranged from below detection limit (nd) to 28.8 ng g⁻¹ on a dry weight (dw) basis, followed by Σ_{12} PBDE (0.01–20.4 ng g⁻¹ dw) and TBC (nd–12.1 ng g⁻¹ dw). Statistically significant linear correlations were found among the three BFRs. Positive correlations were found between BFRs concentrations and lipid content in mollusks. The concentrations tend to decrease with increasing trophic levels of the mollusks, implying trophic dilution rather than biomagnifications of the BFRs in the aquatic food chains of the sampling area. Among the 11 mollusks species,



Mytilus edulis showed higher bioaccumulation capability than others and was therefore considered to be an appropriate bioindicator of contamination by the BFRs in the Chinese Bohai Sea, in agreement with its previous selection for the biomonitoring of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). A dramatic decrease in PBDE concentrations in mollusks of the area was found for the time period from 2003 to 2010, with a half-life of only 2.3 ± 1.7 years, reflecting a rapid response of mollusks to the change in pollution of the marine environment.

■ INTRODUCTION

Brominated flame retardants (BFRs) have been widely utilized for the past several decades around the world. In China, the estimated domestic production of BFRs is 81 000 tonnes in 2006. Among the commercial BFRs, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) and tetrabromobiphenol A (TBBPA) have been manufactured with the highest production volumes both globally and in China.² Due to their adverse effect on the environment and human health, two commercial PBDE products have been included in the list of persistent organic pollutants (POPs) in the Stockholm Convention on POPs, while HBCDs are currently under assessment for possible inclusion.³⁻⁶ A novel BFR, tris(2,3-dibromopropyl) isocyanurate (TBC), has recently been identified in the environment.⁷ The first reported use of TBC as an additive flame retardant in styrene polymers appeared in 1959,8 which was followed by a number of patents in Europe and Japan in the 1970s, although information on its large-scale production in these countries has not been found. A technical product containing TBC is Armoquell FR930 manufactured by AkzoNobel Functional Chemicals headquartered in The Netherlands and operated in many countries. The product is a white to off-white powder specially developed for use in polypropylene, polyethylene, and polystyrene. In China, TBC has been widely applied in polymer products since the mid-1980s. The number of patent applications involving TBC as flame retardant has increased in recent years, mostly from Japan and China. Following the cessation of commercial production of penta- and octa-BDEs worldwide, deca-BDE production has been phased out in Europe and its use in the U.S. will end. Therefore, increasing production and use of TBC may be expected.

Nonetheless, the potential environmental occurrence of TBC has not been given attention until recently. It was recently detected in the water, sediment, and biota samples near a manufacturing factory in southern China⁷ at concentrations as high as 6000 ng/g dry weight in the sediment. However, there

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is no study on the investigation of the environmental occurrence of TBC outside manufacturing source affected areas. With a hexabrominated heterocyclic s-triazine structure (Figure 1), TBC has high thermal stability, durability, and

Figure 1. Molecular structure of tris(2,3-dibromopropyl) isocyanurate (TBC).

resistance to photodegradation based on model calculations.⁷ Its high calculated octanol—water partition coefficient (log $K_{\rm ow}$ = 7.37) and bioconcentration factor (log BCF = 4.30) indicate that TBC is bioaccumulative.¹⁰ The long-range transport potential of TBC is unknown, but the estimated low vapor pressure (1.57 × 10⁻¹³ Pa) and high octanol—air partition coefficient (log $K_{\rm oa}$ = 23.7) suggest it is strongly sorptive to surfaces and particles. Recent studies on zebrafish embryos suggested that exposure to TBC causes a variety of potential reproductive and endocrine disrupting toxic effects, damaging mitochondria and causing defects in gas bladder inflation.^{11,12}

