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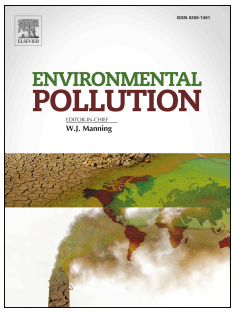

Quantitative and semiquantitative analyses of hexa-mix-chlorinated/brominated benzenes in fly ash, soil and air using gas chromatography-high resolution mass spectrometry assisted with isotopologue distribution computation

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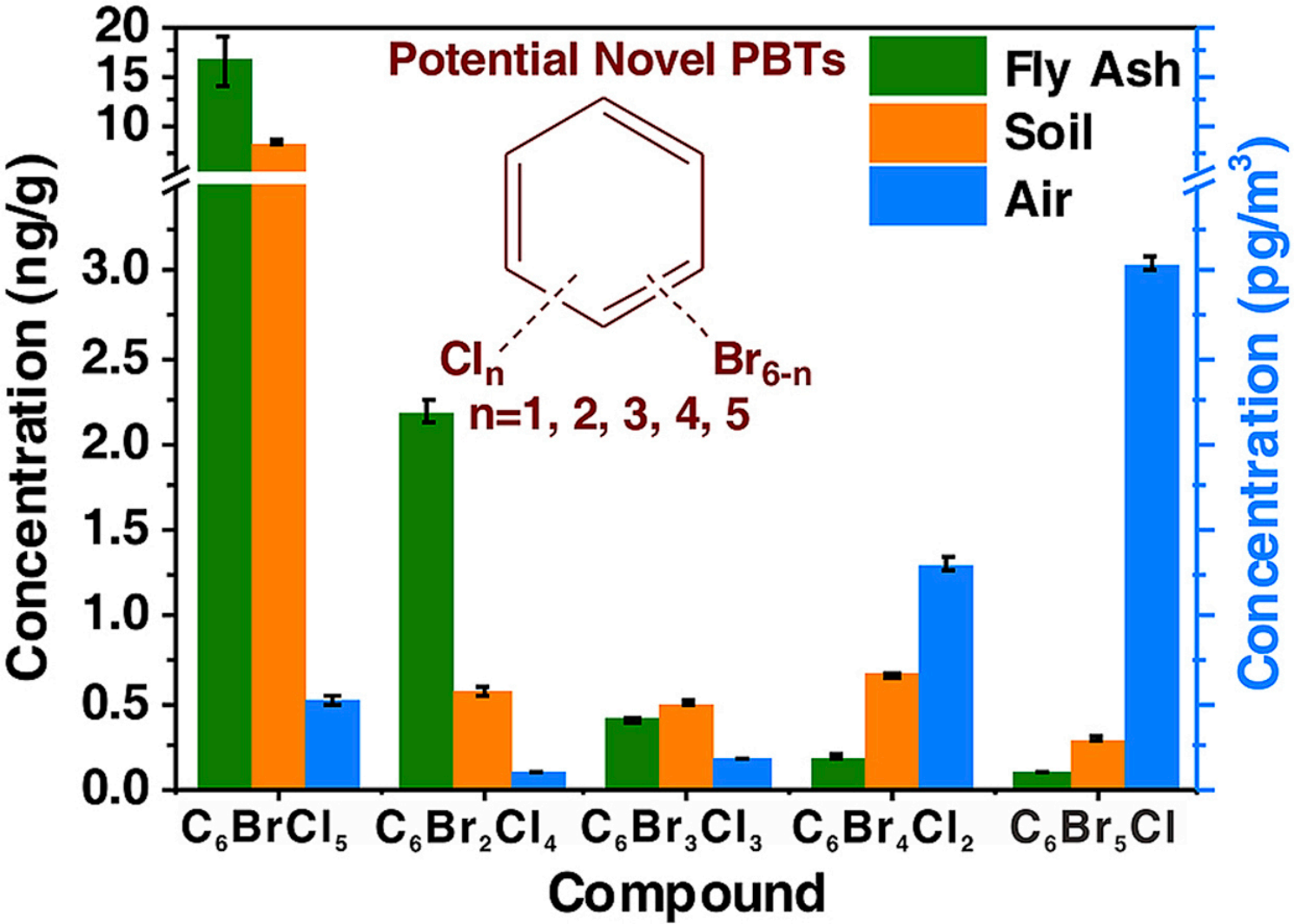
Potential Novel PBTs
Fly Ash
Soil
Air
Cl$_n$
Br$_{6-n}$
n=1, 2, 3, 4, 5
Concentration (ng/g)
Concentration (pg/m^3)
20
15
10
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C$_6$BrCl$_5$
C$_6$Br$_2$Cl$_4$
C$_6$Br$_3$Cl$_3$
C$_6$Br$_4$Cl$_2$
C$_6$Br$_5$Cl
Compound

Quantitative and semiquantitative analyses of hexa-mix-chlorinated/brominated benzenes in fly ash, soil and air using gas chromatography-high resolution mass spectrometry assisted with isotopologue distribution computation

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ABSTRACT

Hexa-mix-chlorinated/brominated benzenes (HXBs), a group of newly found analogues of hexachlorobenzene (HCB) and hexabromobenzene (HBB), may exhibit similar environmental risks and toxicities as HCB and HBB, and therefore possess high interests in environmental and toxicological research. Yet information regarding HXBs in the environment remains scarce. In this study, we developed an isotope dilution method for quantitative and semiquantitative determination of five HXBs in fly ash, soil and air using gas chromatography high resolution mass spectrometry (GC-HRMS) in multiple ion detection mode. The samples were Soxhlet-extracted and purified with multilayer composite silica gel-alumina columns, followed by GC-HRMS detection. Identification of HXBs was conducted by the comparison between theoretical and detected mass spectra using paired-samples T test and cosine similarity analysis. Two HXBs (C_6BrCl_5 and $C_6Br_4Cl_2$) with reference standards were quantitatively determined while the rest three ($C_6Br_2Cl_4$, $C_6Br_3Cl_3$ and C_6Br_5Cl) without reference standards were semiquantitatively analyzed by sharing the calibration curves of C_6BrCl_5 and $C_6Br_4Cl_2$ in cooperation with isotopologue distribution computation. The accuracies for C_6BrCl_5 and $C_6Br_4Cl_2$ were 87.3-107.8% with relative standard deviations (RSD) of 2.8-5.0%. The method limits of quantification of the HXBs were 0.10 ng/g in fly ash and soil samples and 0.09 pg/m^3 in ambient air samples. The recoveries ranged from 42.7% to 102.1% with RSD of 3.7-13.9%. This method has been successfully applied to the analysis of the HXBs in

the environmental samples. The total concentrations of HXBs in the fly ash, soil and ambient air samples were 19.48 ng/g, 10.44 ng/g and 5.13 pg/m^3, respectively, which accounted for 10.6%, 0.4% and 10.8% of the corresponding total concentrations of HCB and HBB. This study provides a reference method for quantitative and/or semiquantitative analyses of novel mix-halogenated organic compounds, and sheds light on the full picture of HXBs pollution in the environment.

Main finding:

HXBs were quantified/semi-quantified for the first time, and found to be non-ignorable pollutants in the environment, particularly the atmosphere.

Keywords:

Hexa-mix-chlorinated/brominated benzenes; Quantification and semi-quantification; Gas chromatography-high resolution mass spectrometry; Environmental samples; Isotopologue distribution

1. Introduction

Halogenated organic pollutants (HOPs) have been raising environmental and public health concerns worldwide due to their persistence, bioaccumulation and potential toxicities (Kamel, 2013; Köhler and Triebskorn, 2013; Oaks et al., 2004). HOPs including man-made and naturally generated are widely present in the environment (Guo et al., 2014; Gribble, 2010). Identification and quantification of novel HOPs in environmental matrices have become emerging and promising research hotspots (Pena-Abaurrea et al., 2014; Shaul et al, 2015; Hilton et al., 2010; Simon et al., 2013; Fernando et al., 2018; Fernandes et al., 2014; Kakutani et al., 2014; Trego et al., 2018; Phillips et al., 2018). Many state-of-the-art techniques such as gas chromatography-high resolution mass spectrometry (GC-HRMS) (Byer et al., 2014; Vetter et al., 2001; Tang and Tan, 2018), comprehensive two-dimensional GC-HRMS (Ieda et al., 2011; Hashimoto et al., 2011; Hashimoto et al., 2013), liquid chromatography tandem mass spectrometry [Qin et al., 2010; Pan and Zhang, 2013; Wang et al., 2018], liquid chromatography HRMS (Peng et al., 2015; Portolés et al., 2009), comprehensive two-dimensional liquid chromatography HRMS (Ouyang et al., 2017), and Fourier transform ion cyclotron resonance HRMS (Taguchi et al., 2010; Jobst et al., 2013) have been applied to identification of novel HOPs. In recent years, a variety of novel HOPs have been identified, e.g., mix-chlorinated/brominated dioxins and dibenzofurans (PXDD/Fs) (Hashimoto et al., 2011; Hashimoto et al., 2013), mix-chlorinated/brominated biphenyls (PXBs) (Fernandes et al., 2014; 2011), halogenated

polycyclic aromatic hydrocarbons (Ieda et al., 2011; Taguchi et al., 2010), mix-chlorinated/brominated polycyclic aromatic hydrocarbons (Ieda et al., 2011), chlorinated polycyclic aromatic sulfur heterocycles (Fernando et al., 2014), mix-polyhalogenated carbazoles (Guo et al., 2014; Vetter et al., 2001), chlorine substituted perfluorocarboxylates (Liu et al., 2015) and mix-chlorinated/brominated diphenyl ethers (PXDEs) (Yu et al., 2011; Bendig et al., 2012). The relevant matrices included fly ash of municipal solid waste incineration (MSWI) (Tang and Tan, 2018), fire debris (Fernando et al., 2014), soil (Yu et al., 2011), sediments (Guo et al., 2014), water, air (Yu et al., 2011), flue gas (Tang and Tan, 2018), food (Phillips et al., 2018) and even biological samples (Ohta et al., 2008; Ohta et al., 2009).

