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Tracing the biotransformation of polychlorinated biphenyls (PCBs) in common carp (*Cryprinus carpio*): Enantiomeric fraction and compound-specific stable carbon isotope analyses

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HIGHLIGHTS

- EFs and CSIA were applied to trace the biotransformation of PCBs in fish exposed to Aroclor 1242.
- No metabolites (OH– or $MeSO_2$-PCBs) were detected.
- Enantioselective biotransformation of the chiral PCB congeners in fish was observed.
- Significant $\delta^{13}C$ change (enrichment or depletion) were observed in some PCB congeners.
- CSIA is a promising method for monitoring the biotransformation of PCBs in biota.

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ABSTRACT

Metabolites of polychlorinated biphenyls (PCBs) in fish are difficult to detect *in vivo* due to the complexity of biometabolism. In the present study, atropisomeric fraction analysis of chiral PCB congeners and compound-specific isotopic analysis (CSIA) were applied to trace the biotransformation of PCBs in fish by exposure of common carp (*Cryprinus carpio*) to the commercial PCB mixture Aroclor 1242. Stereoselective elimination of the chiral PCB congeners 91, 95, and 136 was observed, indicating a stereoselective biotransformation process. The $\delta^{13}C$ values of PCBs 5/8, 18, and 20/33 in fish were increased compared with those in the spiked food, while PCBs 47/48 and 49 showed significant heavy isotope depletion. These results suggested a significant biotransformation of the corresponding individual PCB congeners although the potential PCB metabolites, hydroxylated PCBs (OH-PCBs) and methylsulfone PCBs ($MeSO_2$-PCBs), were not detected in the fish tissue samples throughout this experiment. The results of the present study demonstrated that a combination of chiral analysis and CSIA is a promising new approach for investigating the biotransformation of PCBs in biota.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of 209 anthropogenic chlorinated organic compounds that were formerly used as heat transfer fluids, hydraulic lubricants, and dielectric fluids in transformers and capacitors (Beyer and Biziuk, 2009). Although PCBs were banned for commercial/industrial use in the late 1970s, they remain an important type of legacy contaminant in the environment (O'Sullivan and Sandau, 2013), and their residues are still relevant to human and environmental health. PCBs can bioaccumulate in organisms and biomagnify in the food chain due to their lipophilic properties and higher sorption rates than biotransformation/excretion rates in organisms (Beyer and Biziuk, 2009; Kania-Korwel and Lehmler, 2016). Moreover, PCBs can undergo biotransformation in wildlife and humans, and generate metabolites that may be more toxic than their parent compounds, resulting in a serious threat to the biota (Beyer and Biziuk, 2009; Park et al., 2009; Weijs et al., 2009).

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Even though fish are generally not considered to be capable of efficient biotransformation of PCBs (Boon et al., 1989; Buckman et al., 2004), considerable concentrations of hydroxylated PCBs (OH-PCBs) and methylsulfone PCBs ($MeSO_2$-PCBs), two kinds of PCB metabolites derived from Phase I and/or Phase II biotransformation, have been detected in deepwater sculpin (*Myoxocephalus thompsoni*, 7.04 ± 5.47 ng/g lipid wt of $MeSO_2$-PCBs) and lake trout (*Salvelinus namaycush*, 0.02–0.44 ng/g wet wt of OH-PCBs) collected from the Great Lakes (Campbell et al., 2003; Stapleton et al., 2001), and in northern snakehead (*Channa argus*, 5.82 ± 0.23 ng/g wet wt of OH-PCBs, and 43–370 ng/g lipid wt of $MeSO_2$-PCBs) and mud carp (*Cirrhinus molitorella*, 0.15 ± 0.03 ng/g wet wt of OH-PCBs, and 58–16 g/g lipid wt of $MeSO_2$-PCBs) from an e-waste recycling area in South China (Zeng et al., 2014a; Zhang et al., 2012). Previous work demonstrated that fish do have some capacity to biotransform PCBs (Buckman et al., 2006; Fisk et al., 1998; Wong et al., 2002). The formation of OH-PCBs in rainbow trout (*Oncorhynchus mykiss*) exposed to the environmentally-relevant group of PCB congeners has been reported (Buckman et al., 2006), and a model based on depuration half-life ($t_{1/2}$) to log K_{OW} relationships also has been applied to show biotransformation of PCB in juvenile rainbow trout (Buckman et al., 2006; Fisk et al., 1998). The examination of changes in the enantiomeric fractions (EFs) of chiral PCB congeners provided further evidence of PCB biotransformation in fish (Buckman et al., 2006; Wong et al., 2002). However, metabolites of PCBs are often difficult to detect *in vivo* due to the complexity of biometabolism, and chiral analysis is limited to cases where stereoselective biotransformation occur and would miss achiral biotransformation. Thus, new technologies and methodologies must be applied to trace the metabolism of PCBs in fish.

Compound-specific isotope analysis (CSIA) has been developed into a powerful analytical tool to trace the origin, degradation, and transformation of organic compounds in the environment (Schmidt et al., 2004). Degradation, especially biodegradation, is frequently accompanied by a substantial kinetic isotope effect— the heavier isotopes (here ^{13}C) react more slowly than the light isotopes in most chemical and biological reactions, and thus results in the enrichment of heavier isotopes in the remaining compounds and their depletion in the degradation products (Luo et al., 2013; Schmidt et al., 2004). The fractionation of stable isotopes (e.g., carbon, hydrogen, and nitrogen) generated by abiotic and biotic transformation of a compound is often significantly larger than that induced by phase-transfer processes, providing a unique approach for the identification and quantification of the transformation, and sometimes even for elucidating its mechanism (Schmidt et al., 2004; Zeng et al., 2012). The main benefit of using CSIA is the ability to track the fate of contaminants in complex matrices without the need to identify and quantify intermediates and metabolic products.