Mollusks are invertebrate benthic organisms that are present in marine and fresh water with a wide geographical distribution. They have many characteristics that are suitable as bioindicators of POPs contamination. As water-respiring filter feeders, mollusks have high bioaccumulation potential for POPs due to their relatively high lipid content and low metabolic enzyme activities. Mollusks have good tolerance to a wide spectrum of pollutants which often coexist in polluted water and sediment and are easy to be identified and collected. Mollusks have therefore been commonly used to assess organic pollutant contamination in aquatic systems. 14

This study was carried out to investigate the levels of TBC, HBCD, and PBDEs in mollusks collected from coastal areas along the Chinese Bohai Sea. The objectives are to study the spatial distribution patterns and compare them among different mollusk species as bioindicator. To our knowledge, this is the first study investigating the environmental occurrence of TBC at locations far away from its manufacturing sites. HBCDs show many characteristics of POPs, 15 but there is currently no regulation or control on their production or use in China. Compared to PBDEs, research on the environmental occurrence of HBCDs in China is relatively scarce and mostly in source regions such as e-waste recycling areas and industrial areas. 16,17 No previous examination on HBCD contaminations in mollusks from the study region has been reported. HBCDs are therefore included in this work. The inclusion of PBDEs in this work allowed not only a comparison among the BFRs but also the delineation of temporal trend by combining the data from this work with those of our earlier study. 18

■ EXPERIMENTAL SECTION

Sampling. In August 2009 and 2010, 11 species of mollusks were collected from nine coastal sites (Figure S1, Supporting

Information) near the cities of Dalian (DL), Yingkou (YK), Huludao (HLD), Beidaihe (BDH), Tianjin (TJ), Shouguang (SG), Penglai (PL), Yantai (YT), and Weihai (WH). These sampling locations encompass the near shore areas of four Chinese provinces in the Bohai Bay Rim Economic Zone, which has experienced rapid socioeconomic development in the past decade.

The 11 selected species of mollusks were Neverita didyma (Nev), Rapana venosa (Rap), Mya arenaria (Mya), Cyclina sinensis (Cyc), Chlamys farreri (Chl), Scapharca subcrenata (Sca), Meretix meretrix (Mer), Mytilus edulis (Myt), Crassostrea talienwhanensis (Ost), Amusium veneriformis (Amu), and Mactra veneriformis (Mac). Their denominations are presented in Table S1 (Supporting Information). The mollusks were depurated in filtered water for about 12 h when sampling, transported to the laboratory on ice, and then cleaned by water in the laboratory. The soft tissue of the mollusks was excised by stainless steel scalpel blades and thoroughly rinsed with Milli-Q water to remove extraneous impurities. For each species from a sampling site, about 500-1500 g of wet soft tissue (consisting of 3-30 individuals) was homogenized in a blender to form one composite sample. The samples were kept at -20 °C until analysis. A total of 131 composite soft tissue samples were obtained and analyzed (94 in 2009 and 37 in 2010).

Sample Preparation. The homogenized samples were freeze-dried and sieved. Sample pretreatment was based on our previous methods^{19,20} with modifications. Briefly, 1.5 g of dried sample was mixed with 15 g of anhydrous sodium sulfate; spiked with ¹³C-labeled BDEs 47, 99, and 153 and γ-HBCD (Wellington Laboratories, Guelph, Canada) surrogate standards; and extracted on an accelerated solvent extractor (Dionex ASE350) in a mixture of 1:1 (v:v) dichloromethane:hexane at 150 °C and 1500 psi. About 10 g of acidic silica (30%, w/w) was added into the extract to remove lipid. Then, a glass column filled with anhydrous sodium sulfate was used to filter the extract. The concentrated extract was cleaned up by elution with 100 mL of hexane and 100 mL of DCM on a glass column (20 cm length and 18 mm i.d.) packed with, from bottom to top, 1 g of activated silica gel, 4 g of basic silica gel (1.2% w/w), 1 g of activated silica gel, 8 g of acid silica gel (30%, w/w), 2 g of activated silica gel, and 4 g of anhydrous sodium sulfate. PBDEs were eluted in the first 100 mL fraction while HBCDs and TBC in the second fraction. The two fractions were separately rotary-evaporated and concentrated under a gentle nitrogen flow. Solvents of the first fraction and the second fraction were changed to 20 µL of nonane and 100 µL of methanol, respectively. Finally, [13C12]-labeled 2,2',3,4,4',5'hexachlorobiphenyl (CB138, 1668A-IS) and $[^2H_{18}]$ -labeled γ -HBCD (Wellington Laboratories, Guelph, Canada) were added into the first and the second concentrated fractions, respectively, prior to instrumental analysis.

