APPLICATION OF COMPOUND-SPECIFIC STABLE CARBON ISOTOPE ANALYSIS FOR THE BIOTRANSFORMATION AND TROPHIC DYNAMICS OF PBDES IN FISH

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Introduction

Bioaccumulation and biotransformation of PBDEs is a critical issue in the scientific evaluation of risks that PBDEs may pose to humans and the environment. The congener profiles of PBDE in the biotic samples were generally found to be different from those in the abiotic samples. Different bioavailability among different congeners and biotransformation of BDE congeners occurred in biota were suggested to be responsible for above observation. It appears impossible to distinguish whether a specific PBDE congener in organism is an accumulated contaminant from diet or the metabolic debromination product of higher-brominated congeners using conventional method based on concentration and composition information. Compound specific isotope analysis (CSIA) could be a solution for this issue. Up to date, this technique has been utilized mostly for volatile and several semi-volatile organic compounds[1-3] and no study has been conducted to investigate the changes of stable carbon isotope signatures of PBDE individual congener in biotransformation processes.

In the present study, two predatory/prey relationships were established in the laboratory. Two predatory species, oscar fish (*Astronotus ocellatus*) and redtail catfish (*Phractocephalus hemiliopterus*), were feed on high contaminated tiger barb (*Barbus tetrazona*), which has been exposed to high dose PBDE technical mixtures (TBDE71x). The PBDE congener profile and stable carbon isotopic composition of several key congeners in the fish samples were examined after a period of exposure. The aims of the present study were to investigate the species-specific debromination of PBDE and trophic dynamic process of PBDE and to evaluate if CSIA could be used to track these processes.

Materials and methods

Tiger barb, oscar fish, and redtail catfish were purchased from a aquarium market in Guangzhou, China and were maintained in separate glass tanks (80 cm×35 cm×50 cm) in the laboratory. Fish in all tanks were acclimated to the control diet for a period of 10 days prior to the start of exposure. The first 200 tiger barb were exposed to artificially contaminant food (4g food/day with TBDE71x concentration of 1 mg/g) in one tank for 5 days, then they were stop exposure and transformed at 40 per day into tanks where oscar fish (16 tiger barb/day) or redtail catfish (16 tiger barb/day) were raised. Of the 40 tiger barbs, 8 were collected and stored at -20 ℃ until further treatment. When the first 200 tiger barb had exposed to contaminant food for 5 day, the second 200 tiger barb in another tank began to exposure. After 4 circles (20 days, 800 tiger barb), the oscar fish (n=8), redtail catfish (n=10), and the collected tiger barbs (n=160), were respectively pooled into two composite samples. The entire fish was used for the tiger barb and the carcass without the internal tissues and gills was used for the oscar fish and the redtail catfish. These composite samples were used for PBDE analysis and compound-specific stable carbon isotope analysis. Before exposure, three oscar fish, three redtail catfish, and twenty-one tiger barb (pooled into three composite samples) were used for background level analysis.

The pooled fish samples were freeze-dried, weighted and extracted with 300 mL hexane : acetone (1:1, v/v). An aliquot of extract (one tenth of extract) was used to perform PBDE quantification analysis and to determine lipid content. A detailed description of the method used to purify the extract for PBDE quantification analysis has been given elsewhere.[4] The remainder extract which used for stable carbon isotope analysis was concentrated and solvent exchanged to hexane (90 mL), then treated with concentrated sulfuric acid three times (each time 10 mL). The lipid-free extract was washed with sodium sulfate solution (5%, w/w) to remove residual sulfuric acid. Then the extract was primarily purified on a complex silica gel column packed from bottom to top with 8 cm neutral silica and 8 cm sulfuric acid silica (44%), and 1 cm layer of anhydrous sodium sulfate with 30 mL of 50% dichloromethane in hexane (volume fraction) as eluent. The extract was concentrated to 1 mL and finally cleaned by an alumina/silica column (1:2 by volume). The column was first eluted with 22 mL hexane,

followed by 5 mL hexane and 15 mL dichloromethane / hexane (1:1, v/v). The first 22 mL hexane was discarded and the last two fractions were combined and finally concentrated to 0.5 mL under a gentle N2 stream.

