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Determination of hexabromocyclododecanes in sediments from the Haihe River in China by an optimized HPLC–MS–MS method

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ABSTRACT

Hexabromocyclododecanes (HBCDs), a new type of persistent organic pollutants widely used as brominated flame retardants, have attracted wide attention due to their increasing level and toxicity. A method based on high-performance liquid chromatography mass spectrometry (HPLC–MS–MS) in electrospray ionization mode has been developed by optimization of various parameters, which effectively improved the separation degree and responsive intensity of α-, β- and γ-HBCD isomers. The concentrations and distribution profiles of three HBCD isomers were investigated in sediments from the Haihe River in China. It was observed that the concentrations of HBCDs varied in the range of 0.4–58.82 ng/g, showing a decreasing trend along the flow direction, possibly due to attenuation and biodegradation along the flow direction of the Haihe River. The distribution profile of α-, β-, γ-HBCD was 7.91%–88.6%, 0–91.47%, and 0.62%–42.83%, respectively. Interestingly, α-HBCD dominated in most sample sites. This was different from the distribution profile in commercial industrial products, which might be attributed to the inter-transformation and different degradation rates of the three HBCD isomers. The potential ecological risk of HBCDs in sediment was characterized under the two-tiered procedure of the European Medicines Evaluation Agency for environmental risk assessment. Although the HBCDs in the selected section of the Haihe River presented "no risk" in the sediment compartment, its risk in sediment cannot be neglected since sediment is one of the important sinks and reservoirs of pollutants.

Introduction

Hexabromocyclododecane (HBCD) comprises a 12-carbon ring featuring six bromine atoms substituted for hydrogen, and is the third most widely used brominated flame retardant following tetrabromobisphenol A and decabromodiphenyl ether (Heeb et al., 2005). HBCDs are commonly added to polystyrene foams, upholstery textiles, and electronic devices as they exhibit excellent properties for flame protection purposes in these commercial products. Since the appearance

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of HBCDs on the market in the 1960s, the global market demand for HBCDs has increased yearly, reaching 16,700 tons in 2001. Europe was the primary consumer market, accounting for 9500 tons (Dwyer, 1996).

HBCDs can be released into the environment *via* their production process, use, or waste disposal. In 1998, HBCDs were detected for the first time in fish and sediment samples from the Viskan River (Sellstrom et al., 1998). Recent studies have shown that the presence of HBCDs in the environment has spread to water bodies, birds, and marine mammals (Remberger et al., 2004). As per the report by Guerra et al. (2010), the levels of HBCDs in silt and sediment in the Ebro River in Spain were respectively 0–375 ng/g and 0.8–1850 ng/g. At present, there is sufficient evidence to support the existence of a large difference in the indoor and outdoor levels of HBCDs in dust. Takigami et al. (2008) analyzed dust from different parts of a TV set, and found that the level of HBCDs was 2–3 orders of magnitude higher than the level of HBCDs in air. This analysis indicated that HBCDs can be transferred from household appliances to the atmosphere. Harrad et al. (2009) analyzed 27 water samples from nine lakes in the UK during 2008–2009. The results showed that the total amounts of HBCDs in the different water samples varied in the range of 80–270 pg/L. HBCDs have the potential for long-distance migration and have been detected in animals in the Arctic. Specifically, Verreault et al. (2016) reported the existence of HBCDs in glaucous gull at concentrations ranging from 0.07 ng/g to 1.24 ng/g. HBCDs have also been detected in the adipose tissue of polar bears. According to reports based on environmental toxicity data, HBCDs present a great health hazard to organisms. They have been found to affect the nervous system of rats (Eriksson et al., 2006). Moreover, animal studies have shown that HBCDs can cause a decline in the serum thyroid-stimulating hormone level (Y-Okabe et al., 2005) and neonatal exposure of rats to HBCDs can lead to developmental behavioral defects (Eriksson et al., 2002). Many publications have demonstrated that HBCDs could be found in breast milk (Roosens et al., 2010; Schecter et al., 2008), and the direct intake of HBCDs by infants or young children through breastfeeding may also represent a health risk. Owing to the hazardous effects of HBCDs on human and the environment, HBCDs, as a new type of persistent organic pollutants (POPs), were added to the Stockholm Convention in 2013 (POPRC4, 2016).

The detection of HBCDs in the environment is typically achieved *via* trace component analysis in complex matrices. Examples of some analytical methods include gas chromatography (GC) and gas chromatography mass spectrometry (GC–MS). However, thermodynamic rearrangement between HBCD molecules occurs at 160°C and debromination degradation occurs at 240°C, which can lead to varied and inconclusive results. Thus, GC is not suitable for identifying HBCD isomers; however, it is appropriate for measuring the total amount of HBCDs. Currently, the determination of HBCDs has focused on biological, environmental, and food samples using high-performance liquid chromatography mass spectrometry (HPLC–MS–MS) in electrospray ionization (ESI) mode; the latter analysis affords isomer analysis. Budakowski and Tomy (2003) used liquid chromatography-tandem electrospray ionization mass spectrometry, which affords high sensitivity, selectivity, and a detection limit of 4–6 pg/g. Harrad et al. (2009) evaluated the levels of HBCDs in water, sediment, and fish using ultra high-performance liquid chromatography (UHPLC)–ESI–MS. The samples were extracted by accelerated solvent extraction. The detection limit was 20 pg/g, and the recovery rate was 100%. Lankova et al. (2013) used UHPLC coupled to tandem mass spectrometry (MS/MS) for the quantitative detection of HBCDs in fish. HBCDs were detected in 4 of 30 fish feeds and 3 of 30 home-produced eggs at low-ng/g wet weight levels. Furthermore, determination of the HBCD isomer level in channel catfish, crayfish, hen eggs, and fish feeds in China has been achieved by isotopic dilution liquid chromatography mass spectrometry (LC–MS/MS) (Hu et al., 2011).

The Haihe River is located in the center of the northern part of China, which contains developed industrial enterprises and a large population. This river flows through Beijing, Tianjin, Hebei, Shanxi, Shandong, Henan, Inner Mongolia and Liaoning, where the industry is mainly concentrated in the metallurgy, chemical industry, steel plant, coal electric power and pharmaceutical fields. However, rapid economic growth has also contributed to environmental pollution, such as water pollution. As reported before, some research had been carried out to investigate the distribution and fate of typical POPs in Tianjin, while reports about HBCDs, a new type of POPs, in Haihe River have been rare. The aim of the present study is to implement an effective set of analysis methods for HBCDs by HPLC–MS–MS using accelerated solvent extraction (ASE) as a pretreatment method for the quantification of individual isomers. Moreover, the distribution of HBCDs isomer was estimated and risk assessment of HBCDs concentration levels in the Haihe River was carried out to provide basic information for the implementation of the monitoring of POPs in typical estuarine areas to ensure ecological protection.