Instrumental Analysis. Thirteen PBDE congeners including BDEs 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, and 190 were analyzed using a high-resolution gas chromatograph (Agilent 6890) coupled with a high resolution mass spectrometer (Waters Micromass). Three major HBCD diastereoisomers (α -, β -, γ -HBCD) and TBC were simultaneously analyzed using a high-performance liquid chromatograph (Waters 2695) equipped with a triple-quadrupole mass spectrometer (Waters Micromass). BDEs 47, 99, and 153 and γ -HBCD were quantified by isotope dilution methods, while other target analytes were quantified by the internal standard

Table 1. Concentrations of PBDEs, HBCD, and TBC in Mollusks from Chinese Bohai Sea^a

	Amu	Chl	Сус	Mac	Mer	Mya	Myt	Nev	Ost	Rap	Sca	All
N	11	10	11	7	18	6	10	11	11	25	11	131
trophic level	3.05	2.96	3.07	2.99	2.96	2.66	2.79	3.23	3.02	3.43	3.07	
lipid (%)	11.1	14	7.5	7.9	13.4	11.1	15.3	6.5	15.7	6.7	13.2	
					TBC (DF = 77%)						
ng/g dw												
mean	0.27	0.66	0.42	0.65	0.49	0.19	2.64	0.41	0.54	0.46	0.19	0.60
median	0.08	0.30	0.22	0.22	0.22	0.14	1.01	0.36	0.54	0.12	0.07	0.21
min	nd	nd	nd	nd	nd	nd	nd	nd	0.10	nd	nd	nd
max	1.33	2.72	1.85	2.18	2.31	0.64	12.1	1.25	1.00	5.38	0.54	12.1
ng/g lw												
mean	2.44	4.70	5.59	8.20	3.66	1.71	17.2	6.30	3.44	6.90	1.44	5.60
median	1.08	2.18	3.26	2.02	1.83	1.21	6.68	5.54	3.50	1.81	0.38	2.06
min	nd	nd	nd	nd	nd	nd	nd	nd	0.76	nd	nd	nd
max	13.6	19.4	26.7	29.9	14.6	5.77	139	21.2	6.37	80.3	6.84	139
					\sum HBCD	s (DF = 999	%)					
ng/g dw												
mean	3.78	6.76	2.69	11.8	3.36	8.91	12.1	3.11	9.25	3.28	5.15	5.53
median	4.36	6.99	1.99	7.48	2.14	8.63	11.74	1.67	7.96	2.61	3.24	3.62
min	0.35	1.39	0.33	3.19	nd	2.54	3.63	0.35	1.91	0.23	0.40	nd
max	6.26	14.1	7.83	28.8	12.1	14.7	27.7	15.1	20.0	11.2	15.7	28.8
ng/g lw												
mean	34.2	48.2	35.8	148	25.1	80.2	78.9	47.8	58.9	49.2	39.0	58.7
median	31.0	47.5	26.5	64.4	15.2	75.3	78.1	27.4	58.7	39.0	19.2	39.0
min	7.69	9.93	4.40	nd	nd	25.4	23.37	6.62	12.2	4.20	3.03	nd
max	59.2	147	98.8	370	103	134	166	151	129	162	119	370
					$\sum_{12} PBDE$	s (DF = 100	0%)					
ng/g dw												
mean	0.72	2.09	0.57	1.37	0.95	1.63	5.45	0.40	1.99	0.84	0.79	1.39
median	0.55	2.22	0.35	0.62	0.48	0.86	3.56	0.29	1.44	0.50	0.67	0.76
min	0.19	0.80	0.12	0.45	0.21	0.62	0.01	0.04	0.93	0.15	0.38	0.01
max	1.54	4.26	1.54	4.44	2.54	4.94	20.43	0.78	3.35	4.17	1.53	20.4
ng/g lw												
mean	6.52	14.9	7.59	17.3	7.11	14.7	35.6	6.14	12.7	12.6	5.98	12.8
median	5.61	14.5	4.67	8.50	3.92	7.78	26.2	4.46	10.1	7.46	4.30	7.56
min	1.71	5.71	1.60	5.70	1.57	5.27	0.07	0.62	5.92	3.88	2.88	0.07
max	11.1	30.7	25.2	57.1	21.6	49.4	129	16.1	21.1	91.7	14.7	129