So far, research concerning newly identified HOPs is far from sufficient, owing to the lacking in reference standards of novel HOPs. Limited studies have reported quantitative analysis of novel HOPs, some of which are mix-halogenated organic pollutants (X-HOPs) such as PXDD/Fs and PXBs (Fernandes et al., 2014; Fernandes et al., 2011; Fernandes et al., 2018; Tue et al., 2016). Currently, only a few reference standards of PXDD/Fs and PXBs are commercially available, whereas theoretical congeners of PXDD/Fs and PXBs are far more than those of polychlorinated dioxins and dibenzofurans (PCDD/Fs), polybrominated dioxins and dibenzofurans (PBDD/Fs), polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). For instance, PXDD/Fs have 4600 possible congers whereas PCDD/Fs (or PBDD/Fs) have only 210 congeners (Myers et al., 2012). PXBs possess 9180 congeners, while

the amount of PCBs (or PBBs) congeners is 209 (Haranczyk et al., 2012). In addition, hexa-mix-chlorinated/brominated benzenes (HXBs), a group of analogues of hexachlorobenzene (HCB) and hexabromobenzene (HBB), have 11 congeners whereas both HCB and HBB are uni-structural compounds (Tang and Tan, 2018). If other halogens including fluorine and iodine are introduced, the theoretical congeners of mix-halogenated organic compounds will be massive. Furthermore, the amount of unknown X-HOPs identified in environmental matrices such as fly ash and fire ash is increasing (Fernando et al., 2018; Tang and Tan, 2018; Fernando et al., 2014). Therefore, quantification of each newly identified X-HOP is impracticable.

In this context, semi-quantitative analysis seems an alternative approach to further investigate the pollution status involving approximate concentration information of most novel X-HOPs. Up to now, a few studies have reported the semi-quantification of PXDD/Fs in fire debris (Organtini et al, 2014; 2015) and mix-polyhalogenated carbazoles in lake sediments (Guo et al., 2014). However, no report is available so far for semi-quantitative analysis of other X-HOPs, e.g., HXBs, PXBs and PXDEs. In addition, the available semi-quantification of PXDD/Fs was based on isomeric reference standards, which could limit analysis of analytes without isomeric reference standards.

As typical novel X-HOPs, HXBs have five formulae (i.e., C_6BrCl_5, $C_6Br_2Cl_4$, $C_6Br_3Cl_3$, $C_6Br_4Cl_2$, and C_6Br_5Cl), of which the middle three possess three isomers individually. HXBs are anticipated to exhibit similar properties, environmental

behaviors and toxicities as HCB and HBB which are typical persistent, bioaccumulative and toxic substances (PBTs), and thus of high research interests (Haranczyk et al., 2012; Tang and Tan, 2018). Moreover, HXBs can be generated during incineration and combustion of solid wastes and present as byproducts in synthesized HCB and HBB products (Tang and Tan, 2018), and may find their way into the environment finally. Recently, some HXBs have been identified in MSWI fly ash, flue gas, soil, and ambient air (Tang and Tan, 2018). Furthermore, two reference standards of HXBs, namely 1-bromo-2,3,4,5,6-pentachlorobenzene and 1,2,4,5-terabromo-3,6-dichlorobenze are commercially available at present. Therefore, HXBs merit to be chosen as representative novel X-HOPs to implement quantitative and semi-quantitative analyses in environmental matrices.

In the present study, we developed an isotope dilution method for quantification and semi-quantification of five HXBs in MSWI fly ash, soil, and ambient air using GC-HRMS. Quantification of two HXBs was conducted with commercial reference standards, while semi-quantification of the rest three was performed with the aid of isotopologue distribution computation and the relationship between molar concentration and MS signal intensity (signal response factor). The method has been validated in terms of accuracy, precision, recovery, sensitivity, selectivity and repeatability. Finally, this method has been successfully applied to the quantitative and semi-quantitative analyses of HXBs in the environmental matrices. This study proposes a reference approach for quantification and semi-quantification of novel X-

131 HOPs, and provides new and frontier insights into the pollution status of HXBs in the

132 environment.

2. Materials and methods

2.1. Chemicals and materials

Reference standards 1-bromo-2,3,4,5,6-pentachlorobenzene (C_6BrCl_5, purity ≥ 97%) and 1,2,4,5-terabromo-3,6-dichlorobenze ($C_6Br_4Cl_2$, purity ≥ 97%) were bought from Sigma-Aldrich LLC. (St. Louis, MO, USA) and Bide Pharmatech Ltd. (Shanghai, China), respectively. HBB (100 μg/ml in toluene) was purchased from Accustandard Inc. (New Haven, CT, USA). Two HCB standards were bought from Accustandard Inc. (HCB standard-1, 2 mg/mL in hexane) and Dr. Ehrenstorfer (Augsburg, Germany, HCB standard-2, purity ≥ 99.5%), respectively. Stable isotope-labeled standards $^{13}C_6$-hexachlorobenzene ($^{13}C_6$-HCB, 100 μg/ml in nonane) and $^{13}C_6$-hexabromobenzene ($^{13}C_6$-HBB, 100 μg/ml in toluene) were obtained from Cambridge Isotope Laboratory (CIL) Inc. (Andover, MA, USA). A standard solution containing four $^{13}C_{12}$-polychlorobiphenyls ($^{13}C_{12}$-PCBs), i.e., $^{13}C_{12}$-PCB70, $^{13}C_{12}$-PCB111, $^{13}C_{12}$-PCB138 and $^{13}C_{12}$-PCB170, in nonane (WP-ISS, 1 μg/mL for each standard) was purchased from Wellington Laboratories Inc. (Ontario, Canada). Perfluorotributylamine (FC43) used for calibrating HRMS was obtained from Sigma-Aldrich LLC.

Hexane, dichloromethane and acetone were of HPLC grade and purchased from Merck Crop. (Darmstadt, Germany). Chromatographic grade solvents nonane and isooctane were bought from Alfa Aesar Company (Ward Hill, MA, USA) and CNW Technologies GmbH (Düsseldorf, Germany), respectively. Concentrated sulfuric acid (H_2SO_4) and alumina powder (70-230 mesh) were obtained from Sigma-Aldrich (St.

Louis, MO, USA). Neutral silica gel (60-200 mesh) and potassium hydroxide (KOH) were bought from Merck Crop. (Darmstadt, Germany). Anhydrous sodium sulfate (Na_2SO_4) was purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

Neutral silica gel was activated at 180 ^oC in an oven for 12 hours, and then cooled to room temperature in a vacuum dryer and allowed to stay overnight before use. Acidified silica gel was prepared by mixing concentrated sulfuric acid and activated neutral silica gel at a proportion of silica gel/sulfuric acid = 3:2 (*w/w*). After mixing, the acidified silica gel was kept in a vacuum dryer overnight prior to use. Basified silica gel was prepared by adding saturated KOH aqueous solution into activated neutral silica gel at a proportion of silica gel/KOH solution = 97:3 (*w/w*). Alumina powder was activated at 500 ^oC for 8 hours and then cooled overnight before use. Anhydrous sodium sulfate was baked at 450 ^oC for 4 hours, and then cooled to ambient temperature in a vacuum dryer prior to use.

The purchased standards in powder form including C_6BrCl_5, $C_6Br_4Cl_2$ and HCB standard-2 were accurately weighed, followed by dilution with isooctane to obtain stock solutions at 1 mg/mL. Other purchased standards in solution form were directly used as stock solutions. A cocktail solution containing both C_6BrCl_5 and $C_6Br_4Cl_2$ at 10 μL/mL for individual compounds was prepared by diluting the stock solutions of the two analytes with isooctane. This cocktail solution was used as the calibration working solution of the highest concentration. Other calibration working solutions

175 (5000, 1000, 500, 100, 50, 20 and 10 ng/mL) and quality control working solutions
176 (8000, 400, 25 and 10 ng/mL) were further prepared by serial dilution from the
177 calibration working solution of the highest concentration using isooctane.

178 The HCB standard-1 and HBB standard were employed to prepare the cocktail
179 working solutions of calibration and quality control samples (QCs) for HCB and HBB.
180 The preparation procedures of working solutions for HCB and HBB were the same to
181 those for C_6BrCl_5 and $C_6Br_4Cl_2$. The working solution containing the internal
182 standards $^{13}C_6$-HCB and $^{13}C_6$-HBB was prepared by diluting the corresponding stock
183 solutions to 5 μg/mL for each compound with isooctane. The injection internal
184 standard working solution containing the four $^{13}C_{12}$-PCBs was prepared by diluting
185 the WP-ISS stock solution with isooctane to 200 ng/mL for individual compounds.