1. Materials and methods

1.1. Chemicals

Three individual standards (α-HBCD, β-HBCD, γ-HBCD) and an isotopically labeled HBCD mixture (comprising an unequal mix of the three isomers; $^{13}C_{12}$, 99%) at a concentration of 50 μg/mL in toluene were purchased from AccuStandard Inc. (New Haven, CT, USA). Methanol and acetonitrile were HPLC-grade reagents purchased from Fisher (Fair Lawn, NJ, USA). Dichloromethane, hexane, and acetone were of analytical grade purchased from Dika Technologies (Lake Forest, CA, USA). Analysis grade anhydrous Na_2SO_4, concentrated H_2SO_4, NaOH, and silica gel were purchased from Beijing Chemical Co. (Beijing, China).

1.2. Sample handling

Seven soil samples were collected from various sampling sites (T_1–T_7) distributed near the Haihe River in Tianjin (Fig. 1). The samples were stored in glass beakers, rather than plastic ones, to prevent potential contamination from plastics containing HBCDs. The samples were frozen at −60°C and dried under

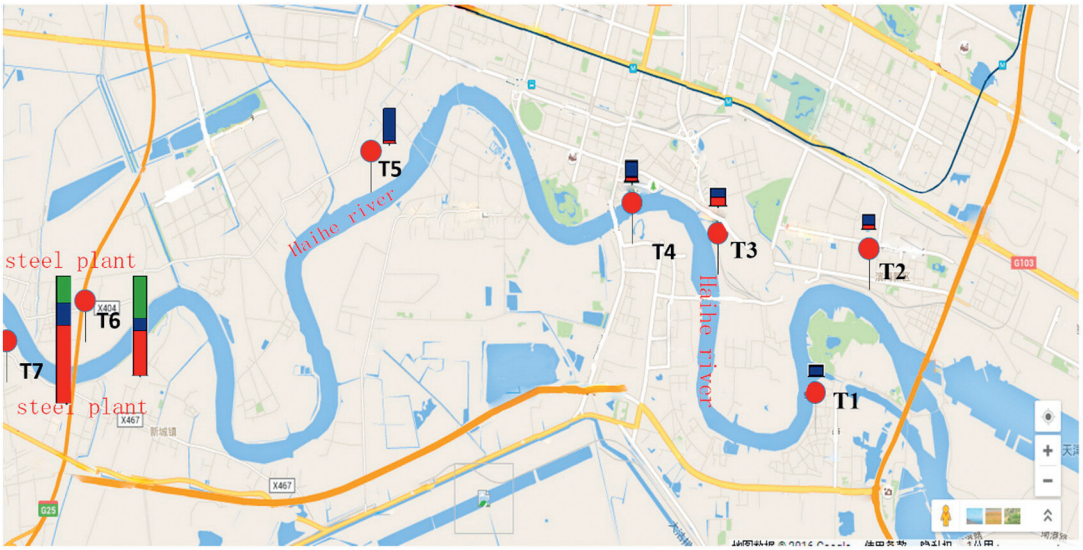

Fig. 1 – Sampling sites along the Haihe River (T1–T7).

vacuum conditions after preliminary air drying. The dried samples were sieved with a 60-mesh screen and stored in wide-mouth bottles for subsequent experiments.

The isolation of HBCDs in each 10 g portion of sample was performed by ASE at 150°C for 30 min using 100 mL dichloromethane/hexane (V/V = 1:1) as the extracting solvent. The solutions were spiked with a mixed $^{13}C_{12}$-internal standard solution comprising $^{13}C_{12}$-α-HBCD, $^{13}C_{12}$-β-HBCD, and $^{13}C_{12}$-γ-HBCD. Then, the extracts were concentrated to 2 mL at 50°C in a heart-shaped flask fitted to a rotary evaporator (Heidolph Hei-VAP Precision made in Germany). The extracts were washed by passing through a multi-layer column comprising, from the base to the top of the column, 1 g silica gel, 4 g alkaline silica gel, 1 g silica gel, 8 g acidic silica gel, 2 g silica gel, and 2 g anhydrous Na_2SO_4. A dichloromethane/hexane (V/V = 1:1) mixture was used as the eluent to ensure desorption of the washed extract from the silica gel column bed. The first fraction (70 mL) was discarded, whereas the second fraction consisting of HBCDs was collected for analysis. The fraction was concentrated to 0.5 mL prior to HPLC–EVOQ–MS/MS analysis.

1.3. HPLC–MS–MS analysis of HBCDs

A Bruker EVOQ Elite LC-triple quadrupole (TQ) mass spectrometer equipped with an ESI source was used to analyze the HBCDs. A C18 column (1.7 μm × 2.1 mm × 50 mm, ACQUITY UPLC BEH, Waters) was used to separate the HBCD isomers. The liquid chromatography (LC) system consisted of two ultra high pressure pumps (pumps A and B), and the mobile phase consisted of a mixed methane/acetonitrile (V/V = 1:1) solution pumped into the system by pump B, and water was pumped into the system by pump A. The gradient elution program used is detailed in Table 1. The column temperature was set at 40°C and allowed to equilibrate for 20 min before injection of a 1-μl test sample.

The mass spectrometer was operated in negative ESI mode based on literature procedures (Brunstrom and Halldin, 2000; Janák et al., 2005). The precursor ions, *m/z* 640.4 $[M - H]^-$ and 657.4 $[M - H]^-$ were detected as per the manufacturer's (Bruker) recommendations. The spray voltage, probe temperature, and cone temperature were optimized. Additionally, the effect of the ammonium acetate concentration (0, 1, 5, 10, 15 mmol/L) on ionization was explored. After optimization, the following source parameters were established: spray voltage, 4400 V; probe temperature, 260°C; and cone temperature, 300°C (Table 2). Multiple reaction monitoring (MRM) was implemented *via* the system-integrated MRM mode, and the digital syringe used was provided by EVOQ (Bruker). The optimal quantitative and qualitative product ions as well as their collision energy are given in the report from EVOQ (Bruker). The quantitative determination used for $^{12}C_{12}$-HBCDs was based on the signal from MRM of *m/z* 640.4 to product ions

Table 1 – Conditions and composition of the mobile phase employed for determination of the hexabromocyclododecane isomers.