To date, limited data are available for carbon isotope studies of PCBs. Horii et al. (2005) and Jarman et al. (1998) reported the $\delta^{13}C$ values of PCB congeners in several different kinds of technical mixtures. Drenzek et al. (2001) investigated whether carbon isotope changes occurred during reductive dechlorination of PCBs, and found that even if no observable stable isotope fractionation occurs in the dechlorination process, stable isotope analysis is still valuable in examining such processes due to the intrinsic trend of ^{13}C depletion with increase in chlorine content that they observed in Aroclor mixtures. Yanik et al. (2003) found that most PCB congeners extracted from biota in the Housatonic River showed isotope enrichment relative to the PCB source materials. In our previous study of an e-waste site, we observed that the isotope fractionation of PCB congeners found in mud carp (*Cirrhinus molitorella*, the prey) to northern snakehead (*Channa argus*, the predator) increased, suggesting a similar metabolic pathway of PCBs in the studied prey/predator relationship, thus indicating that CSIA can provide an insight into the biochemical process and provides a method to trace the fate of PCBs at the trophic level (Zeng et al., 2013b). However, given the fact that fish have little ability to biotransform PCBs and the complexity of matrices in the field, it is difficult to know whether the change in $\delta^{13}C$ values of PCBs in fish is due to their own biotransformation or is inherited from their food. Therefore, a further fish-exposure experiment was needed to investigate the biotransformation of PCBs in fish utilizing CSIA (Zeng et al., 2013b).

In the present study, common carp (*Cyprinus carpio*) individuals were administered a high dose (to ensure sufficient amounts of compounds to perform CSIA) of the commercial PCB mixture Aroclor 1242 in their diet for 28 days, followed by a depuration period of 30 days, during which the carp were fed unfortified, non-spiked food. The PCB congener profiles, two potential metabolites (OH-PCBs and $MeSO_2$-PCB), the EF values of the chiral PCB congeners, and the stable carbon isotopic compositions of several key congeners in the fish samples were then examined. The primary aim of this study was to verify and trace the biotransformation of PCBs in fish by the application of CSIA.

2. Materials and methods

2.1. Standards and reagents

The technical PCB mixture Aroclor 1242 was obtained from AccuStandard (New Haven, CT, USA). $MeSO_2$-PCBs, OH-PCBs and Methoxy PCBs (MeO-PCBs) standard were obtained from Cambridge Isotope Laboratories (Andover, MA, US). Pesticide grade dichloromethane (DCM) and *n*-hexane (Hex) were purchased from CNW Technologies GmbH (Dusseldorf, Germany). Acetone (analytical reagent grade, distilled before use) was purchased from Tianjing Chemical Reagent Factory, China. Guaranteed reagent grade concentrated sulfuric acid (H_2SO_4) and anhydrous sodium sulfate were acquired from Guangzhou Chemical Reagent Factory, China.

2.2. Food preparation

Commercial fish food (protein >40% and crude fat > 4.5%) was obtained from Zhongshan President Enterprise Co. Ltd., and cod liver oil was acquired from Peter Moller (Norway). The PCB mixture was first dissolved in 5 g of cod liver oil, which was then mixed with 400 g of fish food pellets. The initial concentration of PCBs in the food was 15 μg/g dry weight. Non-spiked food, which was used for the depuration phase and control group, was treated in an identical manner but without addition of the PCB mixture. Food was homogenized by mixing in a shaking incubator (24 h, 20 °C), and then air-dried for 24 h and stored in the dark at −20 °C in amber stopper-sealed jars throughout their use. Spiked food samples were collected at the beginning and end of the feeding intervals to confirm the associated PCB concentration.

2.3. Exposure and sampling

Forty common carp (*C. carpio*) with average initial weights and lengths of 61.8 ± 4.7 g (mean ± SD, similarly hereinafter) and 17.4 ± 0.6 cm, respectively, were purchased from a local aquarium market in Guangzhou, China. At the beginning of the experiment, four carp were removed as background samples. The remaining carp (n = 36) were randomly distributed between two rectangular glass aquariums (120 cm × 45 cm × 80 cm). One was designated the control group (n = 12), in which fish were fed non-spiked food throughout the experiment. The other aquarium was for the treated

group (n = 24). Filtered dechlorinated tap water in each aquarium was maintained at a temperature of 22 ± 1 °C and circulated using a submerged pump at a rate of 2.5 L/min. The water in each aquarium was gently aerated to maintain oxygen saturation, and a 12-h light and dark photoperiod cycle was applied. Fish were acclimated to the non-spiked diet in the laboratory for two weeks prior to exposure, and were fed food at a rate of 1% of their average body weight per day.

After 28 days of exposure (uptake period), the fish were fed non-spiked food for 30 days (depuration period). Fish were taken on days 0, 10, 20, and 30 of the depuration period. On each sampling day, six fish were sampled randomly from the exposed group to determine their fork length and weight, and dissected carefully. Blood samples were obtained from the dorsal aorta using syringes, transferred into 5-mL Teflon tubes, and centrifuged at 3000 rpm for 30 min to obtain the serum. Then, the gills and stomach cavity of each fish were removed from the carcass. The carcasses and serum from fish sampled on the same day were weighed and respectively pooled to form three composite samples, which were then frozen at −20 °C prior to analysis. Meanwhile, three fish were collected randomly from the control group on each sampling day for PCB analysis.

Moreover, fish feces were siphoned out and collected each day, and the feces samples collected on day 1 through day 7, day 8 through day 14, day 15 through day 21, and day 22 through day 28 in the uptake period, and day 1 through day 10, day 11 through day 20, day 21 through day 30 in the depuration period, were pooled into seven composite samples.