"DF (%) = detection frequency; nd = below the MDL. \sum_{12} PBDEs = sum of 12 major congeners; \sum HBCDs = sum of α -, β - and γ -HBCD.

method. More detailed descriptions of the instrument operational procedures can be found in our previous papers. 19,20

Quality Control. One procedural blank of 15 g of anhydrous sodium sulfate was simultaneously analyzed with each batch of five samples. Results showed that HBCDs and TBC in blanks were below the detection limits, while PBDEs (mostly BDEs 47 and 28) were <5% of the total PBDEs of real samples. The data reported in this paper were not corrected on the basis of the levels in the blanks.

Six aliquots of a composite mollusk sample were analyzed in replicates, with three of them unspiked and the other three spiked with 100 ng each of the α -HBCD, β -HBCD, γ -HBCD, and TBC native standards. The recoveries (average \pm standard deviation, N=3) were 64.6 \pm 8.0%, 74.9 \pm 16.5%, 106.1 \pm 5.1%, and 84.1 \pm 10.8% for the spiked α -HBCD, β -HBCD, γ -HBCD, and TBC, respectively. For all the mollusk samples (N=131), recoveries of 13 C-labeled BDEs 47, 99, and 153 were 72.2 \pm 18.8%, 42.7 \pm 13.1%, and 46.6 \pm 16.0%, respectively, which are within the range of acceptance criteria of the U.S. EPA Method 1614. The recovery of the 13 C-labeled γ -HBCD was 83.1 \pm 20.2%. Mean method detection limits (MDL), calculated as 3 times the signal-to-noise ratio on the total ion

chromatograph (TIC) of a low level mollusk sample, were 53 pg/g dw for TBC, 0.5–3.3 pg/g for PBDEs, and 115, 41, and 42 pg/g for α -, β -, and γ -HBCDs, respectively.

Mollusks Characterization. The trophic levels of the collected mollusks were determined using stable isotopes ¹⁵N, which were analyzed using a flash EA 1112 elemental analyzer interfaced with a Thermo-Finnigan Deltaplus isotope ratio mass spectrometer. The water content was measured by weight loss after drying.

■ RESULTS AND DISCUSSION

The mean trophic levels and lipid contents of the mollusks are presented in Table 1. The water contents of mollusks ranged from 75.9% to 88.5%, with a mean of 83.3%. Table 1 summarizes the dry weight (dw) based and lipid weight (lw) normalized concentrations of TBC, \sum_{12} PBDEs, and \sum HBCDs in soft tissue of different mollusk species. Concentrations of the individual HBCD diastereoisomers and PBDE congeners are summarized in Table S2 of the Supporting Information. Among the 131 composite samples analyzed in this study, the detection frequency of \sum_{12} PBDEs was 100%, followed by \sum HBCDs (99.2%) and TBC (76.9%). These high detection frequencies