Time (min)	Rate (μl/min)	Percentage A (water)	Percentage B (methane/acetonitrile)
0.00	200	40%	60%
5.00	200	30%	70%
13.00	200	10%	90%
14.00	200	10%	90%
15.00	200	40%	60%
17.00	200	40%	60%

Table 2 – Source parameters employed for detection of the hexabromocyclododecanes (HBCD) isomers.

Source parameter	Optimized value
Spray voltage (V)	4400(–)
Cone temperature (°C)	300
Cone gas flow(0–100)	20
Probe temperature (°C)	260
Probe gas flow(0–100)	60
Nebulizer gas flow(0–100)	60

m/z 79.1 transition, whereas those used for qualitative determination was based on the signal from MRM of *m*/z 640.4 to product ions *m*/z 81.1 transition. Similarly, the quantitative determination used for $^{12}C_{12}$-HBCDs was based on the signal from MRM of *m*/z 657.4 to product ions *m*/z 79.1 transition, whereas those used for qualitative determination were based on the signal from MRM of *m*/z 657.4 to product ions *m*/z 81.1 transition. (Table 3).

1.4. Quality control test

HBCD isotopically labeled mixtures ($^{13}C_{12}$-α-, β-, γ-HBCD) were used to calibrate the quantification of HBCDs, and 10 ng $^{13}C_{12}$ -α-, β-, γ-HBCD were added to obtain a six-point standard calibration curve with concentrations of 0.01, 0.05, 0.1, 0.5, 0.8, and 1 μg/mL, and seven real samples were measured. Each test sample was measured three times, and the data were averaged. The standard deviation was less than 10%. A solvent blank and sample blank subjected to matrix clean-up were also measured. The equipment detection limit corresponded to a signal-to-noise ratio of 3, and the quantitation limit corresponded to a signal-to-noise ratio of 10.

1.5. Total organic carbon analysis

The X_{oc} value of sediments was analyzed on a solid total organic carbon (TOC) analyzer (O.I. Analyzer, College Station, TX, USA). First, a 0.1 g sample was loaded into the combustion cup, which was packed with quartz wool. Then the sample was wet with 5% phosphoric acid and heated at 250°C for 1 min to purge the inorganic carbon. Finally, the signal was detected by non-dispersed infrared detection when flashed at 900°C for 6 min in the combustion house.

2. Results and discussion

2.1. Optimization of HPLC–MS–MS analysis conditions

The mass spectrometer was operated in ESI negative ion mode to examine the ionization of HBCDs. Individual α-, β-, and γ-HBCD standards were used to establish the mass spectral features. In MS scan mode, the $[M - H]^-$ base peak of the HBCD isomers could be observed at *m*/z 640.4. Subsequently, the product ion scan mode was employed to select the product ions and optimize the collision energy. MRM is a method used in tandem mass spectrometry. It includes two stages of mass selection. The mass of the intact analyte (parent ion) is selected at the first stage (MS1). After the parent ions collide with gas atoms, the mass of a specific fragment ion is selected at the second stage (MS2). MS1 and MS2 collectively generate a selected reaction monitoring assay. The MRM mode can simultaneously monitor many pairs of ions, enabling a very specific and sensitive response for the selected analyte. Two specific MRM transitions were used for each compound for qualitative and quantitative analysis. They were 640.4/79.1 and 640.4/81.1 for the three HBCD isomers. The $[M - H]^-$ precursor ion, product ion and MRM transitions with the optimized collision energy for the HBCD isomers are summarized in Table 3. Following development of the MRM method, a series of parameters including spray voltage, probe temperature, cone temperature, and concentration of buffer salt (ammonium acetate) were optimized to attain excellent response signals for the HBCD isomers.

As an example, α-HBCD was used to estimate the influence of the above parameters on the corresponding peak area (Fig. 2). As observed in Fig. 2a, the obtained peak area, corresponding to α-HBCD, initially increased, and then decreased with increasing spray voltage in the range of 1000–4500 V. The spray voltage of 4400 V, which resulted in the largest peak area, was considered as the most suitable spray voltage. The probe temperature also influenced the response signal of the peaks as shown in Fig. 2b. The largest peak area was obtained when the probe temperature was set to 260°C. In contrast, the effect of the cone temperature in the range of 100–420°C on the peak area was not significant when compared with that of the spray voltage and probe temperature (Fig. 2c). However, a considerable decrease in the peak area was noted at cone temperatures greater than 420°C, possibly due to the decomposition of the detected analyte. Thus, a temperature of 300°C was chosen as the optimum cone temperature. In the ESI mode, the added buffer salt could increase the ionic charge of the test solution, thereby enhancing the signal response of the target ion (Morris et al., 2006). Ammonium acetate is the most widely used buffer salt (Hirayama et al., 2009; Kebarle, 2000), owing to its excellent ability to maintain the number of ionic species in the system at a minimum and avoid the formation of moving pH-boundaries in the separation capillary (Vuorensola et al.,

Table 3 – Equipment parameters and target HBCD isomers used for qualitative and quantitative analysis.

Compound	Molecular formula	Molecular mass	Transition ions	Collision energy (eV)	Q1 and Q3 resolution	Retention time (min)
α-HBCD	$C_{12}H_{18}Br_6$	641.7	640.4–79.1[a]	9	2	9.322
			640.4–81.1[b]	7		
β-HBCD	$C_{12}H_{18}Br_6$	641.7	640.4–79.1[a]	9	2	10.031
			640.4–81.1[b]	7		
γ-HBCD	$C_{12}H_{18}Br_6$	641.7	640.4–79.1[a]	9	2	11.055
			640.4–81.1[b]	7		

[a] Transition ions used for quantitative determination.
[b] Transition ions used for qualitative determination.