2.4. Sample preparation and extraction

The carcasses and composite fish feces were freeze-dried, homogenized, and weighed. The feces and a small portion of the carcass samples were used for PCB and $MeSO_2$-PCB quantification analysis, and the sera were analyzed for PCBs and OH-PCBs. The remainder of the carcass material was used for compound-specific stable carbon isotope analysis.

The extraction and quantification analysis procedures used for the fish tissues (serum and carcass) and composite fish feces were similar to those described in previous studies (Gebbink et al., 2008; Zeng et al., 2014a), with minor modifications. Detailed descriptions of the sample extraction and cleanup procedures are given in the supplemental information (SI).

The remainder of the fish carcass samples (approximately 30 g dry weight for each samples) were used for stable carbon isotope analysis. The large sample volumes inevitably lead to complex matrices containing large amounts of lipids that must be dealt with and to interference due to co-elution. Therefore, extensive purification steps are required to attain high-purity extracts before CSIA. The method for purification of PCBs in fish for compound-specific isotope analysis was based on our previous studies (Zeng et al., 2012, 2013a), with minor modifications. Briefly, the remainder of the fish carcass samples used for stable carbon isotope analysis was Soxhelt-extracted with 350 mL Hex/Acetone (1:1 v/v) for 72 h. The extract was concentrated to approximately 1 mL by rotary evaporator and the solvent exchanged with hexane (10 mL). This was poured into a 50-mL screw-top Teflon tube and then treated with 6 × 3 mL H_2SO_4 to remove the lipids. The neutral contaminants were concentrated to approximately 1 ml by rotary evaporator and then primarily purified on a complex silica gel column (i.d. = 1.0 cm) packed from bottom to top with 8 cm neutral silica, 16 cm sulfuric acid silica (56:44 w/w), and a 2-cm layer of anhydrous sodium sulfate. The first elution with 15 mL Hex was discarded, and the target compounds were obtained by eluting with 10 mL Hex. The extract was concentrated to approximately 1 ml by rotary evaporator and cleaned with a packed alumina/neutral silica column (6 cm/12 cm, both 3% deactivated; i.d. = 1.0 cm), topped with a 2-cm layer of anhydrous Na_2SO_4. The first elution with 10 mL Hex was discarded, followed by an elution with 15 mL Hex, which was collected and concentrated to approximately 1 ml by rotary evaporator. The extract was subjected to further separation by an 18 cm Florisil column (i.d. = 1.0 cm) topped with a 2-cm layer of anhydrous Na_2SO_4. The column was first eluted with 10 mL of Hex, which was discarded. The PCBs were obtained in the second fraction eluted with a subsequent 15 mL of Hex. This fraction was concentrated to approximately 1 ml by rotary evaporator, solvent exchanged to isooctane, and finally evaporated to near-dryness under a gentle stream of nitrogen and re-dissolved in 100 μL isooctane. No significant isotope fractionation of the target compounds was observed during the purification process (SI).

2.5. Instrumental analysis

Determination of PCBs was performed using an Agilent 7890 GC coupled with a 5975C mass selective detector in an electron impact (EI) ion source in the selected ion (SIM) mode. A DB-5 MS column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) was used for PCB separation. A Chiralsil-Dex (25 m × 0.25 mm i.d. × 0.25 μm film thickness) column was used for PCB atropisomer determination. Chiral PCB compositions are expressed as EFs, which are defined as follows:

$$EF = A(+)/(A(+) + A(-))$$

where A (+) and A (−) represent the areas of the (+)- and the (−)-atropisomer peaks in the stereoselective chromatograph. The (−)-atropisomer elutes first for PCBs 95, 132, 136, and 149, and the (+)-atropisomer elutes first for PCB 91 (Dai et al., 2014; Morrissey et al., 2007.). $MeSO_2$-PCBs and OH-PCBs (OH-PCBs were derivatized to their methoxy analogues before instrumental analysis) were analyzed using an Agilent 6890 GC coupled with a 5975B mass selective detector in the electron capture negative ionization (ECNI) mode, and were separated with a DB-XLB (30 m × 0.25 mm i.d. × 0.25 μm film thickness) capillary column. Details of the GC conditions and oven temperature programs are given in the SI.

2.6. GC-C-isotope ratio mass spectrometry (IRMS) analysis

The purities of the extracts used for CSIA were first checked using an Agilent 6890 GC-5975MS system with EI ion source in the full scan mode. The Aroclor 1242 mixture was used as a standard for the qualitative analysis of PCBs. The individual congeners of the PCBs were identified by comparing the mass spectrum and the retention time of the target compounds with the calibration standards.

CSIA measurements were performed on a Trace GC Ultra-IsoLink Delta V Advantage isotope ratio mass spectrometer (Thermo-Fisher Scientific, USA). Samples were injected at 290 °C in the splitless mode (split opened after 1 min). PCBs were separated on a DB-5 MS column (60 m × 0.25 mm i.d. × 0.25 μm film thickness). The GC oven was programmed as follows: the initial oven temperature was 120 °C, and was then increased at 6 °C/min to reach 180 °C, at 1 °C/min to reach 240 °C, and finally at 6 °C/min to reach 290 °C, where it was maintained for 17 min. Helium was used as the carrier gas at 1.1 mL/min. The combustion interface was maintained at a temperature of 1050 °C.