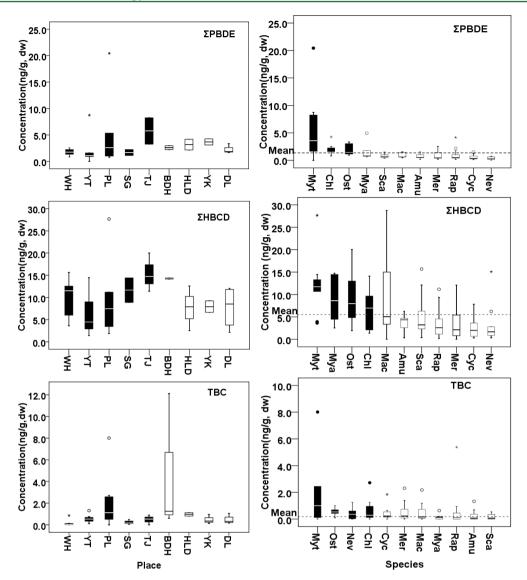


Figure 2. Spatial and species distributions of \sum_{12} PBDEs, \sum HBCDs, and TBC in mollusks. In the left panels, the black boxes represent southern sampling sites, and the white ones represent northern sampling sites. In the right panels, the black boxes represent the mollusk species in which the target compound's concentration is higher than the mean values in all samples. Single values beyond the 150th percentiles of the difference between 25th and 75th percentiles are presented by " \bullet " or "O", while extreme values are presented by " \ast ", which are beyond the triplication of the difference between the 25th and 75th percentiles.

indicated the ubiquitous contamination in the coastal Bohai Sea by the BFRs. Comparisons among sampling locations and among species are illustrated in Figures 2 and S2 (Supporting Information).

TBC. TBC concentrations ranged from under MDL to 12.1 ng/g dw, with means of 0.60 ng/g dw and 6.14 ng/g lw. This concentration range is understandably lower by at least 1 order of magnitude than those found in biota (earthworms and fish) near a TBC manufacturing plant. The results of this work on mollusks, in combination with previous findings with earthworm and fish, further indicate that TBC is bioaccumulative. Because many mollusks species are collected for human consumption, it is interesting to find that, in the mollusks species in which TBC was detected, its concentration was comparable to that of \sum_{12} PBDEs (Table 1). The log K_{ow} and log BCF of TBC, obtained using the Estimation Programs Interface (EPI) Suite v4.10 by the U.S. EPA, are comparable to the estimates for penta-BDE congeners, suggesting similar tendency of bioaccumulation. Noteworthy are the dramatic

differences in estimated vapor pressure and air—water distribution ratio ($K_{\rm aw}$) between the two kinds of BFRs. With a vapor pressure of only 10^{-15} mmHg and an extremely low log $K_{\rm aw}$ of only 16, TBC would preferentially stay in water rather than evaporating into air, in contrast to penta-BDE, which may partition substantially to the atmosphere (vapor pressure = 10^{-6} mmHg and log $K_{\rm aw} = -4$).

Relatively weak but statistically significant positive correlations were found between TBC concentrations and \sum_{12} PBDEs (R=0.24, p=0.015) and \sum HBCDs (R=0.20, p=0.043) (Figure S3, Supporting Information), suggesting that the mollusks in this region were exposed to similar sources of the BFRs. In the left panels of Figure 2, the sampling locations are divided into southern sites and northern sites of the Bohai Sea. Most of the southern sampling sites are in Shandong province, where many BFR manufactures and industrial users are located. However, the average TBC concentration is higher in the north (0.81 ng/g dw) than in the south (0.50 ng/g dw). The highest concentrations of TBC were observed at northern site BDH, a

summer resort known for its beaches (Figures 2 and S2, Supporting Information). Although unknown, the presence of point sources of TBC in this area cannot be excluded.