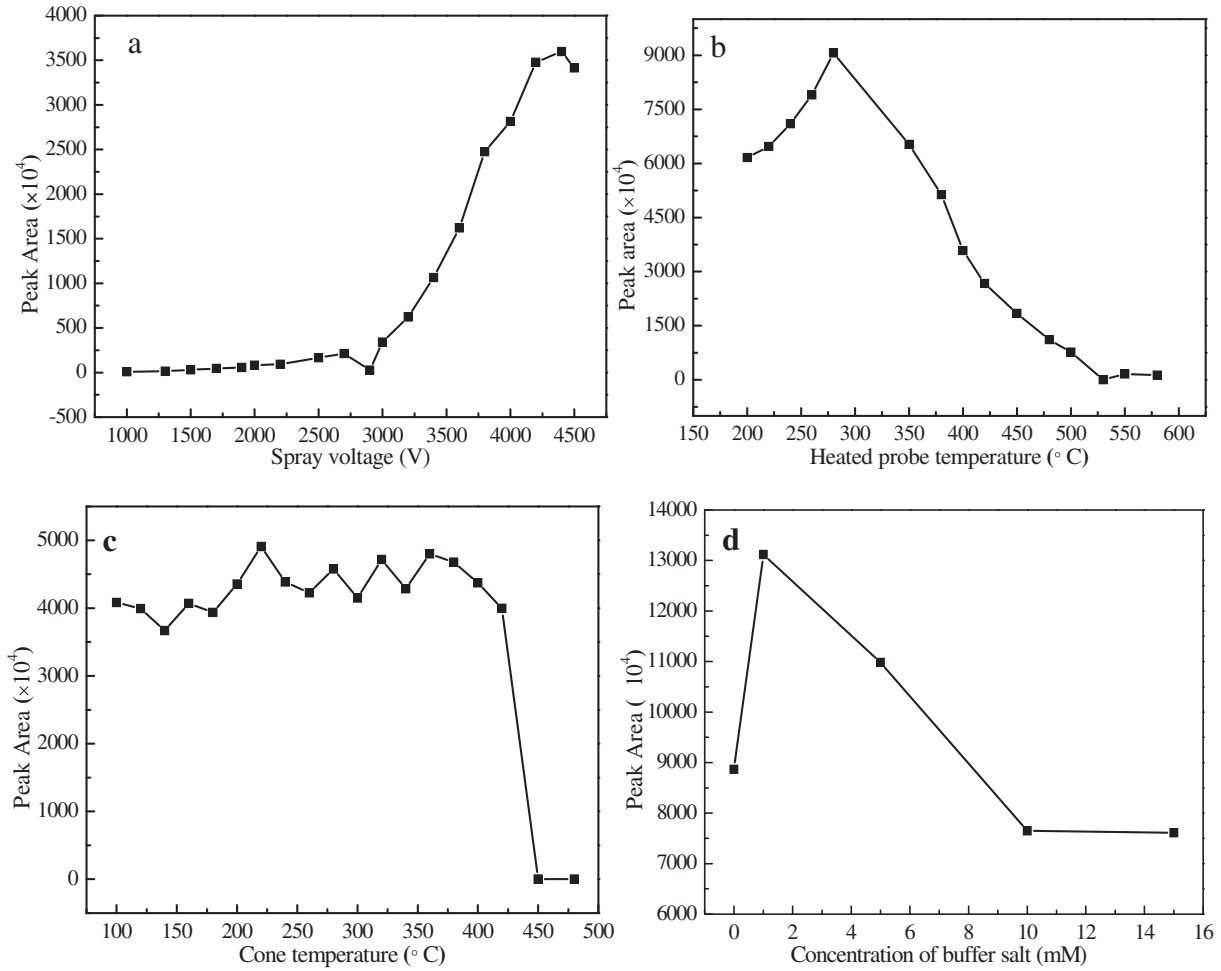

Fig. 2 – Effects of (a) spray voltage, (b) probe temperature, (c) cone temperature, and (d) buffer salt concentration on the peak area of α-HBCD.

2001). The influence of ammonium acetate concentration on the peak area of α-HBCD is shown in Fig. 2d. With increasing concentration, the signal peak intensities increased and then decreased, reaching maximum at a concentration of 1 mmol/L. Therefore, an ammonium acetate concentration of 1 mmol/L was chosen as the optimum buffer salt concentration. The optimized parameters are summarized in Table 2.

To improve the chromatographic separation of the three HBCD isomers, different mobile phase compositions and gradient slopes were examined. Fig. 3 shows the HPLC–MS–MS chromatograms of the HBCD standards using different mobile phase compositions. Regardless of the mobile phase composition, the order of appearance of the HBCD isomer-related signal peaks was consistent. The peak corresponding to α-HBCD was first observed, followed by those corresponding to β- and γ-HBCD. In contrast, different elution results were obtained when different types of mobile phases were used. Specifically, when the mobile phase consisted of methanol and water (Fig. 3a), the retention time (RT) of the α-HBCD-related peak was 10.98 min and those of the β-, and γ-HBCD-related peaks were comparable with each other (*i.e.*, 12.77and 13.23 min, respectively). The latter result may compromise the accuracy of the isomer determination. In contrast, when the mobile phase consisted of acetonitrile and water (Fig. 3b), the RT of the HBCD isomer-related peaks was shorter. Specifically, the RT of the β-HBCD-related peak was greatly shortened and comparable with that of the α-HBCD-related peak. To achieve a better separation of the three HBCD isomers, a mobile phase consisting of methanol/acetonitrile (V/V = 1:1) and water was investigated. As observed in Fig. 3c, α-, β-, and γ-HBCD were separated effectively. Furthermore, the signal response (peak signal intensity) was stronger than that obtained in Fig. 3a and b. Thus, the mixture of methanol/acetonitrile (V/V = 1:1) and water was considered as the optimal mobile phase. Furthermore, the rate of flow of the mobile phase was optimized (200 μl/min). The mobile phase not only ensured effective separation of the three HBCD isomers, but also improved the intensity of the signal response. The separation performance and signal strength obtained in this study were superior to those reported in previous studies (Marvin et al., 2006; Morris et al., 2006; Feng et al., 2010).

2.2. HBCD concentrations in sediment samples

Different concentrations of HBCDs were determined in the sediment samples sourced from seven sites surrounding the