186 The calibration samples (1000, 500, 100, 50, 10, 5, 2 and 1 ng/mL) and reagent QCs
187 (800, 40, 2.5 and 1 ng/mL) were prepared by 10-fold dilution of the calibration and
188 quality control working solutions with isooctane, followed by the addition of 2 μL of
189 the working solution of $^{13}C_6$-HCB and $^{13}C_6$-HBB, and 5 μL of the $^{13}C_{12}$-PCBs
190 working solution. The final volumes of these calibration samples and reagent QCs
191 were 100 μL.

192 *2.2. Sample information and pretreatment*

193 *2.2.1. Sample information*

From 2013 to 2014, ten fly ash samples were collected from either waste incineration power facilities or MSWI plants in three industrialized cities (Guangzhou, Shenzhen, and Foshan) of Guangdong province, China. The incinerator types included Martin furnace, two-stage furnace and pulse furnace. Five soil samples were collected from an electronic waste recycling region (Longtang Town) in Qingyuan City of Guangdong province in 2015. Five ambient air samples were collected from September 2015 to October 2015 at Guangzhou Institute of Geochemistry in Guangzhou, a megacity of China. The ambient air samples were sampled by a high-power air sampler using quartz filter films and polyurethane foam (PUF) cylinders for collection of particle matters and gaseous organic compounds. Ten grams of each fly ash sample were taken and mixed with others to constitute a pooled fly ash matrix. Similarly, 20 grams of the individual soil samples were taken and combined with others to constitute a pooled soil matrix. With respect to ambient air samples, after Soxhlet extraction, the extracted mixtures were combined and evaporated to 80 mL with a rotary evaporator (R-210, Buchi, Switzerland) to obtain a pooled air matrix. These pooled matrices were employed to conduct the method development and validation in this study.

2.2.2. Extraction

One gram of the pooled fly ash/soil matrix was accurately weighed and spiked with internal standard solutions of ($^{13}C_6$-HCB and $^{13}C_6$-HBB) at the concentration of 10 ng/g. The sample was then subjected to Soxhlet extraction. The extraction solvent was

toluene, and activated copper sheets were added for eliminating sulfur during extraction. The total Soxhlet extraction time was two days. All the sampled quartz filters along with PUF cylinders (ambient air samples) were Soxhlet-extracted, and the extraction procedures were similar as those for the fly ash/soil samples. For each parallel ambient air sample, 5 mL out of the 80 mL of pooled mixture was used, which was equivalent to 215.5 m^3 of ambient air at standard conditions.

2.2.3. Sample purification

The extract was rotary-evaporated to near dry followed by the addition of 100 mL of hexane and 30 g of acidified silica gel. Thereafter, this mixture was subjected to magnetic stirring for 2 hours, and then filtered. The filtrate was evaporated to around 1 mL and loaded onto a prepared multilayer composite silica gel-alumina column that was packed with the following materials from bottom to top: 1 g Na_2SO_4, 6 g alumina, 1 g neutral silica gel, 5 g basified silica gel, 1 g neutral silica gel, 12 g acidified silica gel, 1 g neutral silica gel and 1 g Na_2SO_4. The sample mixture was eluted with 120 mL of hexane/dichloromethane (1:1, *v/v*) and concentrated with a rotary evaporator to around 1 mL. Afterwards, the concentrated mixture was transferred to a glass injection vial and further evaporated to near dry with a gentle nitrogen stream, and then 5 μL of the injection internal standard working solution ($^{13}C_{12}$-PCBs in isooctane at 200 ng/mL) was added. The sample residual was reconstituted with 50 μL of nonane and then assigned to instrumental analysis.

2.3. GC-HRMS analysis

The GC-HRMS system comprised dual Trace-GC-Ultra gas chromatographs coupled with a Triplus auto-sampler and a double focusing magnetic-sector high resolution mass spectrometer (DFS-HRMS, Thermo-Fisher Scientific, Bremen, Germany). Chromatographic separation was carried out with a DB-5 MS capillary column (60 m × 0.25 mm, 0.25 μm, J&W Scientific, USA). The temperature program was proceeded as: held at 120 ºC for 2 min, ramped to 220 ºC at 20 ºC/min and held for 16 min, then ramped to 235 ºC at 5 ºC/min and held for 7 min, thereafter, ramped to 260 ºC at 5 ºC/min, and finally ramped to 330 ºC at 30 ºC/min and held for 9.67 min. The carrier gas was ultra-pure helium with a constant flow rate at 1 mL/min. Splitless injection mode was adopted and the injection volume was 1 μL. The solvent delay time was set at 8 min.

The working parameters and conditions of HRMS were provided as the following: ion source was operated in positive electron ionization (EI) mode; electron impact energy was set at 45 eV; ion source temperature was set at 250 °C; multiple ion detection (MID) was used as scanning mode; mass resolution (5% peak-valley definition) was ≥ 10000; MS detection accuracy was set at ±0.001 u; and HRMS was real-timely calibrated with FC43.

Chemical structures of all the analytes and internal standards were depicted with ChemDraw (Ultra 7.0, Cambridgesoft, Cambridge, USA) and exact molecular weights of isotopologues were then calculated at a mass accuracy of 0.00001 μ. For the HXBs, the first six isotopologues with the highest theoretical relative abundances of each

compound were selected. For HCB and HBB, the first four isotopologues with the highest theoretical relative abundances of each compound were chosen. The numbers of selected isotopologues of the isotope-labeled internal standards were 2-5. The exact mass-to-charge ratios (*m/z*) of isotopologue ions on EI source were calculated by reducing the relative mass of an electron from each exact molecular mass. Afterwards, these exact *m/z* values were imported into the MID module of HRMS for detecting the analytes and internal standards. The dwell time of each isotopologue ion was 50 ms. The exact *m/z* values, formulae and theoretical relative abundances of isotopologues of all the involved compounds are listed in Table S-1, and the representative chromatograms of C_6BrCl_5, $C_6Br_4Cl_2$, HCB and HBB are shown in Figure S-1.

2.4. Data processing

The identification procedures have been detailed in our previous study (Tang and Tan, 2018), and also provided in the Supplementary data. Briefly, paired-samples T test using SPSS Statistics 19.0 (IBM Inc., Armonk, USA) and cosine similarity analysis using SPSS or Excel 2010 (Microsoft Company, Seattle, USA) were applied to the identification of analytes, particularly the HXBs without reference standards. The simulated mass spectra (obtained by MassLynx V4.1 (Waters Corp., Manchester, UK)), and the detected mass spectra in HXBs standards, HBB/HCB standards and real samples were evaluated in terms of similarity with the two similarity analysis approaches. If a p-value of paired-samples T test is ≥ 0.05 and coefficient of association (R) is ≥ 0.90 ($p \leq 0.05$), the null hypothesis is accepted, indicating no

significant difference between two mass spectra. In addition, if the cosine similarity value (cos θ) between the two mass spectra is ≥ 0.90, then the two mass spectra are determined to be significantly similar, and the relevant compound of interest in a sample can thus be identified. Because the reference standards of three HXBs were unavailable, the above qualitative analysis procedures were necessary.

Quantification and semi-quantification of the analytes were performed with internal standard method. Eight-point calibration curves were constructed for quantification of C_6BrCl_5, $C_6Br_4Cl_2$, HCB and HBB with reference standards. Semi-quantification of $C_6Br_2Cl_4$, $C_6Br_3Cl_3$ and C_6Br_5Cl were conducted by sharing the calibration curves of C_6BrCl_5 and $C_6Br_4Cl_2$. Specifically, the concentrations of $C_6Br_2Cl_4$ were calculated with the calibration curves of C_6BrCl_5, and those of $C_6Br_3Cl_3$ and C_6Br_5Cl were calculated with the calibration curves of $C_6Br_4Cl_2$. The quantification and semi-quantification procedures were carried out with Xcalibur 2.0 (Thermo-Fisher) and Excel.

2.5. Method validation

2.5.1. Calibration, accuracy and precision

Eight calibration samples of C_6BrCl_5 and $C_6Br_4Cl_2$ from 1 to 1000 ng/mL, along with eight calibration samples of HCB and HBB (1-1000 ng/mL) were analyzed in each batch. Eight-point calibration curves were then established with the weight factor of $1/x^2$ to calculate the concentrations of each analyte. Accuracy and precision of back-calculated concentrations of the analytes were evaluated.

299 Reagent QCs and spiked fly ash QCs were prepared and analyzed for validation of
300 accuracy and precision. In each batch, 1-3 groups of reagent QCs (each group
301 containing four concentration levels of QCs (i.e, 1, 2.5, 40 and 800 ng/mL) were
302 analyzed. The spiked fly ash QCs were prepared by spiking HXB standards into
303 parallel fly ash samples at 60 ng/g for C_6BrCl_5, and at 1 ng/g and 6 ng/g for $C_6Br_4Cl_2$.
304 In addition, precision of the method was also evaluated with replicated analysis of
305 environmental samples and the standards of HBB and HCB. Both intra-batch and
306 inter-batch precisions were evaluated.