A CO_2 reference gas was automatically introduced into the isotopic ratio mass spectrometer in a series of pulses at the beginning and the end of each run. The stable carbon isotope data are reported in the delta (δ) notation, and all values reported are relative to the

international standard, V-PDB (Vienna Pee Dee Belemnite). The reliability of the IRMS equipment for these experiments was verified by injecting a standard mixture containing 10 n-alkanes with a known isotopic composition (provided by Indiana University, Bloomington, USA) at the beginning of the analysis and a co-injected standard, 2,4,6-trichlorobiphenyl (PCB 30), obtained from Ultra Scientific (North Kingstown, RI, USA). The $\delta^{13}C$ of the co-injected standard was determined offline with a Flash 2000 EA-Delta V Plus IRMS (Thermo-Fisher Scientific, USA). The differences between the values of the online-measured ((−29.14‰)–(−28.86‰)) and the offline-measured (−28.80‰) for the co-injected standard (PCB 30) were less than 0.5‰. In addition, thorough re-oxidation was performed after every sequence (minimum once a day) by oxygen backflush for 2 h. A short re-oxidation was performed after every single run for 2–5 min. To ensure the stability of the instrument, the performance of the GC-C-IRMS system was determined regularly by analyzing a PCB standard mixture Aroclor 1242. Each extract was analyzed in triplicate, and the data were only considered if the $\delta^{13}C$ values of the three injections did not vary by more than 0.5‰.

2.7. Statistical analysis

All data are presented as mean ± standard deviation unless otherwise specified. Statistical analyses were performed using the SPSS 21 software for Windows (SPSS). The level of significance was set at $p = 0.05$ throughout the study. The statistical differences in the EFs of chiral PCBs and $\delta^{13}C$ values between different groups of samples were determined by one-way ANOVA with Tukey's Post Hoc test.

3. Results and discussion

3.1. Background levels and quality control

No natural mortality was observed during the experiment. The background concentration of ΣPCBs, i.e., the total concentration of the 81 PCB congeners, was 6.6 ± 1.7 ng/g wet weight (w/w). No OH-PCBs or $MeSO_2$-PCBs were detected in the background or exposed samples at the beginning or end of the experiment. The PCB levels in the background samples were two to three orders of magnitude lower than those in the exposed fish. Thus, the influence of background PCBs on the isotopic composition of PCBs in the exposure group was negligible. The ΣPCB concentration in the spiked food pellet homogenate was 14.8 μg/g, which was slightly lower than the nominal concentration (15 μg/g). This was most likely due to the additional dilution of food pellets with cod liver oil and loss during homogenization.

3.2. Gastrointestinal absorption of PCBs

The dominant PCB congeners in the commercial Aroclor 1242 mixture included PCBs 5/8, 17, 18, 20/33, 22, 28/31, 44, 47/48, 49, 52, 56, 66, 70, and 74, collectively representing 62.6% of the ΣPCBs. The congener profiles of PCBs in the administered food remained the same throughout the experiment, and no differences were seen in the dominant congeners in the PCB profiles of the food and the feces (Figure S1 of the SI).

The ratio of the PCB concentration in the fish feces to that of the food administered in the exposure period can be used to evaluate the food absorption and excretion. If PCBs are absorbed at a lower rate than the food, then the PCB concentration in the feces will increase, and vice versa (Zeng et al., 2014b). The chemical losses from feces to water are insignificant (unpublished data) during 10-h feces accumulation because of the high lipophilic character of the PCBs ($K_{OW} > 10^4$).

The ratios of C_{feces}/C_{food} for each congener ranged from 0.07 to 0.24, 0.10 to 0.24, 0.15 to 0.33, 0.22 to 0.41, and 0.32 to 0.44 for di-, tri-, tetra-, penta-, and hexa-CBs, respectively (Figure S2 of the SI). Significant positive correlation is seen between the number of chlorine atoms and the feces/food ratio for congeners ($p < 0.05$), which indicates that the absorption rate decreases with an increasing number of chlorine atoms.

3.3. PCB congener profile change and potential metabolites

A total of 81 PCB congeners were detected in both the commercial Aroclor 1242 mixture standard and the fish tissue samples. The ΣPCB concentrations in fish tissue and feces over the depuration period are displayed in Fig. 1. The ΣPCB concentrations in carcass were 39.9 ± 1.5 μg/g lipid wt (similarly hereinafter) , 33.6 ± 3.1 μg/g, 29.9 ± 1.6 μg/g, and 20.8 ± 3.0 μg/g on day 0, 10, 20, and 30 of the depuration period, respectively. And in these sampling time points, the concentrations in sera were 17.0 ± 17.1 μg/g, 12.2 ± 0.6 μg/g, 8.9 ± 0.3 μg/g and 3.1 ± 0.1 μg/g, respectively. A significant decrease trend in the PCB concentrations was observed (one-way ANOVA, $p < 0.05$). Particularly, the concentration of PCBs in the feces collected on day 10 of the depuration period (0.5 μg/g) showed a sharp decrease of one order of magnitude compared to the feces in the uptake period (3.1 ± 0.2 μg/g).

The congeners detected in tissues from fish administered the PCB mixture were the same as those in the spiked food, while the congener profile in the fish tissues was different from that in the spiked food (Figure S3 of the SI). The contributions of the lower chlorinated PCBs, i.e., 5/8, 6, 15, 16, 17, 18, 32, and 37, decrease over the depuration period. However, those of the higher chlorinated PCBs, such as 44, 49, 52, 56, 60, 66, 70, 101, 105, 110, and 118, show an increasing trend (Figure S2). This could be attributed to the fact that less chlorinated PCBs were typically more readily metabolized than more chlorinated PCBs. However, the contribution of PCB 28/31, a congener with only three chlorine atoms, showed an increasing trend as well (Figure S2). It is suggested that the position of the chlorine substitution was another critical factor to PCB biotransformation (Megson et al., 2013). PCBs with chlorine bonding in the (2, 4), (2, 3, 4), (2, 4, 5), (3, 4, 5), and (2, 3, 4, 5) positions are often more resistant to biotransformation and therefore are likely to be classed as steady state congeners (Brown and Lawton, 2001; Megson et al., 2013). These congeners include but not limited to PCBs 28, 56, 60, 66, 70, 74, 105, 118, which were found to be recalcitrant to metabolized in our study.