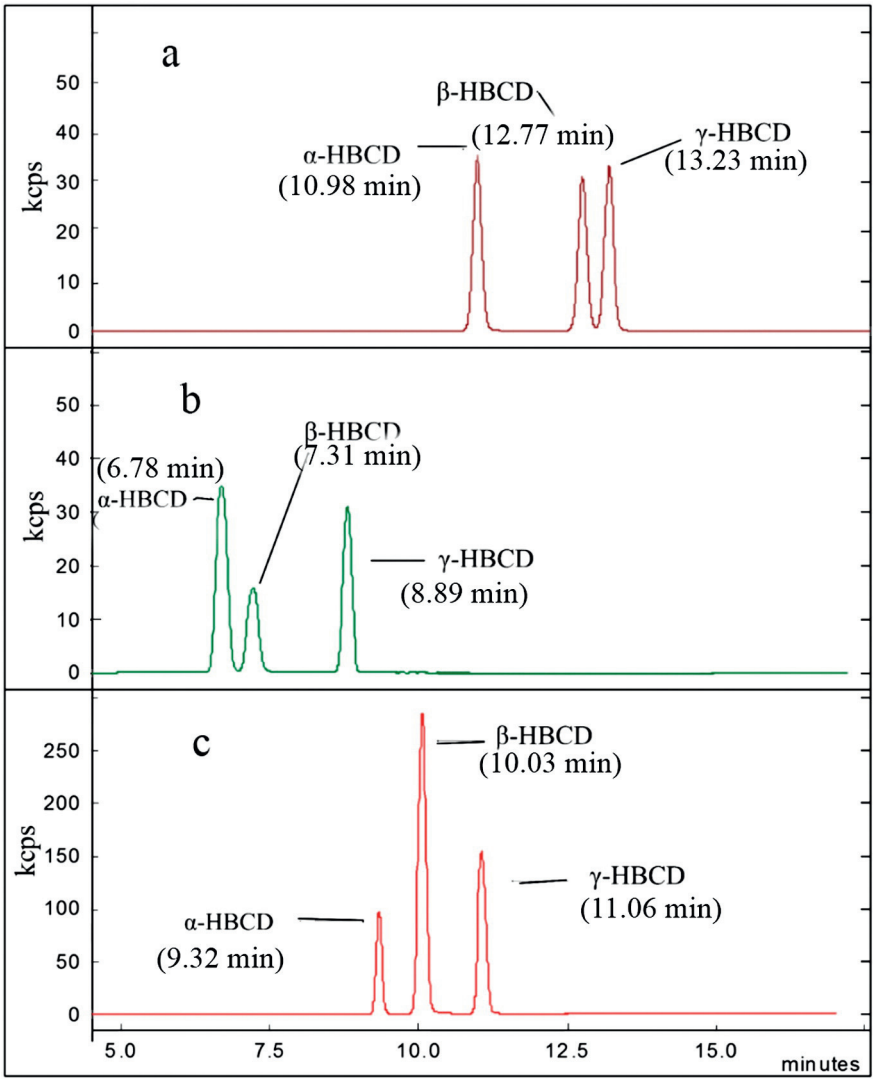

Fig. 3 – Chromatographic separation of the three HBCD isomers using different mobile phases: (a) methanol and water, (b) acetonitrile and water, and (c) methanol/acetonitrile (V/V = 1:1) and water.

Haihe River and sediment samples in Haihe River contained detectable concentrations of HBCDs and limnic organisms were reported before (Zhang et al., 2013a). This indicated that these pollutants are common around the Haihe River in Tianjin. The concentration results for the HBCD isomers and the total amount of the three HBCD isomers per site (ΣHBCD) in the sediment samples are presented in Table 4 and Fig. 4. ΣHBCD varied from 0.4 to 58.82 ng/g, with an average of 15.71 ng/g. As shown in Table 4, the total and average concentrations of HBCDs were higher in the Dagu

Table 4 – Total concentrations of HBCD isomers and ΣHBCD in different sediment samples.

Sample style	Location	Concentration (ng/g) [a]		Quantity	Time	References
		ΣHBCD	$\overline{X}_{HBCD}$			
Sediment	Haihe River, Tianjin	0.4–58.82	15.71	n = 7	2015	This study
Surface sediment	Dagu Drainage Canal, Tianjin	5.59–634	83.7	n = 16	2013	Zhang et al. (2013b)
Sediment	Shanghai, China	0.01–13.7	3.41	n = 40	2015	Tang et al. (2015)
Sediment	Lake Erie, America	0.11–0.71	0.36	n = 6	2015	Letcher et al. (2015)
Sediment	Coastal waters, Korea	0.39–59	10.82	n = 24	2010	Ramu et al. (2010)
Limnic organisms	Haihe River, Tianjin	64.3–1111	–	112 km^2	2013	Zhang et al. (2013a)

HBCD: hexabromocyclododecanes.
[a] ΣHBCD, sum of the amounts of α-, β-, and γ-HBCD isomers detected in one site; $\overline{X}_{HBCD}$, average amount of HBCD determined across the seven samples sites.