HBCDs. Σ HBCDs in mollusks ranged from under MDL to 28.8 ng/g dw, with means of 5.53 ng/g dw and 50.6 ng/g lw. The concentrations of Σ HBCDs in mollusks were several times higher than those of TBC and \sum_{12} PBDEs. The HBCD concentrations found in this study were comparable to mollusks in Japan⁵ and UK, ²² but about 10-100 times higher than those in Korea.²³ A significant linear relationship was found between concentrations of total HBCDs and \sum_{12} PBDEs (R = 0.62, p < 0.001) in mollusks, as shown in Figure S2 (Supporting Information). Similar findings were observed in fish collected from Yangtze River²⁴ and blubber samples of Indo-Pacific dolphins and finless porpoises from Hong Kong in China.²⁵ Among the sampling locations of this work, relatively high levels of HBCDs were observed at sites TJ, BDH, and SG. TJ is the largest industrial city in the Bohai Bay Rim economic zone. SG, within Weifang Prefecture of Shangdong Province, is home to several known BFR manufacturing facilities whose products include technical HBCD.

The technical HBCD is a mixture of several diastereomers, among which α -, β - and γ -HBCDs are the major pairs. The mean percentage contribution of α -HBCD to the Σ HBCDs in the mollusks of this work was 85.1%, followed by γ -HBCD (12%) and β -HBCD (2.9%). This finding is consistent with those from other studies on aquatic organisms where α -HBCD was the most predominant and is opposite to the commercial mixtures where γ -HBCD contributes about 80% to the total HBCDs. The reasons of aquatic organisms having higher ratio of α -HBCD still remain unclear. Some of the possible explanations are that α -HBCD has higher bioaccumulative potential or tends to be more resistant to biotransformation and that β - and γ -HBCD can be isomerized to α -HBCD in biota.

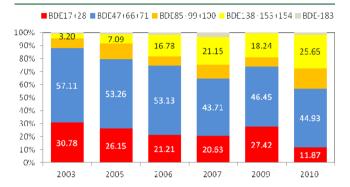
PBDEs. The concentration of BDE190 was below the detection limit in all samples and was thus excluded from the data analysis. The concentration of \sum_{12} PBDE ranged from 0.01 to 20.4 ng/g dw with a mean of 1.39 ng/g dw. The corresponding lipid weight based mean was 12.4 ng/g lw. The concentrations in Ost and Myt found in this work were 10–100 times lower than those in North America (9–64 ng/g, dw),²⁷ Korea (0.38–9.19 ng/g, wet weight),²⁸ Hong Kong (27.0–83.7 ng/g, dw),²⁹ and Singapore (2.0–38 ng/g, dw),³⁰ but comparable to those other Asian countries, such as India, Vietnam, Indonesia. 5,23

The relative abundances of the 12 PBDE congeners were very similar among the 11 mollusks species, with dominant congeners of BDEs 47, 71, 28, and 154, which accounted for $31.4 \pm 7.9\%$, $15.8 \pm 5.3\%$, $15.3 \pm 11.6\%$, and $11.2 \pm 9.7\%$ of \sum_{12} PBDE, respectively. BDEs 71 and 28 showed higher proportions than BDEs 99 and 100 in most samples of this study, which is consistent with the findings from two other studies in this area^{18,31} and suggests the possibility of naturally environmental debromination.

Spatial distribution of PBDEs is shown in Figures 2 and S2 (Supporting Information). No significant differences in average \sum_{12} PBDE as well as \sum HBCD were found between southern and northern sampling sites. This observation may suggest that, although manufacturing facilities are dominant sources of the BFRs to the vicinal environment, ³² their overall impact to the regional aquatic environment might be limited. Among the nine

sampling cities, TJ showed the highest contamination level of PBDEs in the mollusks, similar to the case of HBCDs.