However, neither OH-PCBs nor $MeSO_2$-PCBs were detected in the serum or fish tissues of the exposed carp throughout the experiment. In contrast, it has been reported that fish can selectively biotransform PCBs to OH-PCBs. For example, White et al. (1997) detected two major hydroxylated metabolites (5-OH-tetrachlorobiphenyl and 4-OH-tetrachlorobiphenyl) in the gallbladder, as well as two minor metabolites (6-OH-tetrachlorobiphenyl and 2-OH-tetrachlorobiphenyl) in the bile of marine scup (*Stenotomus chrysops*) exposed to 0.1 mg/kg of PCB 77. Furthermore, Buckman et al. (2006) found that seven hydroxylated metabolites (4-OH-PCB 107, 4-OH-PCB 130, 3-OH-PCB 138, 4-OH-PCB 146, 4′–OH–PCB 172, 3′–OH–PCB 180, and 4-OH-PCB 187) were present in the plasma of rainbow trout exposed to an environmentally relevant group of PCB congeners (i.e., a 1:1:1 mixture of Aroclor 1242, 1254, and 1260, supplemented with PCBs 202 and 209) for 30 days. Moreover, significant concentrations of OH-PCBs have been observed in lake trout from the Great Lakes (Campbell et al., 2003), and mud carp and northern snakehead from an e-waste recycling site (Zeng et al., 2014a). The notable changes in PCB profiles and the lack of detectable OH-PCBs metabolites in the present study

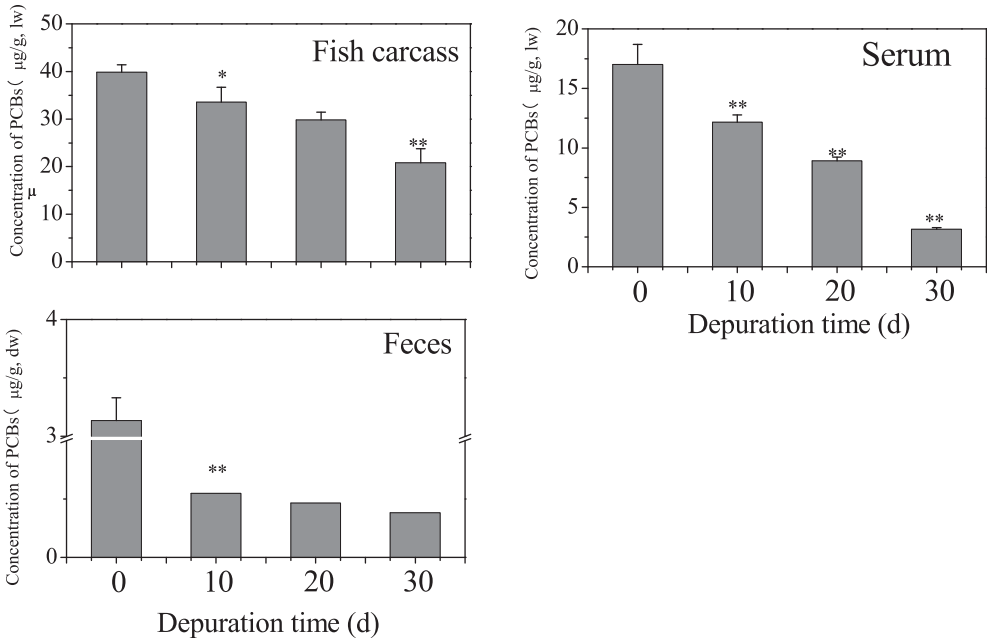

Fig. 1. Concentrations of PCBs in fish tissues and feces over the entire 30-day depuration period ($^{*}p < 0.05$, $^{**}p < 0.01$).

suggests several possibilities in relation to PCB metabolism and metabolite retention. It is possible that OH-PCBs are formed in carp via CYP enzymes and subsequent epoxide hydrolase mediation. Indeed, a previous study found that phase II conjugating processes may be effective in removing hydroxylated polybrominated diphenyl ethers (OH-BDEs) at a rate similar to that at which they are formed (Hakk and Letcher, 2003; Stapleton et al., 2004). The same effective elimination may occur for OH-PCBs due to their similar molecular structure. Another possibility is that OH-PCBs are further biotransformed to di–OH–PCBs and even sulfated metabolites, which have been detected in recent studies where rats were inhalation-exposed to PCB 3 (Dhakal et al., 2012, 2014). However, this is unlikely since fish generally have less ability to biotransform PCBs than rats. Additionally, it is possible that carp do not have the capacity to produce OH-PCBs in the short period of time used in the present study, while arene oxide intermediates—the initial step in PCB metabolism—may have been generated, but cannot be detected by the method used.

As for $MeSO_2$-PCBs, even though significant concentrations have been detected in wild fish species (Stapleton et al., 2001; Zeng et al., 2014a; Zhang et al., 2012), no $MeSO_2$-PCBs have been discovered in laboratory studies so far. A previous study suggested that fish do not possess appreciable sulfone-forming capacity (Letcher et al., 2000). Compared with biotransformation to OH-PCBs, $MeSO_2$-PCBs were not the major biotransformation route for PCB metabolism. The extremely slow metabolism of the PCB parent compounds to $MeSO_2$-PCBs by administrated fish may indicate that the concentrations observed in the wild fish may be the result of accumulation from exogenous sources over long periods of time (years).