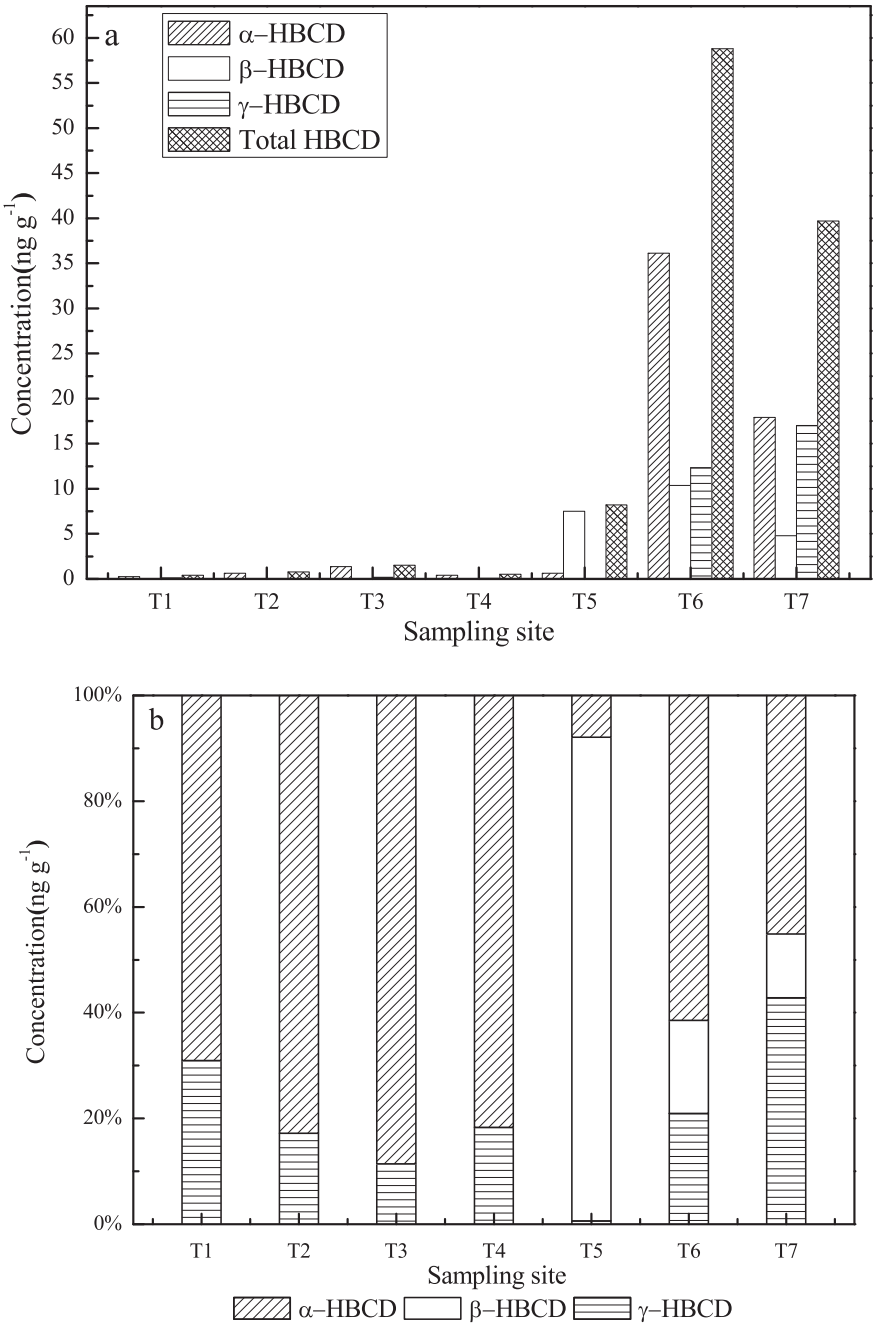

Fig. 4 – Concentration of (a) isomers and total of HBCD, and (b) distribution profiles of α-HBCD, β-HBCD, and γ-HBCD in sediment samples from the Haihe River.

Drainage Canal of Tianjin (Zhang et al., 2013b) when compared with those obtained in this study. The Dagu Drainage Canal is the main sewage drainage canal in Tianjin, which receives wastewater from wastewater treatment plants that treat 50% of the wastewater from the whole central area of Tianjin City (Sun et al., 2012). Thus, the results obtained therein would be influenced accordingly. Despite the existence of some industrial parks around the Haihe River, most of the examined sediments had not been affected yet. In contrast, the HBCD concentrations were lower in surface sediments from river drainage basins of Shanghai (Tang et al., 2015) when compared with those obtained in the present study. Likewise, the HBCD concentrations previously reported from other countries and regions in the Detroit River of North America (Letcher et al., 2015) and in coastal waters of Korea (Ramu et al., 2010) were lower than those obtained in this study.

Among all the sampling sites T_1–T_7 investigated, the two highest HBCD concentrations were determined at T_6 (58.82 ng/g) and T_7 (39.71 ng/g)(Fig. 4a), which are located near steel manufacturing plants. These concentrations differed considerably from those obtained at sites T_1–T_5 located along the Haihe River, which flows from west to east. The average concentration of HBCDs detected at T_1–T_5 sites was 2.29 ng/g,

with the T_5 sample featuring the highest concentration of 8.21 ng/g. This result indicated that the flow direction of the river may contribute to the dilution of the concentrations of HBCDs in Haihe River. Other factors, such as those linked to urban and industrial activities, could have contributed to that result, as reported in the study examining sediment samples from the Detroit River (Marvin et al., 2006).

The profile of the HBCD diastereomers in the sediment samples of the Haihe River is shown in Fig. 4b. As observed for sampling site T_7, α-HBCD contributed 61.44% of the total HBCDs isomers, followed by γ-HBCD (42.83%) and β-HBCD (17.61%). The same trend was observed for sample site T_6, *i.e.*, α-HBCD (45.09%), followed by γ-HBCD (20.95%) and β-HBCD (12.08%).

α-HBCD and γ-HBCD were detected at all seven sampling sites, whereas β-HBCD was detected at sampling sites T_5–T_7 only. Individual isomers of HBCD were detected at maximum concentrations of 36.14 ng/g for α-HBCD, 10.36 ng/g for β-HBCD, and 17 ng/g for γ-HBCD. The contribution of α-HBCD, β-HBCD, and γ-HBCD to ΣHBCD was 7.91%–88.6%, 0–91.47%, and 0.62%–42.83%, respectively (Fig. 4b). The distribution of the HBCD isomers at sampling sites T1–T4, which are far away from the steel plants, was similar, with α-HBCD being the most abundant individual isomer, whereas γ-HBCD was the least abundant. In contrast, α-HBCD was the dominant isomer at sampling sites T6 and T7 (which are located near the steel plants), and the amounts of the remaining two isomers were relatively high. However, the result obtained for sampling site T5 showed an interesting phenomenon, whereby β-HBCD contributed greatly to the total concentration when compared with α-HBCD and γ-HBCD. Nevertheless, it could generally be summarized that the concentration of the three isomers in samples near the Haihe River followed the sequence α- > γ- > β-HBCD. This is the same order in composition for sewage treatment plants and water from Japanese Rivers reported by Ichihara et al. (2014) and English lake water reported by Harrad et al. (2009). However, it is different from the distribution pattern in commercial industrial commercial products, which follows the sequence γ- > α- > β-HBCD (Covaci et al., 2006). The different amounts observed among the HBCDs isomers in the sediment samples may be attributed to inter-transformation between isomers and different degradation rates for the three HBCD isomers (Gereche et al., 2006).

2.3. Ecological risk assessment of HBCDs in sediment

The potential ecological risk (PER) of HBCDs in sediment was characterized under the two-tiered procedure of European Medicines Evaluation Agency (EMEA) for environmental risk assessment (Guidance for industry, 1998). In the development of environmental risk assessment guidelines, the PER has been the basic protocol internationally adopted. Bound et al. reported risk assessment strategies of pharmaceuticals in the aquatic environment based on EMEA (Bound and Voulvoulis, 2011). Hernando et al. (2006) also presented the risk assessment studies of pharmaceutical residues in various environmental compartments according to this procedure. In the present study, the first-tier approach was employed from the levels of measured environmental concentrations (MECs) (Schowanek and Webb, 2002). Furthermore, the concentration range causing adverse effects on organisms is significant information in risk assessment. Thus, the second-tier procedure included the physico-chemical and toxicological data on HBCDs. The predicted no-effect concentrations (PNEC) are estimated from LC50 or EC50 values obtained by standard acute toxicity measurements on algae, daphnia and fish. Following the above approach, the risk quotients (RQs) (the ratio of an MEC to its PNEC) to organisms in the environment can be applied to assess ecological risk; and this ecological risk of a selected compound can be divided into four levels based on RQ, *i.e.*, no risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), medium risk (0.1 < RQ < 1), and high risk (RQ > 1) (Zhu and Chen, 2014).

The PNEC for sediment ($PNEC_{sed}$) was estimated according to Eq. (1):

$$PNEC_{sed} = \left((PNEC_{wat} \times K_p)/d\right) \times f \quad (1)$$

$$PNEC_{wat} = EC_{50}/f \quad (2)$$

$$K_p = K_{oc} \times X_{oc} \quad (3)$$

where $PNEC_{wat}$ (μg/L) is the PNEC in water, which was determined by Eq. (2); K_p, the sediment–water partition coefficient, was calculated following Eq. (3); d (g/m^3) is the sediment density; and f is an assessment factor (1000), representing the possible variations between acute and chronic conditions (Bound and Voulvoulis, 2011; Hernando et al., 2006). The value of EC_{50} was referred to the growth inhibition test with *Skeletonema costatum* (72hr-EC50 52 μg/L) (Li et al., 2013). K_{OC} represents the organic carbon partition coefficient, and its value was obtained from the literature (Davis et al., 2005). X_{oc} (g/kg) is the organic carbon fraction in sediment.