The \sum_{12} PBDE levels in this work combined with those in our previous study¹⁸ illustrated the temporal trend in mollusks of the Chinese Bohai Sea, as shown in Figure 3. Concentrations



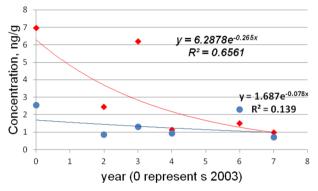


Figure 3. Temporal trends of PBDE profiles and concentrations. In the lower panel, the red dots show the mean whereas the blue dots represent the median values.

of \sum_{12} PBDE in mollusks decreased from 7.0 ng/g dw (singular values are excluded) in 2003 to 1.0 ng/g dw in 2010. During this time period, the half-life of \sum_{12} PBDEs was 2.3 \pm 1.7 years, which was calculated by applying, to the annual means, the first-order kinetic function $C = C^0 e^{-kt}$, where C is $\sum_{12} PBDE$ at a given time t; C^0 is the concentration of \sum_{12} PBDE in 2003, and the k is the rate constant. This trend may be ascribed to the discontinued usage of penta-BDEs and octa-BDEs technical products on a global scale from 2004 and the restricted usage of PBDEs in China since 2006. Temporal trend studies from Europe, Asia, and the Arctic have also shown declining PBDE concentrations in aquatic organisms between the mid-1990s and the mid-2000s. ^{6,25,33} On the other hand, congener pattern analysis in this study clearly indicated that the proportion of heavier PBDE congeners in the mollusks has increased gradually from 2003 to 2010. The percentage of hexa-BDEs (the sum of BDEs 138, 153, and 154) in the \sum_{12} PBDEs increased by 7 times between 2003 (3.20%) and 2010 (25.7%), while that of tri-BDEs (sum of BDEs 17 and 28) decreased by 61.4% (Figure 3).

Bioindicator Selection. There are numerous recognized species of marine mollusks that are highly diverse in body size, composition, and structure. In our previous work, we have shown that Myt and Ost could be more suitable than other mollusks as bioindicators of contamination by OCPs, PCBs, and PBDEs in the Chinese Bohai Sea. ¹⁸ In the present work, we further evaluated the possibility of these species as

Component Plot in Rotated Space

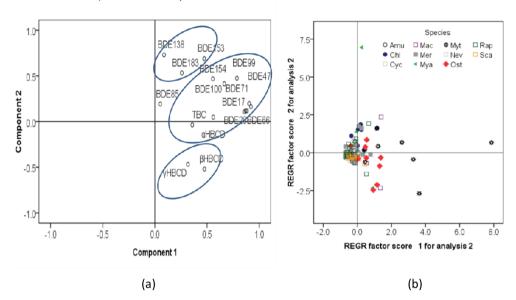


Figure 4. Loading (a) and score plots (b) of PCA by the database of BFRs (PBDEs, HBCDs, and TBC) in mollusks collected from the Chinese Bohai Sea in 2009 and 2010.

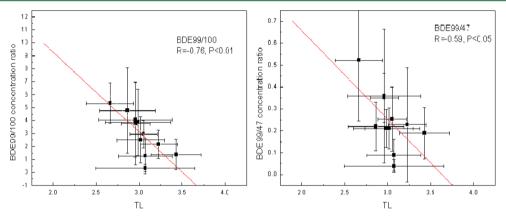


Figure 5. Relations between concentration radios of PBDEs congeners and TL in mollusks from the Bohai Sea.

bioindicators of contamination by TBC, HBCDs, and PBDEs in this area.

The linear correlation of concentrations (Figure S2, Supporting Information) and the similarity of calculated octanol-water partition coefficient (Kow) among penta-BDEs (log $K_{\text{ow}} = 7.66$), HBCDs (log $K_{\text{ow}} = 7.74$), and TBC (log K_{ow} = 7.37)⁷ suggested that the three BFRs may share similar mechanisms of bioaccumulation in aquatic organisms. Consequently, it is likely that Myt and Ost would also be more suitable in reflecting the extent of pollution by TBC and HBCDs in the Chinese Bohai Sea, since these two species seem to have higher bioaccumulative abilities than other selected mollusks. In order to test this hypothesis, principal component analysis (PCA) was applied to the measured concentration data. The results showed that the first two PCs represented 55.7% of the total variances of BFR concentrations in mollusks (Figure 4). Combined with the loading and score plots, TBC, the lower brominated PBDE congeners, and α -HBCD were congregated into one group and more heavily accumulated in Myt than in other mollusks. Higher brominated PBDE congeners such as BDEs 153, 138, and 183 were located in upper y-axis (Figure 4a) and accumulated more in Chl and Myt,

while γ -HBCD and β -HBCD were primarily accumulated in Myt and Ost. On the basis of the trends from the PCA plots, Myt was found to be the most sensitive and appropriate specie for indicating the contamination of TBC, PBDEs, and HBCDs in the Bohai Sea.