3.4. Chiral data

Five chiral PCB congeners (PCBs 91, 95, 132, 136, and 149) were selected for chirality analysis in this work, and the EFs of PCBs 91, 95, 132, 136, and 149 in the spiked food were 0.526 ± 0.004, 0.496 ± 0.004, 0.470 ± 0.09, 0.498 ± 0.005, and 0.498 ± 0.004, respectively. Compared with the values for the spiked food, the EFs of PCBs 91, 95, and 136 in the carp changed remarkably (one-way ANOVA, $p < 0.05$), providing evidence of PCB biotransformation in fish. The relative abundances of the (−) atropisomers of PCBs 91 and 136, and the (+) atropisomer of PCB 95, increased with time, indicating the preferential metabolism of (+)-PCB 91, (−)-PCB 95, and (+)-PCB 136 in carp (Fig. 2). This result was consistent with our previous field study on PCBs in mud carp and/or northern snakehead from an e-waste site (Zeng et al., 2014a), but was slightly different to a previous exposure experiment using rainbow trout by Buckman et al. (2006). In their study, rainbow trout biotransformed (+)-PCB 91 (E_1-PCB 91) and (+)-PCB 136 preferentially, while PCB 95 was found to be racemic throughout the course of the experiment (Buckman et al., 2006). Species-specific metabolism of chiral PCBs was suggested as a cause for this observation.

The EF values for PCBs 132 and 149 in the common carp over the course of this experiment are the same as those in the spiked food, which implies that either no metabolism of these two congeners occurs, or that both atropisomers of these congeners are biotransformed at the same rate, i.e., that their biotransformation is achiral (Buckman et al., 2006). In a study of the exposure of rainbow trout to PCBs, Buckman et al. (2006) also found that PCBs 132 and 149 remained racemic. Their previous study demonstrated that PCB congeners with vicinal hydrogen atoms at meta-para positions were readily biotransformed (Buckman et al., 2006). Both PCB 132 and PCB 149 have vicinal hydrogen atoms at meta-para positions, as do PCBs 91, 95, and 136; therefore, it was expected that PCBs 132 and 149 would have a biotransformation potential similar to that of PCBs 91, 95, and 136. It has been suggested that using chiral analysis in the determination of biotransformation in fish may be a more sensitive tool than direct measurements of metabolite products, particularly when biotransformation is slow (Buckman et al., 2006; Wong et al., 2002). However, this hypothesis implicitly assumes stereoselective biotransformation and would be unsuitable for the study of achiral biotransformation (Buckman et al., 2006; Wong et al., 2002).

3.5. PCB carbon isotope data

Although 81 PCB congeners were detected in the Aroclor 1242 standard and the fish samples, accurate $\delta^{13}C$ values can only be

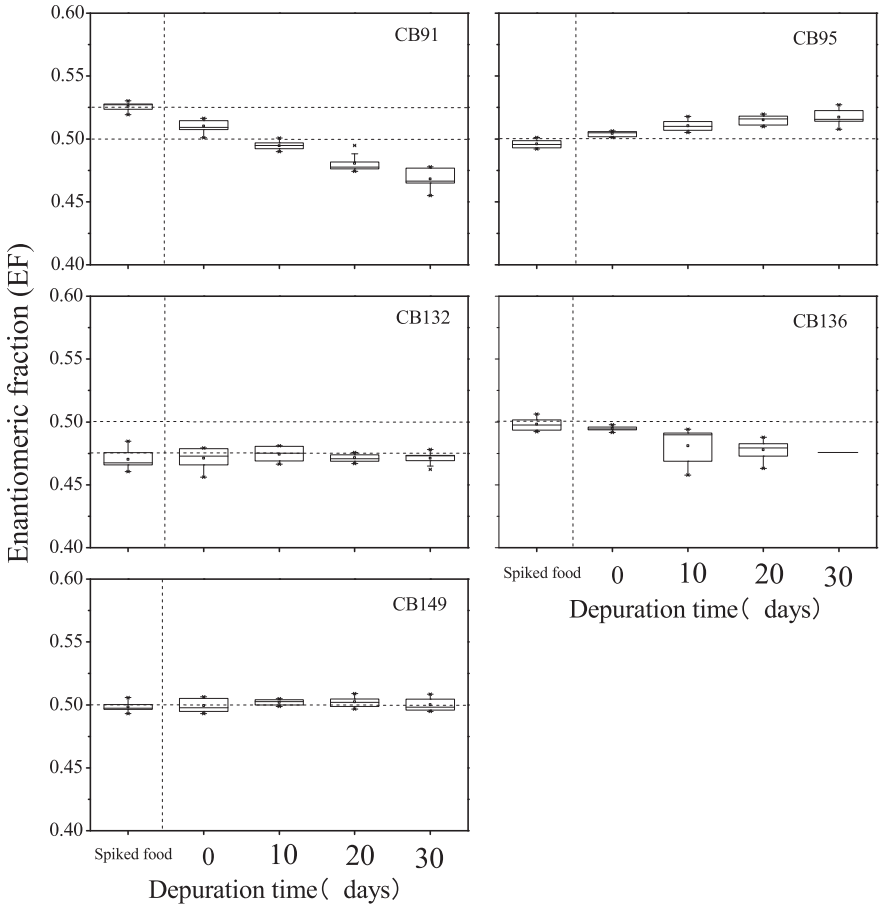

Fig. 2. Enantiomer fractions (EFs) of the chiral PCBs in the spiked food (n = 6), and common carp samples over the depuration period.

obtained for those congeners with well-resolved peaks and high concentrations. For a precise isotope measurement, at least 1 nmol carbon of a given compound is needed for commercially available GC/IRMS instruments (Schmidt et al., 2004). As for PCBs, the carbon content ranged from 28% (deca-chlorinated biphenyl) to 76% (mono-chlorinated biphenyls), so that it can be assumed that approximately 30 ng of individual congener would be required for appropriate measurements (Vetter et al., 2008). The requirement of high concentrations of target compounds was a major limitation of using CSIA.