The X_{oc} value measured in sediment samples was 1.15 g/kg dried sediment. Combined with the MEC values, calculated values of K_p, $PNEC_{wat}$, $PNEC_{sed}$ were 0.0014, 0.059 μg/L and 48,920 ng/g, respectively. It should be noted that the PNEC values of HBCDs in water and sediment display a considerable difference. The $PNEC_{wat}$ value of HBCDs was below 0.1 μg/L, suggesting the high toxicity of HBCDs to aquatic organisms (POPRC4, 2016). According to the calculated RQs of HBCDs in all sampling sites, sample site T6 obtained the highest RQ value (0.0012), while the values in other sampling sites were below 0.001. These results indicated that HBCDs in sediments in the selected part of the Haihe River presented "no risk" in this compartment. However, its risk in sediment cannot be neglected, since sediment is one of the important sinks and reservoirs of pollutants. HBCDs are easily adsorbed on organic matters and then enter the aquatic environment through deposition of atmospheric particles, soil erosion and surface runoff, due to their hydrophobic characteristics and low volatility (Li et al., 2013). Furthermore, Remberger et al. (2004) found that HBCDs in sediments deposited several decades ago could still be detected, which indicated that they are fairly persistent during sediment diagenesis. According to the present study, other rivers containing high concentrations of HBCDs, such as the Dagu Drainage Canal in Tianjin, should draw attention regarding risk assessment.

3. Conclusions

A method was developed using HPLC–MS–MS and used to analyze HBCD isomers. The source parameters of the MS–MS including spray voltage, probe temperature and cone temperature were optimized to obtain excellent response for HBCDs, when they were respectively 4400 V, 260°C, and 300°C. The solvent composition and gradient slope of the mobile phase were also investigated. A satisfactory separation effect was obtained when using methanol–acetonitrile (1:1, V/V) and water as the mobile phase in HPLC. The optimal method was applied to detect the concentrations of total HBCDs and three individual isomers (α-, β-, γ-HBCD) in seven sediment samples in the Haihe River. The concentrations of HBCDs ranging from 0.4–58.82 ng/g exhibited a decreasing trend along the flow direction, which was attributed to attenuation and biodegradation along the flow direction of the Haihe River. The distribution profile of α-, β-, and γ-HBCD was 7.91%–88.6%, 0–91.47%, and 0.62%–42.83%, respectively. The α-HBCD isomer dominated in all samples except T_5. This differed from the distribution pattern in commercial industrial products, indicating the inter-transformation of isomers and different degradation rates for the three HBCDs isomers. The risk assessment of HBCDs was carried out and it was calculated that the RQ value of every site is below 0.0012, which indicated "no risk" in this study.

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REFERENCES

Bound, J.P., Voulvoulis, N., 2011. Pharmaceuticals in the aquatic environment—a comparison of risk assessment strategies. Chemosphere 56 (11), 1143–1155.

Brunstrom, B., Halldin, K., 2000. Ecotoxicological risk assessment of environmental pollutants in the Arctic. Toxicol. Lett. 112-113 (5), 111–118.

Budakowski, W., Tomy, G., 2003. Congener-specific analysis of hexabromocyclododecane by high-performance liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun. Mass Spectrom. 17 (13), 1399–1404.

Covaci, A., Gerecke, A.C., Law, R.J., Voorspoels, S., Kohler, M., Heeb, N.V., Leslie, H., Allchin, C.R., Eboer, J.D., 2006. Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. Environ. Sci. Technol. 40 (40), 3679–3688.

Davis, J.W., Gonsior, S., Marty, G., Ariano, J., 2005. The transformation of hexabromocyclododecane in aerobic and anaerobic soils and aquatic sediments. Water Res. 39 (6), 1075–1084.

Dwyer, T.O., 1996. Tracking the distribution of persistent organic pollutants. Environ. Sci. Technol. 11 (1), N2–N3.

Eriksson, P., Viberg, H., Fischer, C., Wallin, M., Fredriksson, A., 2002. A comparison on developmental neurotoxic effects of hexabromocyclododecane, 2,2′,4,4′,5,5′-hexabromodiphenylether (PBDE153) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153). Organohalogen Compd. 57 (2), 389–392.

Eriksson, P., Fischer, C., Wallin, M., Jakobsson, E., Fredriksson, A., 2006. Impaired behavior, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCD). Environ. Toxicol. Pharmacol. 21 (3), 317–322.

Feng, J.Y., Wang, Y.W., Ruan, T., Qu, G.B., Jiang, G.B., 2010. Simultaneous determination of hexabromocyclododecanes and tris (2,3-dibromopropyl) isocyanurate using LC–APCI–MS/MS. Talanta 82 (5), 1929–1934.

Fourth meeting of the Persistent Organic Pollutants Review Committee (POPRC4), 2016. http://chm.pops.int/Convention/POPsReviewCommittee/POPRCMeetings/POPRC7/POPRC7Documents/tabid/2267/language/en-US/Default.aspx.

Gereche, A.C., Giger, W., Hartmann, P.C., Heeb, N.V., Hans-Peter, E., Schmid, K.P., Zennegg, M., Kohler, M., 2006. Anaerobic degradation of brominated flame retardants in sewage sludge. Chemosphere 64 (2), 311–317.

Guerra, P., Eljarrat, E., Barcelo, D., 2010. Simultaneous determination of hexabromocyclododecane, tetrabromobisphenol A, and related compounds in sewage sludge and sediment samples from Ebro River basin (Spain). Anal. Bioanal. Chem. 397 (7), 2817–2824.

Guidance for industry, 1998. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070561.pdf).

Harrad, S., Abdallah, M.A.-E., Rose, N.L., Turner, S.D., Davidson, T.A., 2009. Current-use brominated flame retardants in water, sediment, and fish from English lakes. Environ. Sci. Technol. 43 (24), 9077–9083.

Heeb, N.V., Schweizer, W.B., Kohler, M., Gerecke, A.C., 2005. Structure elucidation of hexabromocyclododecanes—a class of compounds with a complex stereochemistry. Chemosphere 61 (1), 65–73.

Hernando, M.D., Mezcuaa, M., Fernández-Albaa, A.R., Barcelób, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69 (2), 334–342.