307 *2.5.2. Limits of quantification and detection*

308 The lower limit of quantification (LLOQ) for all analytes were 1 ng/mL in the
309 calibration samples and reagent QCs. The signal-to-noise (S/N) ratios for LLOQ
310 samples should be ≥ 10. The instrumental limit of detection (LOD) was determined as
311 the concentration in a sample which can generate a signal with the S/N ratio ≥ 3.

312 *2.5.3. Recovery, carryover effect and selectivity*

313 The recovery of the method was estimated with the standards of C_6BrCl_5, $C_6Br_4Cl_2$,
314 $^{13}C_6$-HCB, $^{13}C_6$-HBB, HCB and HBB, along with the rest three HXBs found in the
315 HBB standard. The recovery was calculated as the ratio of the relative signal intensity
316 of a compound to an injection internal standard in a spiked sample with pretreatment
317 relative to that in a neat solution at the same nominal concentration. Specifically, for
318 calculating the recoveries of C_6BrCl_5 and $C_6Br_4Cl_2$, the relative signal intensities in

the non-spiked samples should be subtracted from those in the spiked samples. The recoveries of $^{13}C_6$-HCB and $^{13}C_6$-HBB were calculated with all samples with pretreatment, and those of C_6BrCl_5 and $C_6Br_4Cl_2$ were calculated with the spiked and the non-spiked fly ash samples along with the spiked reagent samples with pretreatment. While the recoveries of HCB, HBB and the rest three HXBs found in the HBB standard were merely evaluated with the spiked reagent samples with pretreatment.

The carryover effect was evaluated with the ratio of the signal intensity of an analyte in a reagent blank which was injected just next to injection of the highest-concentration calibration sample (1000 ng/mL) relative to that in an LLOQ sample (1 ng/mL). The carryover effect should be $\leq$ 20%. All the used glass apparatuses were carefully cleaned and baked at 450 °C for 4 hours for removing possible interferences before use. At least one procedure blank was deployed in each analytical batch. None of the analytes was detectable in the blank samples. Mutual interferences between native and isotope-labeled compounds were investigated, and the isotopologue ions used for quantification were ensured to be free from mutual interferences.

2.6. Statistical analysis

The concentration differences among HXB congeners and matrices were examined with independent-samples T test by SPSS Statistics 19.0. If a p-value (2-tailed) is less than 0.05, then the null hypothesis (e.g., no difference between two concentrations) is rejected, demonstrating a significant difference indeed existent.

3. Results and discussion

3.1. Sample pretreatment

In this study, we aimed to develop a method for quantification and semi-quantification of HXBs in environmental matrices including fly ash, soil and ambient air. Presently, no study has reported the quantitative and/or semi-quantitative analyses of HXBs. Since HXBs are planar halogenated organic compounds like PCDD/Fs, we thus referred to the pretreatment procedures for analysis of PCDD/Fs in previous studies (Li et al 2007; 2008) to develop the pretreatment method for HXBs.

Due to the complexity of environmental samples and the requirements of high-purification injection mixtures of GC-HRMS, acidified silica gel beds with magnetic stirring were used to remove major organic substances and interference compounds, followed by multilayer composite silica gel-alumina columns which were used to further clean up samples. Sample mixtures should be evaporated to near dry before reconstitution with nonane, which could help to obtain satisfactory chromatographic peak shapes and reasonable retention times for the analytes and internal standards. After the pretreatment, satisfactory chromatographic peaks free of inseparable interferences were obtained (Figure 1).

3.2. Optimization of GC-HRMS analysis

HXBs have never been intentionally synthesized by human beings, thus their concentrations may be at trace levels in environmental matrices, while GC-HRMS can provide high sensitivity and selectivity for analysis of HXBs in the environment.

Since some HXBs have no reference standard, the purity of chromatographic peaks is critical to identify these compounds. Hence, a long GC column with a slow temperature program was applied to the separation for eliminating possible interferences. Nine MS scanning windows were assigned to monitor different compounds (Table S-1), which enhanced the dwell times for individual ions without extending the total time of a scanning cycle. This could improve the MS signal intensities of individual ions and smooth the chromatographic peaks.

3.3. Data treatment

3.3.1. Qualification

Data treatment is crucial for this study. Due to the lack of reference standards for three HXBs, i.e., $C_6Br_2Cl_4$, $C_6Br_3Cl_3$ and C_6Br_5Cl, the identification of these compounds should be cautiously performed. As a consequence, we used the quasi-targeted analysis strategy proposed in our previous study to implement the identification of HXBs in the present study (Tang and Tan, 2018). Both paired-samples T test and cosine similarity analysis were employed to evaluate mass spectral similarity.

The similarities between detected and simulated mass spectra of HXBs found in the HBB standard were primarily evaluated with both molecular ions and full ions (including both molecular and dehalogenation product ions). As shown in Figure S-2, the detected mass spectra of HXBs in the HBB standard match the simulated mass spectra very well. And the detailed similarity analysis results also show that the

similarities between the detected and the simulated mass spectra are significant with all the analysis parameters fulfilling the criteria (Table S-2). These results indicate that the identified compounds in the HBB standard are really the HXBs of interest.

Afterwards, we implemented the similarity analysis between simulated and detected mass spectra of C_6BrCl_5 and $C_6Br_4Cl_2$ in the reference standards of C_6BrCl_5, $C_6Br_4Cl_2$ and HBB, as well as the similarity analysis between the detected mass spectra of the two HXBs in the HXBs standards and those in the HBB standard. As shown in Figure 2, the three types of mass spectra of individual HXBs are very similar. Furthermore, as documented in Table S-3, the detailed similarity analysis data show that all the analysis parameters fulfil the requirements for significant similarity, demonstrating that any two types of mass spectra are similar. These results in association with the alignment of retention times (Figure S-1 and Figure 1) confirm that the compounds identified in the HBB standard are exactly the HXBs of interest, which means that the identification approach are competent in identification of HXBs with the lack of reference standards. In addition to the HBB standard, we also found C_6BrCl_5 in the two HCB standards (HCB standard-1 and standard-2), of which the chromatograms and mass spectra are shown in Figure S-3 and Figure S-4, respectively.

The five HXBs found in the HBB standard were then regarded as references to carry out similarity analysis between the reference mass spectra and the detected mass spectra in fly ash, soil and ambient air samples. As shown in Figure 3, the detected mass spectra of the HXBs in fly ash, soil and ambient air samples are apparently

similar to those in the HBB standard. Additionally, as shown in Table S-4, all the similarity analysis parameters meet the requirement of significant similarity, except the p-value of association coefficient between the reference mass spectra and the detected mass spectra of $C_6Br_3Cl_3$ in the air samples. This p-value (> 0.05) could be ascribed to the relatively low abundance of $C_6Br_3Cl_3$ in the air samples. In summary, these similarity analysis results in association with retention time comparison demonstrate that the candidate HXBs identified in the environmental samples are exactly the HXBs of interest.

3.3.2. Quantification and semi-quantification

In this study, C_6BrCl_5, $C_6Br_4Cl_2$, HCB and HBB were quantified with internal standard method. Application of isotope dilution could enhance the accuracy and precision of the analytical method. Semi-quantification was performed for $C_6Br_2Cl_4$, $C_6Br_3Cl_3$ and C_6Br_5Cl, due to the unavailability of reference standards for these compounds. The semi-quantification of these HXBs also conducted with internal standard. The internal standard for C_6BrCl_5, $C_6Br_2Cl_4$ and HCB was $^{13}C_6$-HCB, and that for $C_6Br_3Cl_3$, $C_6Br_4Cl_2$, C_6Br_5Cl and HBB was $^{13}C_6$-HBB. It can be deduced that MS signal intensities positively correlate with molar concentrations for analytes. We deduced that the adjacent HXBs (e.g., C_6BrCl_5 and $C_6Br_2Cl_4$) might have similar calibration curve parameters in an analytical batch. Therefore, the HXBs without reference standards could be semi-quantified with the calibration equations of their adjacent HXBs with reference standards as expressed by Eq (S-37) in the

Supplementary data. Due to that different HXBs have different isotopologue distributions, the relative abundances of the isotopologue ions used for semi-quantification and quantification (the highest-abundance ions) should be taken into consideration. The calibration equations applied to semi-quantification were adjusted with the theoretical relative abundances of the semi-quantification and quantification isotopologue ions, which is detailed in the Theory section in the Supplementary data.

It is noteworthy that the highest-abundance isotopologue ions of $^{13}C_6$-HCB (*m/z* 289.8297) and $^{13}C_6$-HBB (*m/z* 557.5235) were not applied in the quantitative and semi-quantitative analyses, due to the possible interferences caused by the ions *m/z* 289.8008 of HCB and *m/z* 557.4972 of HBB. Instead, *m/z* 295.8209 of $^{13}C_6$-HCB and *m/z* 559.5214 of $^{13}C_6$-HBB, which are were free of interferences from HCB and HBB, were employed to perform the analyses of HXBs along with HCB and HBB. On the other hand, *m/z* 281.8126 of HCB and *m/z* 549.5054 of HBB were applied to quantifying HCB and HBB, respectively, to get rid of possible interferences triggered by isotopologue ions of $^{13}C_6$-HCB and $^{13}C_6$-HBB.