The cleaned extracts used for PBDE quantification were analyzed by GC/MS (Agilent 6890A /5975B MSD; Agilent Technology, CA) with electron capture negative ionization (ECNI) source in a selective ion monitoring (SIM) mode. The extracts used for CSIA were first checked for the purity by GC/MS with an electron impact (EI) ion source in the full scan mode. Then the extracts and the commercial PBDE mixture were analyzed for compound specific isotopic composition with a 6890N- GC coupled to a GV Isoprime isotopic ratio mass spectrometer (IRMS) (GV Instruments UK) via a modified GC combustion interface.

Results and discussion

PBDE Levels in Background Fish. Congeners of BDE 47, 100, and 154 were detected in background tiger barb at concentrations of 5.3 ± 0.83, 1.3 ± 0.16, and 0.77 ± 0.11 ng/g lipid. Only BDE 47 and BDE 154 were detected in background oscar fish with mean concentrations of 9.5 ± 8.3 and 0.95 ± 0.82 ng/g lipid. BDE 28, 47, 100, 99, 154, and 183 were detected in the background redtail catfish at concentrations of 1.2 ± 1.0, 43 ± 20, 6.0 ± 1.6, 9.8 ± 0.95, 6.3 ± 2.5, 2.1 ± 1.9 ng/g lipid, respectively. The influence of the background PBDEs on the stable carbon isotope ratio measurement could be small or neglect because the concentrations of PBDEs in the exposed fish were 4-6 orders of magnitude higher than those in the background fish.

Precise and Accurate Compound Specific Carbon Isotope Analysis of PBDEs. The method applied in the present study has been verified to be efficiency in isolation and purification of PBDEs in fish in our previous study and no significant isotopic fractionation of PBDEs was observed during the purification procedure.[5] Sample concentration is one of the most important operating parameters for precisely and accurately measuring of stable carbon isotopic composition. PBDEs in the technical mixtures and the environmental matrixes are composed of many congeners and the abundance of each congener significantly differs from each other. Some key congeners are 10 or even100 folds more abundance than minor congeners. Therefore, an optimum concentration was needed to measure the stable carbon isotopic ratio for as much PBDE congeners as possible.

In the present study, a wide TBDE-71x concentration range, from 100 mg/L to 4000 mg/L, was tested to determine the optional concentration range for precise and accurate congener-specific carbon isotope measurement. As can been seen in the Figure 1, the data points of 3000 mg/L and 4000 mg/L for six key congeners of TBDE-71x located in the lower right of the diagonal line, which could likely attribute to incomplete combustion. The subtle offset to more 13C-depleted $\delta^{13}C$ for samples of 3000 and 4000 mg/L, an

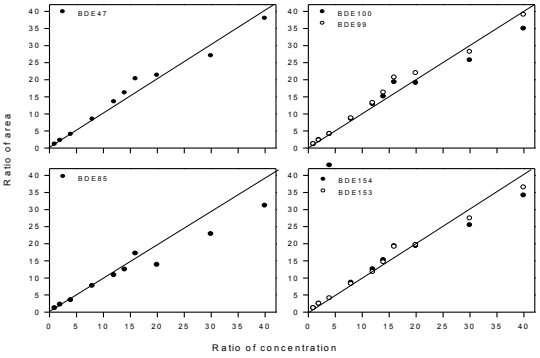

Figure 1 : The diagonal plot of the combustion state of PBDEs in the combustion oven.

expected result of isotopic fractionation due to incomplete combustion of PBDEs, confirmed the above statement (Figure 2). When the PBDE concentration was lower than 400 mg/L, it can be seen from Figure 2 that the deviations of $\delta^{13}C$ (from 0.15 to 1.38‰) of replicate analyses for three key congeners were higher than those for samples with concentration above 400 mg/L (from 0.01 to 0.61‰). The standard deviations (from ±0.08‰ to ±0.58‰) were also higher than those in high concentration samples (from ±0.01‰ to ±0.28‰). Considering the low abundance of congeners of BDE85, 154, and 153 (less than 4%) in the TBDE-71x, the total concentration of PBDEs in sample should excess 1500 mg/L to obtain reliable $^{13}C/^{12}C$ ratio for these minor congeners. Therefore, the data measured in concentration of 2000 mg/L has been

chosen as the final choice of $\delta^{13}C$ determination for each congener in the TBDE-71x (Table 1). The stable carbon isotope values for TBDE-71x ranged from about -30.60 to -23.12‰. Similar to the study by vetter