Trophic level (TL) of the biota is often used in studies on biomagnification in aquatic environment. In this work, TLs of the mollusks were estimated on the basis of their nitrogen isotope ratios $(\delta^{15}\mathrm{N})$, ³⁴ which ranged from 2.12 to 4.09 (Table 1). Among the 11 species, Sca had the widest range while Rap had the highest TL with a mean of 3.43 (Figure S4, Supporting Information, and Table 1), which were consistent with the findings from others studies. ^{18,35} Results from multiple linear regression analysis (stepwise regression) showed the lipid content had stronger relationships with \sum_{12} PBDEs and \sum HBCDs than TL. No significant correlation was found between TBC concentration and lipid content (R = -0.147, p > 0.05) or TL (R = 0.122, p > 0.05) (Table S3, Supporting Information), although Ruan et al. ⁷ indicated TBC was inclined to be enriched in the fat-rich organs of fish.

In order to explore the correlation of BFR concentrations and TL without the influence of lipid content, regression

analysis was performed for the lipid-normalized concentrations of BFRs against TL (Figure S5, Supporting Information). Although the correlations were statistically insignificant (p > 0.05), negative trends were observed for all the three BFRs, indicating potential trophic dilution rather than magnification in the portion of the marine food web involving mollusks. In the Bohai Sea, trophic dilutions have been previously reported for PAHs, PCDD/Fs, PCBs, HCHs, and PBDEs. ^{18,36,37} However, some other POPs with comparable log $K_{\rm ow}$ values, such as DDT, HCB, and coplanar PCBs, exhibited opposite trophodynamics in the same marine food web of the Bohai Sea, with their trophic magnification factor (TMF) values being all higher than 1.37,38

 $\log K_{ow}$ of the chemicals is not the only key factor governing the bioaccumulation potential. Their metabolic transformation rates, half-lives in organisms, and assimilation efficiencies may vary significantly and should also be taken into account.³⁹ For example, the PBDE congener ratio BDE99/100 in most abiotic samples is approximately 80:20, which is comparable to the mean of 84:16 measured for industrial products Bromkal 70-5DE, while the ratio is about 30:70 in biotic samples.⁴⁰ In the present study, large differences in the ratio BDE99/100 were observed among mollusks species, with the lowest in Sca (25:75) and the highest in Mya (84:16). The strong negative dependence of this ratio on the TL (R = -0.76, p < 0.01), as shown in Figure 5, reveals the increase in bioaccumulation of BDE100 or biodegradation of BDE99 with the increasing TL among the mollusks. A similar negative correlation (R = -0.59, p < 0.05) between the congener ratio BDE99/47 and the mollusks TL was obtained (Figure 5), indicating that mollusks species at higher TL in the food chain tend to be more capable of biotransforming BDE99 than BDE47. Future studies are warranted to investigate these phenomena.

In recent years, HBCDs have been proven to be globally ubiquitous. In the Persistent Organic Pollutants Review Committee meeting of 2011 (POPRC 7), HBCDs were considered for Annex F, implying that they could be recommended by POPRC to be listed under the Stockholm Convention in the foreseeable future. With the expected tightening of restrictions on HBCDs, their concentrations in the environment may start to decline. Correspondingly, due to the demand of the global market, the production and usages of novel flame retardants have started to increase. Accordingly, the detection of some new flame retardant chemicals in the environment have already been reported in the recent years. 41–45

ASSOCIATED CONTENT

S Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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