3.4. Validation results

3.4.1. Accuracy and precision

The accuracy and precision of the instrumental method were validated with the calibration samples and reagent QCs. As provided in Table S-5, the accuracies of back-calculated concentrations in the calibration samples for HCB, C_6BrCl_5, $C_6Br_4Cl_2$ and HBB were 95.6-103.5% (with relative standard deviations (RSD) of 1.1-1.9%),

96.2-103.3% (RSD: 1.9-4.3%), 95.3-103.8% (RSD: 1.7-5.4%) and 97.0-104.3% (RSD: 1.5-2.8%), respectively, showing excellent accuracies and precisions. The intra-batch and inter-batch accuracies and precisions of C_6BrCl_5 and $C_6Br_4Cl_2$ in reagent QCs at four concentration levels are shown in Table 1. The intra-batch accuracies of C_6BrCl_5 and $C_6Br_4Cl_2$ were 87.6-99.0% (RSD: 0.4-2.6%) and 91.5-107.2% (RSD: 0.5-5.9%), respectively. The inter-batch accuracies of C_6BrCl_5 and $C_6Br_4Cl_2$ were 89.2-99.0% (RSD: 1.3-4.8%) and 94.0-103.7% (RSD: 3.8-6.9%), respectively. These results indicate satisfactory accuracy and precision of the instrumental method.

The accuracy and precision of the analytical method were validated with the spiked fly ash QCs by spiking the standard solutions of C_6BrCl_5 and $C_6Br_4Cl_2$ into parallel fly ash samples. The spiking concentrations of C_6BrCl_5 and $C_6Br_4Cl_2$ were at least three times higher than those of corresponding compounds in the fly ash samples. As shown in Table 2, the mean accuracy for C_6BrCl_5 at the spiking concentration of 60 ng/g was 87.3% with RSD of 4.1%. The mean accuracies for $C_6Br_4Cl_2$ at the spiking concentrations of 6 ng/g and 1 ng/g were 107.8% (RSD: 5.0%) and 97.2% (RSD: 2.8%), respectively (Table 2). These results demonstrate good accuracy and precision for analysis of HXBs in environmental matrices (e.g., fly ash).

3.4.2. Linearity, and limits of quantification and detection

The linearities of HCB, C_6BrCl_5, $C_6Br_4Cl_2$ and HBB within the quantification range of 1-1000 ng/mL were excellent with the correlation coefficients (R^2) $\geq$ 0.9996 (Figure S-5). Furthermore, the calibration curves of C_6BrCl_5 ($y = -0.0061 + 0.2234x$)

and $C_6Br_4Cl_2$ ($y = -0.0121 + 0.1135x$) show somewhat similar parameters (Figure S-5), indicating that the response factors of C_6BrCl_5 and $C_6Br_4Cl_2$ were similar to some extent. Since the formula difference between C_6BrCl_5 and $C_6Br_4Cl_2$ (three Br and three Cl atoms) is much larger than that between two adjacent HXBs (e.g., C_6BrCl_5 and $C_6Br_2Cl_4$), it can be anticipated that the response factors of two adjacent HXBs were much more similar than those of C_6BrCl_5 and $C_6Br_4Cl_2$. As a result, in this study, the semi-quantification on the basis of sharing the calibration curves of the HXBs with reference standards with their adjacent HXBs without reference standards was rational and to some extent accurate and reliable. As shown in Figure S-1, the chromatographic peaks of HCB, C_6BrCl_5, $C_6Br_4Cl_2$ and HBB in the LLOQ samples exhibit S/N ratios far higher than 10. Thus, the instrumental limits of quantification (LOQs) of all analytes were determined to be 1 ng/mL and the method LOQs were 0.1 ng/g for fly ash and soil samples. For the ambient air samples, if the concentration of an analyte in an injection sample mixture was high than 20% of the instrumental LOQ and the S/N ratio of the chromatographic peak of the analyte was $\geq$ 10, then this analyte in the sample was regarded as quantifiable. Accordingly, the method LOQs of the analytes in the ambient air samples was determined as 0.09 pg/m^3, given the lowest-concentration analyte, i.e., $C_6Br_2Cl_4$ (0.095±0.007 pg/m^3) showing an S/N ratio of chromatographic peak > 10 (Figure 1D). The instrumental LODs for all the HXBs were estimated to be less than 0.3 ng/mL based on the signal intensities in the

LLOQ samples, and the method LODs in fly ash and soil samples were determined to be 0.03 ng/g.

3.4.3. Recovery

As shown in Table 3 and Table S-6, the recoveries of the two internal standards $^{13}C_6$-HCB and $^{13}C_6$-HBB were ≥ 55.4% (RSD: 12.4%) and ≥ 71.3% (RSD: 10.8%), respectively. The recoveries of C_6BrCl_5 in spiked fly ash samples and in spiked reagent samples were 42.7% (RSD: 9.4%) and 84.7% (RSD: 10.0%), respectively. The recoveries of $C_6Br_4Cl_2$ in spiked fly ash samples were ≥ 72.2% (RSD: 13.9%) and that in the spiked reagent samples was 89.5% (RSD: 7.2%). The recoveries of $C_6Br_2Cl_4$, $C_6Br_3Cl_3$, C_6Br_5Cl, HCB and HBB in the spiked reagent samples were 82.5-102.1% (RSD: 3.7-11.6%). Since isotope-dilution approach was applied in this study, some relatively less satisfactory recoveries such as that of C_6BrCl_5 in the spiked fly ash samples (42.7%) were acceptable, provided the MS signal intensities of analytes were sufficient for quantification.

3.4.4. Selectivity

Due to the high separation performance of GC and high resolution power of HRMS, none inseparable interference was observed for each analyte in any sample (Figure 1). However, a few separable interference chromatographic peaks could be observed in the MID channels for some analytes in some samples. For example, a separable interference chromatographic peak at retention time of 27.05 min was observed in the MID channels of C_6Br_5Cl whose retention time was 27.91 min in the same soil

sample (Figure S-6). This interference chromatographic peak might lead to misidentification of the real analyte C_6Br_5Cl, due to the relatively close retention times and instability of retention time possibly caused by matrix differences of different samples. Fortunately, this misidentification can be avoided by the similarity analysis between mass spectra. As illustrated in Figure S-6, the mass spectrum of the interference compound is apparently different from those of C_6Br_5Cl detected in the HBB standard and the soil samples, whereas the latter two mass spectra are significantly similar (Table S-4). This result further indicates the necessity of similarity analysis between mass spectra for identification of HXBs.

3.5. Comparison with other semi-quantification methods

Previous studies have reported semi-quantification of unknown isomers of PXDD/Fs in fire debris (Organtini et al, 2014; 2015) and soil (Tue et al., 2016) using commercially available reference standards of PXDD/Fs based on an assumption that the reference standards and the respective isomers share the same MS signal response factors. However, these methods may be limited by availability of reference standards, since most X-HOPs have no commercial reference standards. Thus, semi-quantitative analysis of most X-HOPs cannot be achieved with this semi-quantification scheme. Guo et al. (2014) carried out semi-quantification of 15 unknown polyhalogenated carbazoles including seven novel mix-halogenated carbazoles in sediments using two commercial reference standards (3,6-dibromocarbazole and 1,3,6,8-tetrabromocarbazole). Specifically, three mix-halogenated carbazoles were semi-

528 quantified by the calibration curve of 3,6-dibromocarbazole and the rest four were
529 semi-quantified by that of 1,3,6,8-tetrabromocarbazole according to the nearness in
530 GC retention times. The MS signal intensities of bromine ions ($^{79}Br^-$ or $^{81}Br^-$) were
531 applied to the semi-quantification, which masked isotopologue distribution
532 differences between the reference standards and corresponding mix-halogenated
533 carbazoles. In our study, we used the calibration curve of C_6BrCl_5 standard to semi-
534 quantify $C_6Br_2Cl_4$, and applied that of $C_6Br_4Cl_2$ standard for semi-quantifying
535 $C_6Br_3Cl_3$ and C_6Br_5Cl. This scheme is to some extent similar to that in the literature
536 (Guo et al., 2014), which can alleviate the dilemma caused by the lack of reference
537 standards. Moreover, we took into account the isotopologue distributions of the HXB
538 reference standards and the HXBs without reference standards, allowing the semi-
539 quantification results as accurate as possible (details referring to the Theory section in
540 the Supplementary data).