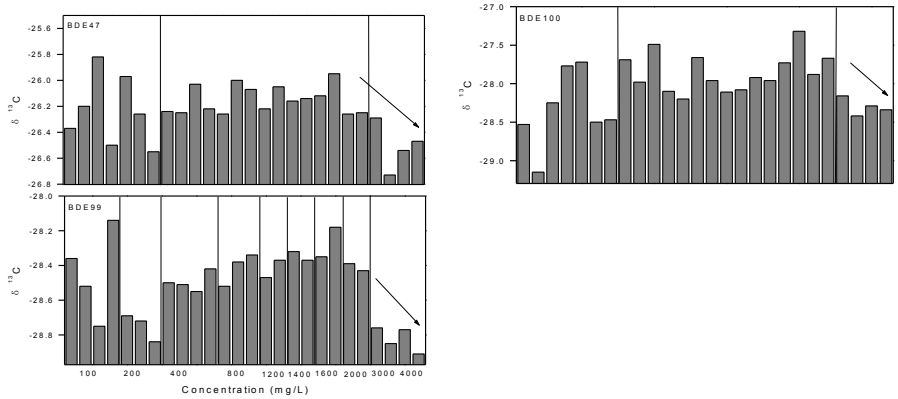

Figure 2: The effects of sample concentration on the determination of δ13C for three key congeners in the technical penta-BDE mixtures (TBDE-71x)

et al., [6] a trend of isotopic depletion with increasing bromine content was observed for TBDE-71x. As for fish sample, the final extracts were adjusted by enrichment or dilution of the analytes to an appropriate concentration to obtain a precise and accurate carbon isotope measurement.

Species-specific Debromination and Trophic Dynamics of PBDE. Total 39 PBDE congeners were detected in the carcasses of fish. The proportion of BDE47 to total PBDEs in the tiger barb increased by 13.9% compared to that in the Penta-BDE mixture, in corresponding with a decrease by 11.5% for BDE99. The PBDE congener profile in the redtail catfish was basically identical to that of the tiger barb, implying that PBDEs were just accumulated from the tiger barb without or with a negligible biotransformation. In the oscar fish, however, a further debromination was observed when compared with the tiger barb. The contribution of BDE99 to total PBDEs decreased from 32.5% in the tiger barb to 3.4% in the oscar fish. Conversely, the contribution of BDE47 increased from 46.1% to 67.2%. The difference in PBDE congener profile between the oscar fish and the red tail catfish obviously demonstrated a species-specific metabolism of PBDEs in fish.

The calculated biomagnification factors (BMFs) of PBDE congeners from the tiger barb to the redtail catfish were between 7.2 and 20.6. Most of the congeners showed uniform BMF values around of 8-9, similar to the BMF value of the total PBDEs (8.1). Conversely, the BMF values of PBDE congeners from the tiger barb to the oscar fish showed great variation ranging from 0.01 to 89.3 with of BMF of 3.5 for total PBDEs. The high lipid content and biomass for oscar fish were the reasons for the low BMF for the total PBDE. When the effects of lipid content and biomass were removed, the BMFs of the total PBDE (8.4) from the tiger barb to the oscar fish was similar to that from the tiger barb to the redtail catfish (8.1). Therefore, the BMF for each congener from two predatory/prey relationships can be directly compared with each other. A group of congeners such as BDE42, 85, 99,138, 139 showed significantly lower BMFs in the oscar fish than in the redtail catfish. These congeners were thought to debrominate in the oscar fish. Some congeners (BDE49, 91, 100, 104, 154, and 155) in the oscar fish show similar to or slight high BMF in the redtail catfish. These congeners could be thought of as direct accumulation from the tiger barb. The other congeners, such as BDE17, 28, 75, 47, 66 etc showed remarkable high BMF in the oscar fish than in the redtail fish. These congeners accumulated through both absorption from the tiger barb and debromination of higher brominated congeners.