A subset of 20 PCB congeners in the Aroclor 1242 standard have measurable $\delta^{13}C$ values, but only 12 congeners (PCBs 5/8, 18, 20/33, 22, 28/31, 44, 47/48, 49, 52, 66, 70, and 74) in the fish samples were available for detailed isotope analysis (Figure S4 of the SI). The $\delta^{13}C$ values of the PCBs for the Aroclor 1242 mixture range from −28.2‰ to −24.4‰ (Table 1). Similar to the studies by Jarman et al. (1998) and Yanik et al. (2003), a trend of isotope depletion with increasing chlorine content was observed.

For most PCB congeners in the carp samples, including PCBs 22, 28/31, 44, 52, 66, 70, and 74, there is some isotope agreement with the PCBs in the spiked food, as shown in Fig. 3 (isotopic differences between the fish and the source materials less than 1‰ were regarded as insignificant, considering that the uncertainty in CSIA measurement is typically 0.5‰ (Badea and Danet, 2015)). A previous study demonstrated that PCBs 28, 44, 52, and 66 are more resistant to transformation than the others due to their maximum chlorine substitution in the meta and para positions of the biphenyl rings, and thus should not exhibit significant biotransformation (Buckman et al., 2006).

Three PCB congeners (PCBs 5/8, 18, and 20/33) from the carp samples are isotopically enriched relative to the spiked food during the depuration period (Fig. 3). These results are in accordance with our previous study on sediment and fish collected from an e-waste site (Zeng et al., 2013b). In that study, most PCB congeners from the fish samples showed isotopic enrichment compared to those obtained from the sediment, a major source of pollutants for fish. Similar observations were reported for fish and duck samples collected from the Housatonic River, Massachusetts (Yanik et al., 2003). Given that other physical or chemical processes, such as chemical transport, distribution and sorption, generally not or only little isotope fractionating (Philp, 2007; Schmidt et al., 2004), isotopic enrichment in the fish samples indicates that some isotope fractionation occurs during biotransformation, which results in an isotopic enrichment in the remaining PCB reservoir (Mook and Rozanski, 2000; Zeng et al., 2013b). PCBs with vicinal hydrogen atoms at the meta-para position can readily be metabolized by fish, as reported in a previous study (Buckman et al., 2006). The PCB congeners that show significant $\delta^{13}C$ enrichment compared to the source materials, i.e., a $\Delta\delta^{13}C > 1‰$, including PCBs 5/8, 18, and 33/20 (Fig. 2), all have vicinal hydrogen atoms at the meta-para position in their molecular structure. PCBs 91 and 95 also exhibit meta-para vicinal hydrogens and, based on changes in their EFs, are found to be biotransformed. However, PCB 95 is co-eluted with PCB 66, and exhibits an order of magnitude lower contribution. Thus, the change of $\delta^{13}C$ values for PCB 95 could be diluted by those of PCB 66. The low concentration of PCBs 91 and 136 restricted its availability for isotope analysis. As discussed above, the absence of metabolized products could be attributed to the biotransformation

Table 1
Summary of the isotopic values of PCBs in PCB mixture Aroclor 1242, the spiked food and the common carp during the depuration period.

	Aroclor 1242	Spiked food	Depuration time				$\Delta\delta^{13}C$ (‰)[a]
			0 days	10 days	20 days	30 days	
PCB 30 (I.S)	−29.0 ± 0.1	−28.9 ± 0.1	−28.9 ± 0.1	−28.8 ± 0.1	−28.9 ± 0.1	28.9 ± 0.1	
PCB 5/8	−25.4 ± 0.1	−25.4 ± 0.2	−25.2 ± 0.2	−24.8 ± 0.2	−24.7 ± 0.2	−24.4 ± 0.2	1.0
PCB 18	−25.8 ± 0.2	−25.6 ± 0.2	−24.3 ± 0.4	−23.1 ± 0.4	−21.3 ± 0.2	−21.4 ± 0.3	4.2
PCB 20/33	−26.5 ± 0.1	−26.6 ± 0.2	−26.3 ± 0.1	−26.3 ± 0.3	−25.2 ± 0.2	−25.2 ± 0.5	1.4
PCB B22	−26.0 ± 0.3	−26.0 ± 0.3	−26.3 ± 0.2	−26.6 ± 0.1	−26.5 ± 0.1	−26.5 ± 0.2	−0.5
PCB 28/31	−26.9 ± 0.2	−26.9 ± 0.1	−27.1 ± 0.1	−27.2 ± 0.1	−27.2 ± 0.1	−27.4 ± 0.2	−0.4
PCB 44	−28.0 ± 0.4	−28.0 ± 0.2	−27.8 ± 0.1	−28.2 ± 0.2	−28.2 ± 0.3	−28.1 ± 0.3	−0.1
PCB 47/48	−27.2 ± 0.2	−27.1 ± 0.3	−27.5 ± 0.2	−27.7 ± 0.3	−28.0 ± 0.2	−28.3 ± 0.2	−1.2
PCB 49	−26.8 ± 0.3	−26.8 ± 0.3	−27.6 ± 0.2	−28.3 ± 0.2	−28.5 ± 0.1	−28.9 ± 0.1	−2.1
PCB 52	−27.6 ± 0.3	−27.8 ± 0.3	−27.8 ± 0.2	−28.1 ± 0.3	−28.2 ± 0.3	−28.2 ± 0.1	−0.4
PCB 66/95	−28.2 ± 0.3	−28.1 ± 0.2	−28.4 ± 0.4	−28.3 ± 0.1	−28.3 ± 0.4	−28.2 ± 0.3	−0.1
PCB 70	−28.0 ± 0.1	−28.0 ± 0.1	−28.0 ± 0.3	−28.3 ± 0.1	−28.6 ± 0.1	−28.7 ± 0.1	−0.7
PCB 74	−27.8 ± 0.2	−27.9 ± 0.3	−28.4 ± 0.1	−28.2 ± 0.2	−28.3 ± 0.3	−28.2 ± 0.1	−0.3
PCB 6	−24.4 ± 0.3	−24.4 ± 0.2	/	/	/	/	/
PCB 7/9	−24.9 ± 0.3	−24.7 ± 0.2	/	/	/	/	/
PCB 10/4	−25.4 ± 0.3	−25.4 ± 0.1	/	/	/	/	/
PCB 15	−25.6 ± 0.4	−25.6 ± 0.2	/	/	/	/	/
PCB 17	−27.3 ± 0.2	−27.1 ± 0.2	/	/	/	/	/
PCB 19	−26.7 ± 0.3	−26.7 ± 0.3	/	/	/	/	/
PCB 40/103	−26.5 ± 0.3	−26.4 ± 0.4	/	/	/	/	/
PCB 45	−26.0 ± 0.4	−26.0 ± 0.2	/	/	/	/	/