541 *3.6. Application and environmental implications*

542 The developed method has been successfully applied to the quantitative and
543 semiquantitative analyses of HXBs in fly ash, soil, ambient air, and commercial
544 reference standards of HBB and HCB. As shown in Table 4, all the HXBs were
545 detected in the environmental matrices. The concentrations of HXBs in the fly ash,
546 soil and ambient air samples were 0.11±0.003 to 16.60±2.64 ng/g, 0.29±0.01 to
547 8.43±0.35 ng/g, and 0.09±0.01 to 3.04±0.04 pg/m^3, respectively, with RSDs ranging
548 from 1.2% to 14.8%, indicating good repeatability of the method. The concentration

orders of HXBs in the fly ash, soil and ambient air samples were C_6BrCl_5 > $C_6Br_2Cl_4$ > $C_6Br_3Cl_3$ > $C_6Br_4Cl_2$ > C_6Br_5Cl, C_6BrCl_5 > $C_6Br_4Cl_2$ > $C_6Br_2Cl_4$ > $C_6Br_3Cl_3$ > C_6Br_5Cl, and C_6Br_5Cl > $C_6Br_4Cl_2$ > C_6BrCl_5 > $C_6Br_3Cl_3$ > $C_6Br_2Cl_4$, respectively. This result demonstrates evident different congener distributions of HXBs in these environmental matrices. In both the fly ash and soil, the dominant HXB congener was C_6BrCl_5, which accounted for 85.2% and 80.8% of the total HXBs concentration in the fly ash and soil, respectively, and had significant higher concentrations than the other congers ($p \leq 0.001$). The congener distribution of HXBs in the air samples was more distinctive in comparison with others in the fly ash and soil. The predominant HXB congener in the air samples was C_6Br_5Cl, presenting a percentage of 59.3% relative to the total HXBs concentration and a significant higher concentration than the rest congeners ($p < 0.001$). To the contrary, C_6Br_5Cl exhibited the lowest concentrations in the fly ash and soil, which were significantly lower than those of other congeners ($p < 0.001$). In addition, all the HXBs were detected in the HBB standard, presenting the concentrations from 65.83±1.52 to 5193.83±30.96 μg/g with RSDs of 0.6-3.1%. The concentration order of HXBs in the HBB standard was C_6Br_5Cl > $C_6Br_4Cl_2$ > $C_6Br_3Cl_3$ > $C_6Br_2Cl_4$ > C_6BrCl_5, which was completely opposite to that in the fly ash and apparently different from that in the soil, but relatively less different from that in the air samples. The dominant HXB congeners in the HBB standard were C_6Br_5Cl and $C_6Br_4Cl_2$, whose total concentration accounted for 92.4% of the total concentration of all the HXBs, with the concentrations

570 significantly higher than those of the rest congeners ($p < 0.001$). On the other hand,
571 merely C_6BrCl_5 was detected in the two HCB standards (Figure S-3 and Figure S-4),
572 with the concentrations of 751.54±20.27 μg/g and 6.60±0.98 μg/g in the HCB
573 standard-1 and HCB standard-2, respectively (Table S-7).

574 We deduce that the contents of HXB byproducts are dependent on the synthetic
575 reactions and final products, in accordance with the findings of HXBs in the HBB and
576 the HCB standards. When the final product is HBB, the HXB byproducts with more
577 Br atoms are more liable to be generated, because the main reactant is Br_2; if the final
578 product is HCB, the branch reactions favor the production of the HXB byproducts
579 with more Cl atoms due to that the major reactant is Cl_2. Therefore, it can be
580 anticipated that the concentration order of HXBs in HBB industrial products is
581 $C_6Br_5Cl > C_6Br_4Cl_2 > C_6Br_3Cl_3 > C_6Br_2Cl_4 > C_6BrCl_5$ and that in HCB industrial
582 products is $C_6BrCl_5 > C_6Br_2Cl_4 > C_6Br_3Cl_3 > C_6Br_4Cl_2 > C_6Br_5Cl$. With regard to the
583 HXBs found in the fly ash and the soil samples, HCB and HBB might partially
584 contribute to the generation of HXBs by acting as original reactants during MSWI and
585 E-waste combustion processes, where Cl ↔ Br exchange reactions might occur. The
586 yields of HXB congeners in the MSWI and E-waste combustion processes might
587 depend on concentrations of HCB and HBB, reaction conditions (e.g., temperatures
588 and catalysts), and reaction rate constants of the Cl ↔ Br exchanges. As a result,
589 HXBs from different sources are probably in possession of different congener
590 distributions, which could be helpful in source identification and apportionment for

591 HXB pollutants in the environment. Comparing the congener distribution of HXBs in
592 the air samples with those in other samples, we infer that the higher brominated
593 congeners ($C_6Br_4Cl_2$ and C_6Br_5Cl) might be mainly contributed by HBB industrial
594 products, and the higher chlorinated congeners, particularly C_6BrCl_5, could be mainly
595 derived from HCB industrial products and waste incineration and/or combustion.

596 In the fly ash samples, the concentrations of C_6BrCl_5, $C_6Br_2Cl_4$ and $C_6Br_3Cl_3$ were
597 0.8-74.4 times higher than that of HBB, and those of $C_6Br_4Cl_2$ and C_6Br_5Cl accounted
598 for 85.9% and 47.8% of that of HBB (Figure 4A, Table S-8), respectively. The
599 relative contents of the HXBs to HCB and to the sum of HCB and HBB (HCB+HBB)
600 in the fly ash samples were similar and ranged from 0.1% to 9.1%. In the soil samples,
601 all the relative contents of the HXBs were fairly low, within the range of 0.01-1.3%
602 (Figure 4B). In the ambient air samples, the relative contents of the HXBs to HBB
603 were from 2.1% to 66.5% and significantly higher than those of the HXBs to HCB
604 (0.2-7.0%) and to HCB+HBB (0.2-6.4%, Figure 4C). The relative contents of the
605 HXBs to HBB in the HBB standard were 0.01-0.5% (Figure 4D).

606 The total concentrations of HXBs in the fly ash, soil and ambient air samples were
607 19.48 ng/g, 10.44 ng/g and 5.13 pg/m^3, respectively, accounting for 10.6%, 0.4% and
608 10.8% of the corresponding total concentrations of HCB+HBB (Table S-8). The total
609 concentration of HXBs in the fly ash was significantly higher than that in the soil ($p =$
610 0.003), although the total concentration of HCB+HBB in the fly ash merely accounted
611 for 6.6% of that in the soil. This observation indicates that the MSWI may be more

612 efficient to yield HXBs in contrast with the E-waste combustion. In addition, we
613 calculated some physicochemical properties, bioactivities, toxicities and
614 environmental behaviors of HXBs, HCB and HBB using computational toxicology
615 and environmental simulation, indicating similar environmental hazards of HXBs
616 compared with HCB and HBB (Table S-9 and Table S-10). These results manifests
617 that HXBs are not negligible pollutants in the environment in contrast to the
618 conventional pollutants HCB and HBB, especially in fly ash and ambient air. Besides,
619 the total concentration of HXBs in the HBB standard was 9331.51 μg/g, i.e., the HXB
620 byproducts accounted for 0.9% of HBB by weight. C_6BrCl_5 was also found in the two
621 HCB standards in the form of byproduct (Figure S-3 and Figure S-4). Therefore, the
622 HXB byproducts in HCB and HBB industrial products can be inferred as an important
623 source of HXB pollutants in the environment.

4. Conclusions

A GC-HRMS method has been developed for quantitative and semiquantitative analyses of five HXBs in environmental matrices including fly ash, soil and ambient air, in combination with isotope dilution and isotopologue distribution computation. C_6BrCl_5 and $C_6Br_4Cl_2$ were quantitatively analyzed, and the other HXBs (i.e., $C_6Br_2Cl_4$, $C_6Br_3Cl_3$ and C_6Br_5Cl) were semiquantitatively analyzed by using the calibration curves of C_6BrCl_5 and $C_6Br_4Cl_2$ in combination with isotopologue distribution computation. The accuracy, precision, recovery, sensitivity, selectivity and repeatability of the method were validated, and satisfactory validation results were obtained. This method has been successfully applied to the quantification and semi-quantification of HXBs in the environmental matrices. All the HXBs were detected in all the matrices, and their concentrations and potential hazards indicated that HXBs are non-ignorable pollutants in the environment, especially in fly ash and ambient air. This study not only offers a reference strategy for quantitative and semiquantitative analyses of novel X-HOPs in environmental matrices, but also for the first time sheds light on the pollution status of HXBs in the environment. The main limitation of the present study is the lack of reference standards for the three semi-quantified HXBs, which might compromise the accuracy of the analysis results. Our future studies will be working on the preparation of more reference standards of HXBs to fulfil the requirements of accurate quantification of HXBs, and the revelation of underlying causes of the different congener distributions in different

environmental matrices. In addition, further quantitative studies are warranted to investigate the pollution and risks caused by more X-HOPs in more environmental compartments, including water, sediments and biological tissues.

Appendix A. Supplementary data

The Supplementary data is available on the website at http://pending.

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Figure legends

Figure 1. Representative chromatograms of HXBs, HCB and HBB detected in the HBB standard (A), fly ash (B), soil (C) and ambient air (D) samples.

Figure 2. Simulated mass spectra of two HXBs (A) and detected mass spectra of the two HXBs in reference standards of C_6BrCl_5, $C_6Br_4Cl_2$ (B) and HBB (C).

Figure 3. Representative mass spectra of HXBs detected in HBB standard (A), fly ash (B), soil (C) and ambient air (D) samples.

Figure 4. Relative contents of HXBs to HCB, HBB and the sum of HCB and HBB (HCB+HBB) measured in fly ash (A), soil (B), ambient air (C) and HBB standard (D) samples.