Compound Specific Stable Carbon Isotope Signature of PBDEs. The stable carbon isotopic composition can be only accurately determined for congeners BDE28, 47, 100, and 99 in carcass of the three fish species. These four congeners represent three types of congener: debrominated congener (BDE 99), debromination product (BDE 28 and 47), and congener accumulated from diet (BDE 100), respectively. The $\delta^{13}C$ values of BDE100 have no significant differences among TBDE-71x (-27.78 ± 0.15‰), the tiger barb (-27.90 ± 0.11‰ and -27.88 ± 0.25‰), the oscar fish (-27.83 ± 0.23‰ and -27.78 ± 0.22‰), and the redtail catfish (-27.82 ±

0.12‰ and -27.83 ± 0.19‰). The stability of the $\delta^{13}C$ of BDE100 was in line with the expectation PBDE congener accumulated directly from diet would have same isotope signature as those in their diet.

BDE99 experience debromination in the tiger barb and the oscar fish according to the PBDE congener profiles. The $\delta^{13}C$ values of BDE99 in the oscar fish was undetectable due to the low concentration. So only the $\delta^{13}C$ values of BDE99 in the tiger barb can be used to compare with that in the TBDE-71x. The stable carbon isotope ratios of BDE99 in the tiger barb were slight higher than that in the TBDE-71x (-28.16‰ vs -28.41‰). However, the differentiations were within 0.5‰. No remarkable isotopic fractionation was reported for the reductive dechlorination of polychlorinated biphenyl. [7] The main reason for this absence of isotopic fractionation is the dilution of signal of other eleven carbons in the biphenyl that are not involved in reaction.

As a congener accumulated by both absorption from diet and in vivo debromination of higher brominated congeners, the $\delta^{13}C$ value of BDE47 show a decreasing trend with the increasing trophic level (from -26.25‰ in PBDE mixtures to -27.33‰ in the tiger barb to -27.76‰ in the ocsar fish). BDE 99 and BDE 85, two congeners were thought to debrominated to BDE 47, have more negative $\delta^{13}C$ values than BDE 47 in the technical Penta-BDE mixtures. Thus, the formation of BDE 47 due to debromination would result a decrease of $\delta^{13}C$ value in BDE 47 pool in the biota. Previous studies has documented that BDE 47 is not readily debrominated in fish 22, 26 and hydroxylation of PBDE in fish was minor (less than 1%).22, 36 Thus, the decrease of δ13C of BDE47 can be linked with the degree of metabolic debromination of PBDE in biota even as a quantitative indicator. Analogously, the δ13C value of BDE28 also showed a decreased trend with increasing trophic level (from -24.20‰ in PBDE mixtures to -26.44‰ in the tiger barb to -27.686‰ in the ocsar fish), indicating that this congener may derived mainly from the debromination of highly brominated congeners. No significant differences in $\delta^{13}C$ values of BDE47 and BDE28 were observed between the tiger barb and redtail catfish, verifying that these congeners in redtail catfish accumulated from the tiger barb not from metabolic debromination of high brominated congeners.

Summarily, species-specific debromination was observed in the laboratory exposure experiment. The apparent trophic dynamic behaviour of individual PBDE congener was controlled by this species-specific debromination. No remarkable fractionation of stable carbon isotope was found in the debromination processes of highly brominated congeners. However, the metabolic debromination of PBDEs in biota will create congeners with more depleted $\delta^{13}C$ values than its native PBDE congener. Such information may provide additional evidence for the occurrence of this process and aid in further understanding the biogeochemistry of these compounds. The results of the present study show that the CSIA is a powerful method for monitoring debromination of PBDE in biota and for source fingerprinting purposes.

Acknowledgements

This work was financially supported by the National Nature Science Foundation of China (No. 20890112, 40873074), the Chinese Academy of Sciences (No. KZCX2-EW-QN 105) and the National Basic Research Program of China (No. 2009CB4216604).

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