[a] $\Delta\delta^{13}C = \delta^{13}C$ (30 days) − $\delta^{13}C$ (standard).

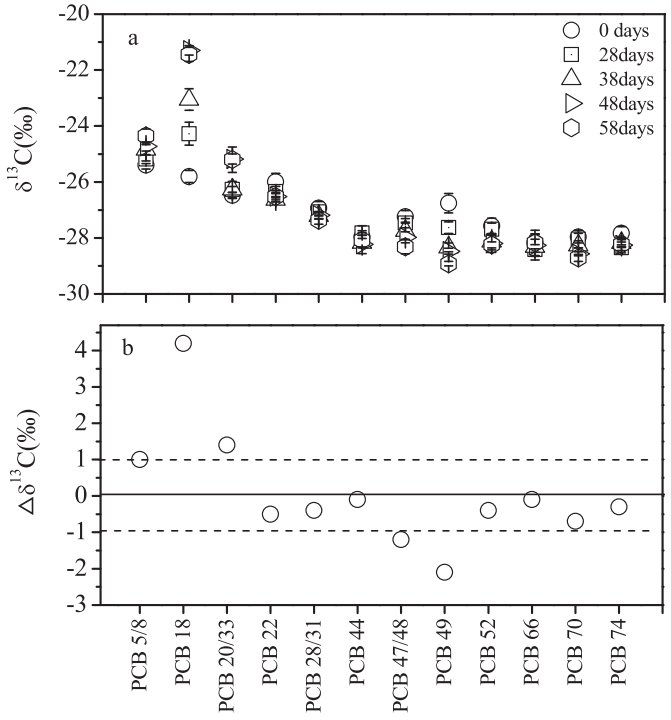

Fig. 3. Comparison between the isotopic values ($\delta^{13}C$) of the determined individual PCB congeners in the spiked food and the common carp (a); $\Delta\delta^{13}C$ was expressed as the isotopic differences of PCB congeners in fish relative to the spiked food (b).

of PCBs to metabolites that cannot be detected by the analysis methods used in the present study.

The $\delta^{13}C$ values of PCB 47/48 and 49 are isotopically depleted compared to those in the spiked food (Fig. 3 and Table 1), which might be attributed to the contribution of dechlorinated products from highly chlorinated congeners (Zeng et al., 2013b). As shown in Fig. 3 and Table 1, the $\delta^{13}C$ values of PCB congeners in the PCB standard mixture generally decrease with increasing chlorine content. Therefore, reductive dechlorination would generate congeners with more negative $\delta^{13}C$ values than native PCB congeners with the same degree of chlorination, resulting in a lower $\delta^{13}C$ for the original PCB congener pool (Yanik et al., 2003; Zeng et al., 2013b). However, it should be noted that the reductive dechlorination pathways are mainly induced by microbiological degradation (Lake et al., 1992), while has not been established in fish. Furthermore, the changes in the $\delta^{13}C$ values of PCB could also be related to mass-independent isotope fractionation, which occurs mainly in spin-forbidden reactions (Zakon et al., 2013). Therefore, further studies are needed to clarify whether the depletion of $\delta^{13}C$ values observed in these congeners is caused by the dechlorination processes in fish.

4. Conclusions and implications

In summary, we investigated the biotransformation of PCBs in fish indirectly by measuring the EF values of chiral PCBs and $\delta^{13}C$ values of some PCB congeners. The metabolites of PCBs are not detectable, but the modification of EFs in chiral PCBs provided evidence of PCB biotransformation in fish. However, chiral analysis implicitly assumes stereoselective biotransformation and does not take into account achiral biotransformation. The $\delta^{13}C$ values of some PCB congeners in fish tissues increased compared with those in the spiked food, suggesting a significant biotransformation process in fish. Some other PCB congeners in fish tissues showed an apparent isotope depletion, which may be attributed to the dechlorination of higher chlorinated congeners. Such information provides additional evidence for the occurrence of biotransformation processes and aids in further understanding the biotransformation of the compounds. The results of this study suggest that CSIA is a powerful method for monitoring the biotransformation of PCBs in biota.

The chiral PCB congeners detected in the present study were not available for CSIA measurement due to their low concentrations. It was not definitely ascertained whether the racemic chiral congeners in fish were biometabolized or not. However, in recent years, the combination of enantiospecific analysis and CSIA has become a promising new approach that can provide insight into the stereoselective fates and source apportionment of environmental organic contaminants. Therefore, further work is needed to identify the

achiral biotransformation of chiral PCBs by combined enantiomer analysis and CSIA.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.06.053.

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