Hirayama, A., Kami, K., Sugimoto, M., Sugawara, M., Toki, N., Onozuka, H., Kinoshita, T., Saito, N., Ochiai, A., Tomita, M., Esumi, H., Soga, T., 2009. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Res. 69 (11), 4918–4925.

Hu, X.Z., Hu, D.C., Song, Q., Li, J., Wang, P., 2011. Determinations of hexabromocyclododecane (HBCD) isomers in channel catfish, crayfish, hen eggs and fish feeds from China by isotopic dilution LC–MS/MS. Chemosphere 82 (5), 698–707.

Ichihara, M., Yamamoto, A., Takakura, K.-I., Kakutani, N., Sudo, M., 2014. Distribution and pollutant load of hexabromocyclododecane (HBCD) in sewage treatment plants and water from Japanese Rivers. Chemosphere 110 (9), 78–84.

Janák, K., Covaci, A., Voorspoels, S., Becher, G., 2005. Hexabromocyclododecane in marine species from the western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation. Environ. Sci. Technol. 39 (7), 1987–1994.

Kebarle, P., 2000. A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry. J. Mass Spectrom. 35 (7), 804–817.

Lankova, D., Kockovska, M., Lacina, O., Kalachova, K., Pulkrabova, J., Hajslova, J., 2013. Rapid and simple method for determination of hexabromocyclododecanes and other LC–MS–MS amenable brominated flame retardants in fish. J. Anal. Bioanal. Chem. 405 (24), 7829–7839.

Letcher, R.J., Lu, Z., Chu, S.G., Haffner, G.D., Drouillard, K., Marvin, C.H., Ciborowski, J.J.H., 2015. Hexabromocyclododecane flame retardant isomers in sediments from Detroit River and Lake Erie of the Laurentian Great Lakes of North America. Bull. Environ. Contam. Toxicol. 95 (1), 31–36.

Li, H.H., Shang, H.T., Wang, P., Wang, Y.W., Zhang, H.D., Zhang, Q.H., Jiang, G.B., 2013. Occurrence and distribution of hexabromocyclododecane in sediments from seven major river drainage basins in China. Environ. Sci. 25 (1), 69–76.

Marvin, C.H., Tomy, G.T., Alaee, M., MacInnis, G., 2006. Distribution of hexabromocyclododecane in Detroit River suspended sediments. Chemosphere 64 (2), 268–275.

Morris, S., Bersuder, P., Allchin, C.R., 2006. Determination of the brominated flame retardant, hexabromocyclodocane, in sediments and biota by liquid chromatography–electrospray ionisation mass spectrometry. TrAC Trends Anal. Chem. 25 (4), 343–349.

Ramu, K., Isobe, T., Takahashi, S., Eun-Young, K., Byung-Yoon, M., Sung-Ug, W., Tanabe, S., 2010. Spatial distribution of polybrominated diphenyl ethers and hexabromocyclododecanes in sediments from coastal waters of Korea. Chemosphere 79 (7), 713–719.

Remberger, M., Sternbeck, J., Palm, A., Kaj, L., Strömberg, K., Lundén, E.B., 2004. The environmental occurrence of hexabromocyclododecane in Sweden. Chemosphere 54 (1), 9–21.

Roosens, L., Hollander, W.D., Bervoets, L., Reynders, H., Campenhout, K.V., Cornelis, C., Heuvel, R.V.D., Koppen, G., Covaci, A., 2010. Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk. Environ. Pollut. 158 (8), 2546–2552.

Schecter, A., Harris, T.R., Shah, N., Musumba, A., Päpke, O., 2008. Brominated flame retardants in US food. Mol. Nutr. Food Res. 52 (2), 266–272.

Schowanek, D., Webb, S., 2002. Exposure simulation for pharmaceuticals in European surface waters with GREAT-ER. Toxicol. Lett. 131 (1–2), 39–50.

Sellstrom, U., Kierkegaard, A., Wit, C.D., Jansson, B., 1998. Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. Environ. Toxicol. Chem. 17 (6), 1065–1072.

Sun, H.W., Zhang, X.Z., Wang, L., Zhang, T., Li, F., He, N., Alder, A.C., 2012. Perfluoroalkyl compounds in municipal WWTPs in Tianjin, China—concentrations, distribution and mass flow. Environ. Sci. Pollut. Res. 19 (5), 1405–1415.

Takigami, H., Suzuki, G., Hirai, Y., Sakai, S.I., 2008. Transfer of brominated flame retardants from components into dust inside television cabinets. Chemosphere 73 (2), 161–169.

Tang, L., Shao, H.Y., Zhu, J.Y., Xu, G., Han, T., Peng, B.Q., Wu, M.H., 2015. Hexabromocyclododecane diastereoisomers in surface sediments from river drainage basins of Shanghai, China: occurrence, distribution, and mass inventory. Environ. Sci. Pollut. Res. 22 (16), 11993–12000.

Verreault, J., shaogang, gabrielsen, G.W., Chu, S., Muir, D.C.G., Andersen, M., Hamaed, A., Letcher, R.J., 2016. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. Environ. Sci. Technol. 39 (16), 6021–6028.

Vuorensola, K., Kokkonen, J., Sirén, H., Ketola, R.A., 2001. Optimization of capillary electrophoretic–electrospray ionization-mass spectrometric analysis of catecholamines. Electrophoresis 22 (20), 4347–4354.

Y-Okabe, T., Sakai, H., Kashima, Y., Y-Okabe, H., 2005. Modulation at a cellular level of the thyroid hormone receptor mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4′-diiodobiphenyl (DIB), and nitrofen (NIP). Toxicol. Lett. 155 (1), 127–133.

Zhang, Y.W., Sun, H.W., Liu, F., Dai, Y.Y., Qin, X.B., Ruan, Y.F., Zhao, L.J., Gan, Z.W., 2013a. Hexabromocyclododecanes in limnic and marine organisms and terrestrial plants from Tianjin, China: diastereomer- and enantiomer-specific profiles, biomagnification, and human exposure. Chemosphere 93 (8), 1561–1568.

Zhang, Y.W., Ruan, Y.F., Sun, H.W., Zhao, L.J., Gan, Z.W., 2013b. Hexabromocyclododecanes in surface sediments and a sediment core from rivers and harbor in the northern Chinese city of Tianjin. Chemosphere 90 (5), 1610–1616.

Zhu, S.C., Chen, H., 2014. The fate and risk of selected pharmaceutical and presonal care products in wastewater treat plants and a pilot-scale multistage constructed wetland system. Environ. Sci. Pollut. Res. 21 (2), 1466–1479.