# DETERMINATION OF HYDROXYLATED POLYBROMINATED DIPHENYL ETHERS IN SEWAGE SLUDGE COLLECTED FROM WASTEWATER TREATMENT PLANTS IN SHANDONG PROVINCE, CHINA

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**Introduction**

As structural analogues of PBDEs, hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have attracted more interest in recent years. OH-PBDEs have been confirmed as natural products in marine environment[1], but they have also been suggested to be metabolites of PBDEs[2]. OH-PBDEs have been found in red algae[3], marine animals[4], as well as in human[5]. However, there are no reports on the levels of OH-PBDEs in sewage sludge although 2'-OH-BDE-28 were unexpectedly found in effluent of wastewater treatment plants (WWTPs)[6] when it was used as internal standard for quantification of triclosan. Moreover, PBDEs were found in the sewage sludge of WWTPs in previous work of our group[7], indicating the possible accumulation of OH-PBDEs in sewage sludge.

**Materials and methods**

**Materials.** Standards of hydroxylated polybrominated diphenyl ethers, including 4-OH-BDE-42, 4'-OH-BDE-49, 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 2'-OH-BDE-68, 6-OH-BDE-85, 5'-OH-BDE-99, and 6'-OH-BDE-99, were purchased from AccuStandard (USA). All solvents were HPLC grade or pesticide grade and were obtained from J. T. Baker (Phillipsburg, NJ, USA). Silica gel (100-200 mesh size, Merck, Darmstadt, Germany) were activated at 140 °C for 7 h. Acid silica gel was prepared by mixing 56 g activated silica with 44 g concentrated $H_2SO_4$.

**Sample Preparation**. Eight sewage sludge samples were collected from eight cities of Shandong province, China in 2011 (Figure 1). Freshly digested sludge samples were packed in aluminum foil and sealed in Ziplock bags, and then immediately delivered to the laboratory. All samples were freeze-dried and homogenized. Matrix solid phase dispersion (MSPD) method was developed for OH-PBDEs analysis. Briefly, the mixture of sludge sample (2 g, spiked with surrogate standard) and acid silica (4 g) at a 1:2 sample/sorbent mass ratio was ground in a glass mortar for ten minutes. A glass column was loaded from bottom to top with anhydrous sodium sulfate (2 g), acid silica gel (8 g), and the sample-sorbent mixture. The mortar and the pestle were rinsed by DCM which was then used as elution solvent. The eluate was concentrated to dryness and reconstituted with 100 μL of acetonitrile for LC-MS/MS injection.

**Instrument**. Analysis of OH-PBDEs was performed using a previously developed method[8]. An Agilent 1290 Series LC system coupled with an Agilent 6460 Triple Quadrupole MS/MS system (Agilent Technologies, Palo Alto, CA) was used. Mass spectrometric detection was performed using ESI source in the negative ion multiple-reaction monitoring (MRM) mode. A C18 column (100 mm × 2.1 mm, 2.2 μm particle size, Thermo Fisher Scientific, USA) was selected for reversed-phased separation. The mobile phase consisting of acetonitrile (Solvent A) and water (Solvent B) was used with a gradient elution of A: B from 55:45 to 75:25 in 20 min with a flow rate of 0.38 mL $min^{-1}$. The volume injected onto the column was 10 μL and the column temperature was set at 40 ºC.

**Results and discussion**

Three of the nine target OH-PBDE congeners, 3-OH-BDE-47, 4-OH-BDE-42 and 6'-OH-BDE-99 were not found in the sludge samples. 6-OH-BDE-47 was the most predominant congener with highest concentration and detected frequency, followed by 2'-OH-BDE-68 and 4'-OH-BDE-49. Significant higher ΣOH-PBDEs and also total 6-OH-BDE-47 and 2'-OH-BDE-68 were found at Rizhao and Qingdao which are both coastal cities. Among the detected OH-BDE congeners, 6-OH-BDE-47 and 2'-OH-BDE-68, which have a hydroxyl group in the *ortho* position relative to the ether bond, have been considered as naturally occurring OH-PBDEs in marine

organisms, while the congeners like 4'-OH-BDE-49 with hydroxyl group in the *meta* and *para* positions have been considered as metabolites from PBDEs[9]. Therefore, it is supposed that the higher ΣOH-PBDEs in sludge from coastal area attributed to seafood processing and consuming. The presence of 6-OH-BDE-47 and 2'-OH-BDE-68 in sludge can be a consequence of accumulation from wastewater influent containing human waste and that from seafood industrial facilities. Wastewater treatments usually contain aeration oxidation or disinfection procedures. The occurrence of 4'-OH-BDE-49, 5-OH-BDE-47, 6-OH-BDE-85 and 5'-OH-BDE-99 in WWTPs could originate from transformation of PBDEs during strong oxidation processes. Besides, an abundant number of microorganisms present in sludge may also play an important role in biotransformation of PBDEs.

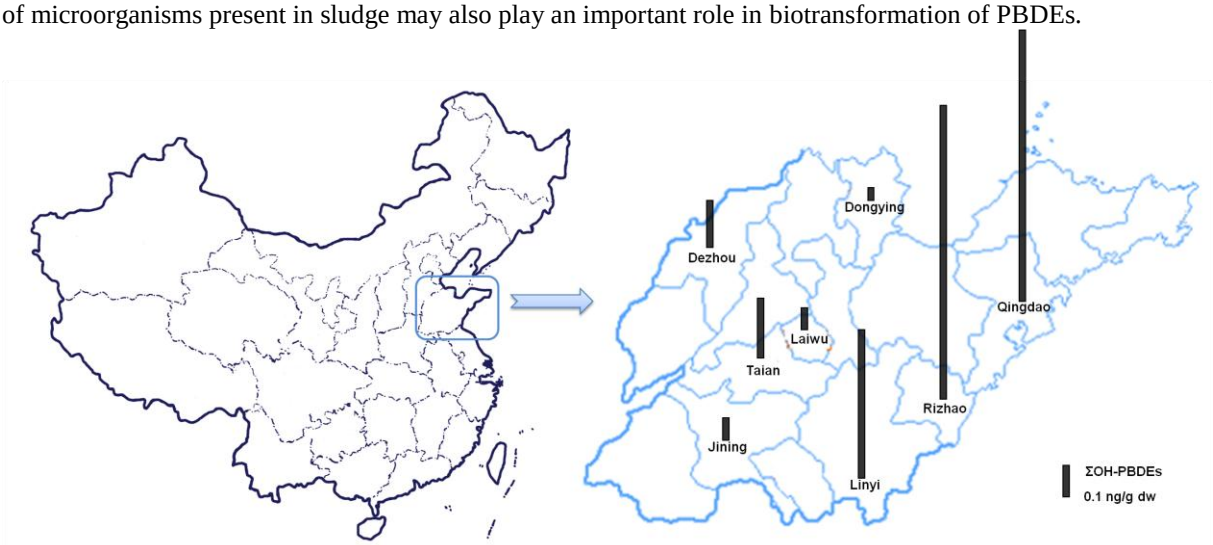


Figure 1. Map of sampling locations and concentrations of ΣOH-PBDE in sludge from WWTPs in Shandong province, China.

**Acknowledgements**
This work was jointly supported by the National Basic Research Program of China (No. 2009CB421605), the National Natural Science Foundation of China (No. 20907062), and Important Directional Item of the Chinese Academy of Sciences (No. KZCX2-EW-QN409), and National Key Water Program (No. 2009ZX07527-005).

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