Table captions

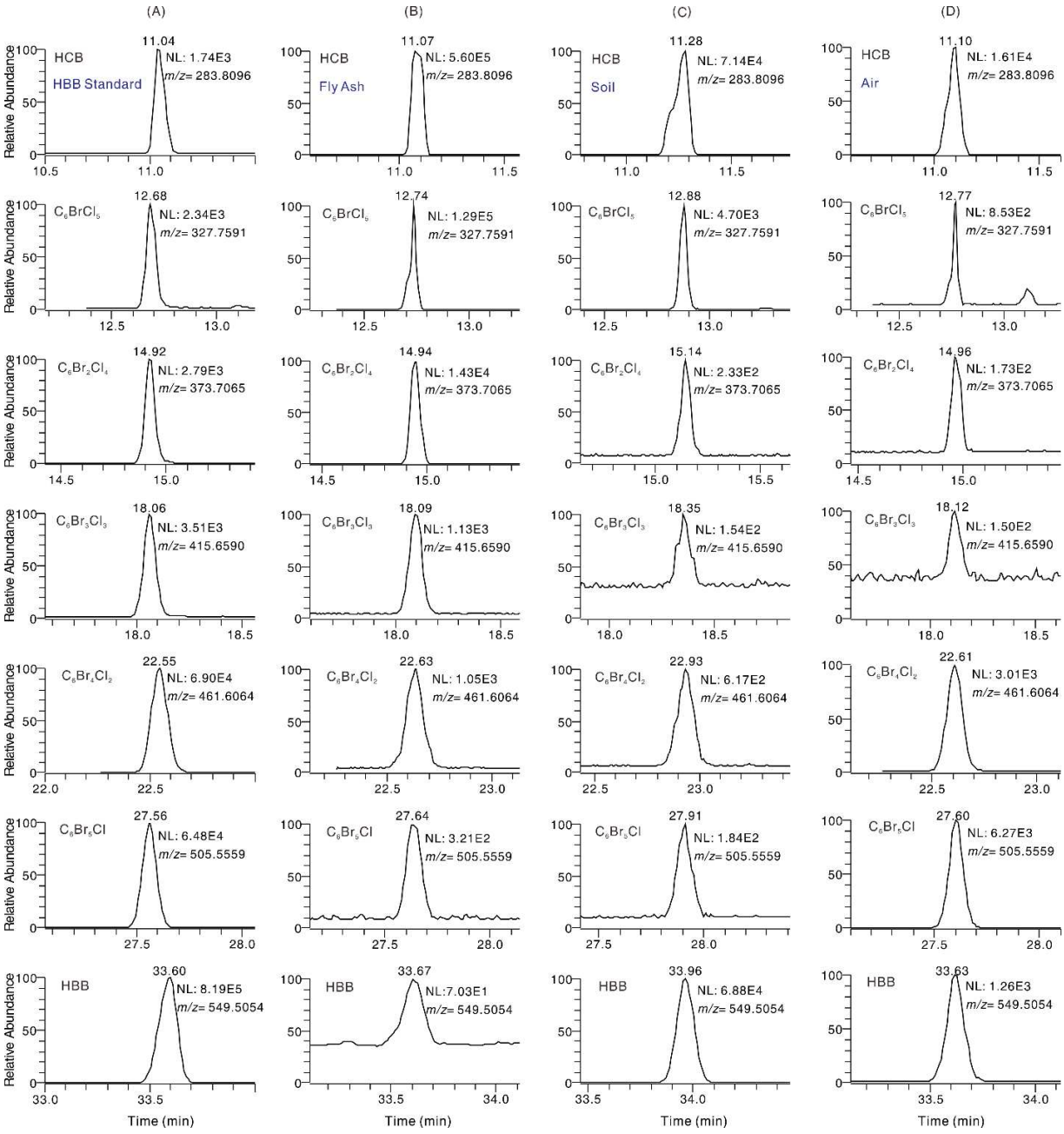
Table 1. Accuracy and precision for the analysis of C_6BrCl_5 and $C_6Br_4Cl_2$ in the reagent quality control samples at four concentration levels.

Table 2. Accuracy and precision for the analysis of C_6BrCl_5 and $C_6Br_4Cl_2$ in spiked quality control samples (fly ash).

Table 3. Recovery of $^{13}C_6$-HCB, $^{13}C_6$-HBB, C_6BrCl_5 and $C_6Br_4Cl_2$ in spiked fly ash quality control samples and spiked reagent samples with pretreatment.

Table 4. Detected concentrations along with detection repeatability of HXBs, HCB and HBB in the fly ash, soil and ambient air samples and in the HBB standard.

Figures



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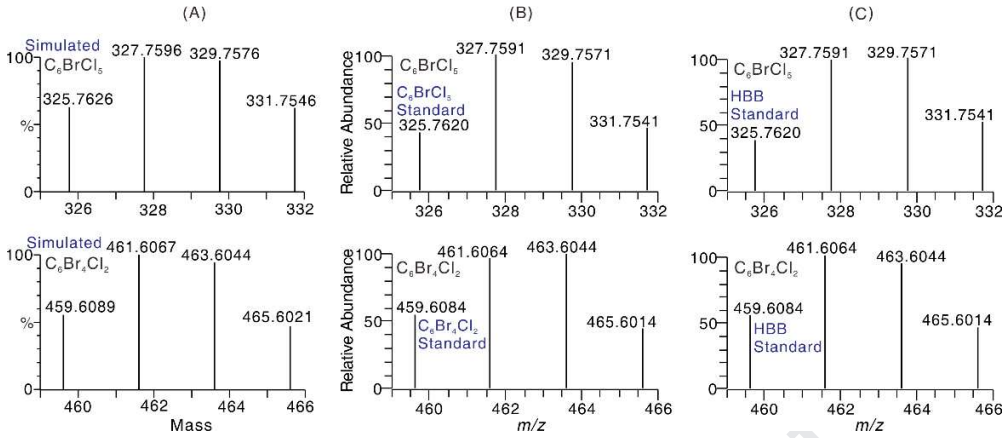

833

834 **Figure 2.** Simulated mass spectra of two HXBs (A) and detected mass spectra of the two HXBs

835 in reference standards of C_6BrCl_5, $C_6Br_4Cl_2$ (B) and HBB (C).

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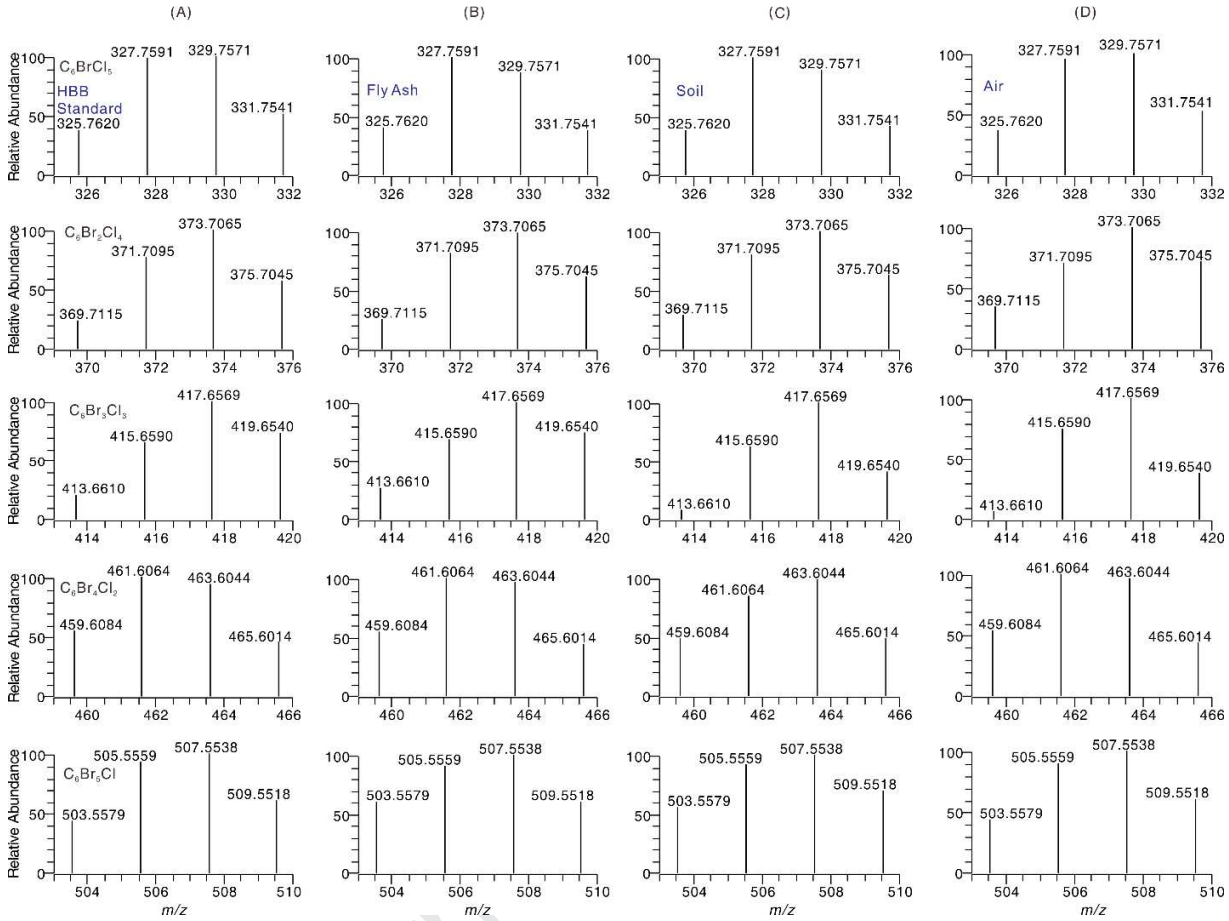

837

838 **Figure 3.** Representative mass spectra of HXBs detected in HBB standard (A), fly ash (B), soil

839 (C) and ambient air (D) samples.

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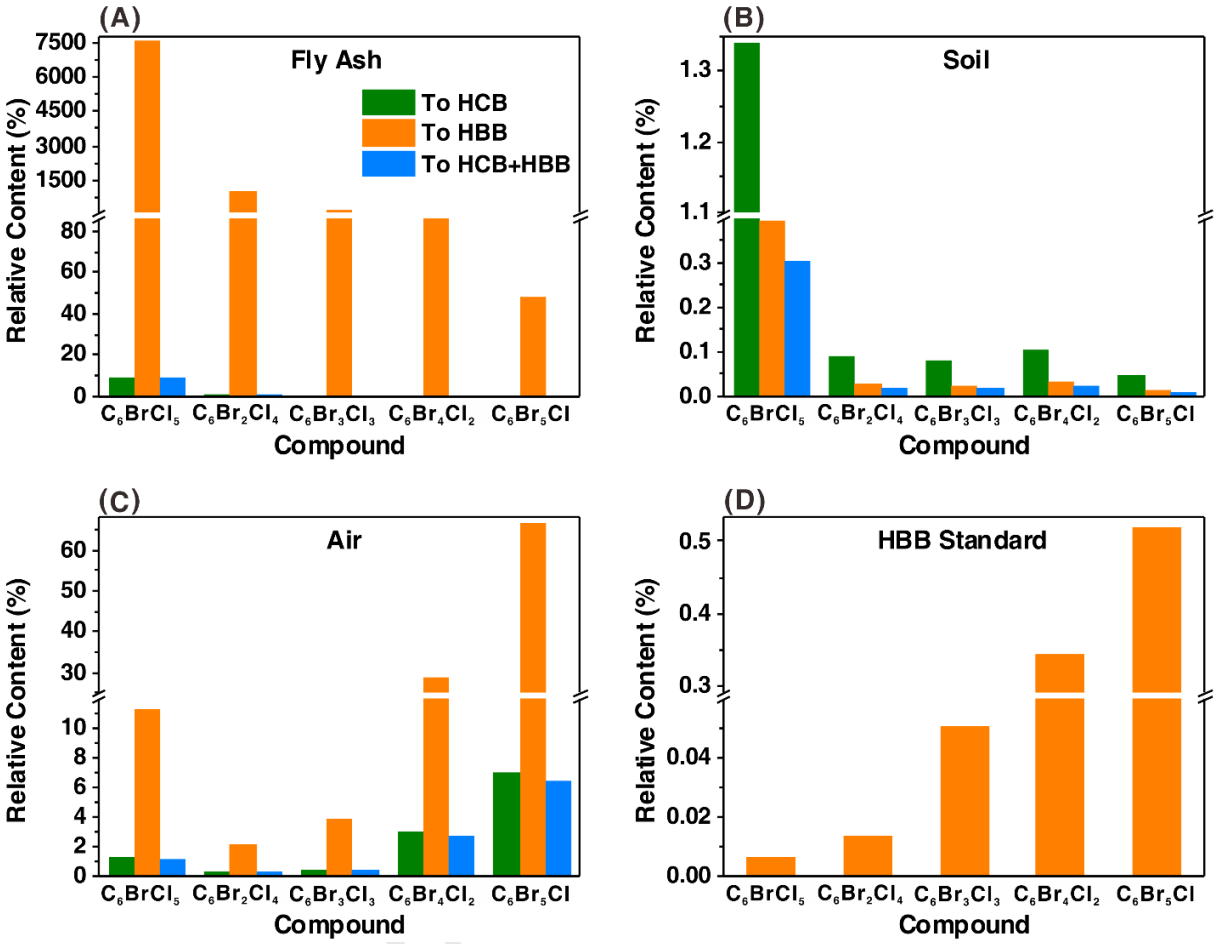

841

842 **Figure 4.** Relative contents of HXBs to HCB, HBB and the sum of HCB and HBB (HCB+HBB)
843 measured in fly ash (A), soil (B), ambient air (C) and HBB standard (D) samples.

Tables

Table 1. Accuracy and precision for the analysis of C_6BrCl_5 and $C_6Br_4Cl_2$ in the reagent quality control samples at four concentration levels.

Analyte	Sample type	Nominal concentration (ng/mL)	Intra-batch (n=3)			Inter-batch (n=6)		
			Mean calculated concentration (ng/mL)	Mean accuracy (%)	RSD (%)	Mean calculated concentration (ng/mL)	Mean accuracy (%)	RSD (%)
C_6BrCl_5	HQC	800	740.28	92.5	2.1	739.86	92.5	1.3
	MQC	40	37.56	93.9	1.5	37.44	93.6	1.8
	LQC	2.5	2.19	87.6	0.4	2.23	89.2	4.0
	LLOQ-QC	1	0.99	99.0	2.6	0.99	99.0	4.8
$C_6Br_4Cl_2$	HQC	800	893.00	104.0	5.9	845.59	101.5	3.8
	MQC	40	39.72	99.3	1.5	38.52	96.3	4.8
	LQC	2.5	2.29	91.5	0.5	2.35	94.0	6.9
	LLOQ-QC	1	1.07	107.2	1.8	1.04	103.7	5.7

Note, RSD: relative standard deviation; HQC, MQC, LQC and LLOQ-QC denote high quality control sample (QC), middle QC, low QC and lower limit of quantification QC, respectively.

849 **Table 2.** Accuracy and precision for the analysis of C_6BrCl_5 and $C_6Br_4Cl_2$ in spiked quality
850 control samples (fly ash).

Analyte	Spiked concentration (ng/g)	Detected concentration (mean, n=3, ng/g)	Mean accuracy (%)	RSD (%)
C_6BrCl_5	60	52.37	87.3	4.1
$C_6Br_4Cl_2$	6	6.47	107.8	5.0
	1	0.97	97.2	2.8

851

852 **Table 3.** Recovery of $^{13}C_6$-HCB, $^{13}C_6$-HBB, C_6BrCl_5 and $C_6Br_4Cl_2$ in spiked fly ash quality
853 control samples and spiked reagent samples with pretreatment.

Compound	Fly ash	n=3-6		Spiked reagent	n=3	
	Spiked concentration (ng/g)	Mean recovery (%)	RSD (%)	Spiked concentration (ng/mL)	Mean recovery (%)	RSD (%)
$^{13}C_6$-HCB	10	55.4	12.4	10	71.6	19.4
$^{13}C_6$-HBB	10	89.8	8.4	10	95.1	7.3
C_6BrCl_5	60	42.7	9.4	50	84.7	10.0
$C_6Br_4Cl_2$	6	72.2	13.9	50	89.5	7.2
	1	98.3	3.6			

854 **Table 4.** Detected concentrations along with detection repeatability of HXBs, HCB and HBB in the fly ash, soil and ambient air
855 samples and in the HBB standard.

	Fly ash			Soil			Air			HBB Standard		
	Intra-batch	n=3		Intra-batch	n=3		Intra-batch	n=3		Intra-batch	n=3	
Compound	Concentration (mean, ng/g)	SD	RSD (%)	Concentration (mean, ng/g)	SD	RSD (%)	Concentration (mean, pg/m^3)	SD	RSD (%)	Concentration (mean, μg/g)	SD	RSD (%)
C_6BrCl_5	16.60	2.46	14.8	8.43	0.35	4.1	0.52	0.02	4.2	65.83	1.52	2.3
$C_6Br_2Cl_4$	2.18	0.06	2.8	0.56	0.03	4.8	0.09	0.01	7.6	135.89	3.87	2.8
$C_6Br_3Cl_3$	0.40	0.01	1.8	0.49	0.01	1.7	0.18	0.01	4.2	508.49	15.66	3.1
$C_6Br_4Cl_2$	0.19	0.01	3.7	0.66	0.02	2.7	1.30	0.03	2.7	3427.47	30.40	0.9
C_6Br_5Cl	0.11	0.003	2.6	0.29	0.01	2.2	3.04	0.04	1.2	5193.83	30.96	0.6
HCB	183.03	6.92	3.8	629.41	37.92	6.0	43.17	1.22	2.8	236.29	29.06	12.3
HBB	0.22	0.01	5.0	2149.90	86.44	4.0	4.57	0.20	4.4			

856 Note, SD: standard deviation.

Research Highlights

- Six HXBs in fly ash, soil and air were quantified/semi-quantified by GC-HRMS.
- Isotopologue distributions were involved in identification and semi-quantification.
- The accuracies, precisions, recoveries and limits of quantification were satisfying.
- HXBs are non-ignorable pollutants in light of their concentrations and latent risks.
- The study for the first time reports the pollution status of HXBs in the environment.

Declaration of Interest Statement

The authors have declared no conflict of interest.

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