Environmental Pollution 222 (2017) 165–174

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Bioaccumulation of short chain chlorinated paraffins in a typical freshwater food web contaminated by e-waste in south china: Bioaccumulation factors, tissue distribution, and trophic transfer☆

Runxia Sun [a, b], Xiaojun Luo [a, *], Bin Tang [a, b], Laiguo Chen [c], Yu Liu [a, b], Bixian Mai [a]

[a] State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing, 100049, People's Republic of China
[c] Urban Environment and Ecology Research Center, South China Institute of Environmental Sciences (SCIES), Ministry of Environmental Protection, Guangzhou 510655, People's Republic of China

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form
20 December 2016
Accepted 22 December 2016
Available online 28 December 2016

Keywords:
Short chain chlorinated paraffins
Aquatic organisms
Bioaccumulation factors
Tissue distribution
Trophic transfer

ABSTRACT

Short chain chlorinated paraffins (SCCPs) are under review for inclusion into the Stockholm Convention on Persistent Organic Pollutants. However, limited information is available on their bioaccumulation and biomagnification in ecosystems, which is hindering evaluation of their ecological and health risks. In the present study, wild aquatic organisms (fish and invertebrates), water, and sediment collected from an enclosed freshwater pond contaminated by electronic waste (e-waste) were analyzed to investigate the bioaccumulation, distribution, and trophic transfer of SCCPs in the aquatic ecosystem. SCCPs were detected in all of the investigated aquatic species at concentrations of 1700–95,000 ng/g lipid weight. The calculated bioaccumulation factors (BAFs) varied from 2.46 to 3.49. The relationship between log BAF and the octanol/water partition coefficient (log K_{OW}) for benthopelagic omnivorous fish species followed the empirical model of bioconcentration, indicating that bioconcentration plays an important role in accumulation of SCCPs. In contrast, the relationship for the benthic carnivorous fish and invertebrates was not consistent with the empirical model of bioconcentration, implying that the bioaccumulation of SCCPs in these species could be more influenced by other complex factors (e.g., habitat and feeding habit). Preferential distribution in the liver rather than in other tissues (e.g., muscle, gills, skin, and kidneys) was noted for the SCCP congeners with higher log K_{OW}, and bioaccumulation pathway (i.e. water or sediment) can affect the tissue distribution of SCCP congeners. SCCPs underwent trophic dilution in the aquatic food web, and the trophic magnification factor (TMF) values of SCCP congener groups significantly correlated with their corresponding log K_{OW} values ($p < 0.0001$). The present study results improved our understanding on the environmental behavior and fate of SCCPs in aquatic ecosystem.

1. Introduction

Short chain chlorinated paraffins (SCCPs) are a synthetic complex mixture of chlorinated *n*-alkanes ranging from C_{10} to C_{13} with degrees of chlorination of 40–50% by mass weight (Fiedler, 2010). They are used as flame retardants and plasticizers in rubber compounds and polymers, additives in metal working fluids, paints, sealants, and leather treatment agents, as well as in extreme-pressure lubricants. SCCPs have generated worldwide concern in the last decade owing to their persistence in the environment, high potential for long-range transport, bioaccumulation, and toxicity to organisms (Basconcillo et al., 2015; Geng et al., 2015; Ma et al., 2014a, 2014c; Zeng et al., 2011b; Zhang et al., 2016). The manufacture and use of SCCPs is restricted or banned in the European Union, Japan, Canada, and the United States (UNEP, 2015b). Furthermore, SCCPs are currently candidates for classification as persistent organic pollutants (POPs) under the Stockholm Convention (UNEP, 2015a).

SCCPs are high production volume chemicals, and have been produced in many countries, especially China, which is the largest producer and consumer of chlorinated paraffins in the world

☆ This paper has been recommended for acceptance by Charles Wong.
* Corresponding author.
E-mail address: luoxiaoj@gig.ac.cn (X. Luo).

http://dx.doi.org/10.1016/j.envpol.2016.12.060
0269-7491/

(Sverko et al., 2012; van Mourik et al., 2016). In 2013, China's annual production of chlorinated paraffins was equal to 1.05 million tons (WCC, 2014), which accounted for 15% of the total global chlorinated paraffins production (van Mourik et al., 2016), although specific information on the production volumes of SCCPs are limited. The release of SCCPs into the environment can occur during production, storage, transportation, usage, and disposal or recycling of SCCPs and SCCP-containing products. Even in countries where SCCPs are prohibited, discharge into ecosystems continues to occur by means of old materials that are still in use or during disposal of these materials (van Mourik et al., 2016). Studies indicate that SCCPs are ubiquitous in the environment and are routinely detected in the air, soil, water, sediment, and in aquatic and terrestrial wildlife (Chen et al., 2011; Huang et al., 2016; Luo et al., 2015; Ma et al., 2014b; van Mourik et al., 2016; Wang et al., 2013). Notably, concentrations of SCCPs are generally present in higher levels than are other organohalogen compounds such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethanes (DDTs) in biotic and abiotic compartments (Bytingsvik, 2015; Li et al., 2014; Strid et al., 2013; Sun et al., 2016; Tan et al., 2016). Thus, it is important to improve our understanding of the environmental fate, behavior, and potential risks posed by SCCPs.

Limited data is available on the bioaccumulation and trophic transfer of SCCPs, which are the key criteria for assessing their potential risk. To our knowledge, the trophic magnification factors (TMFs) for SCCPs have only been reported in three freshwater food webs from Gaobeidian Lake (China) (Zeng et al., 2011b), and Lake Ontario and Lake Michigan (Canada) (Houde et al., 2008), and two marine food webs from the Bohai Bay (China) (Ma et al., 2014b; Yuan et al., 2012) and the Arctic (Herzke, 2013). The results on the bioaccumulation potential and trophic transfer of SCCPs in the organisms from these studies are inconsistent. Houde et al. (2008) reported that the biomagnification for SCCPs was found between *Diporeia* (prey) and sculpin (*Cottus cognatus*) (predator) in Lake Ontario and Lake Michigan, while lake trout (*Salvelinus namaycush*), a top predatory fish, in both lakes had lower SCCP concentrations than did several of their prey based on lipid content. SCCPs exhibited trophic dilution in shelled benthic species (bivalves, gastropods, and crustaceans) in the Bohai Sea in China, but showed biomagnification in trophic transfer from zooplankton-shrimp-fish in this region (Ma et al., 2014b; Yuan et al., 2012). These complex results indicate that further research is necessary to provide understanding on the bioaccumulation potential of SCCPs in different food webs. Additionally, limited information is available on tissue-specific accumulation and the pathways for aquatic organisms to accumulate SCCPs.

Electronic waste (e-waste) recycling sites have been demonstrated as hot spots for SCCP contamination (Chen et al., 2011; Luo et al., 2015). In comparison to large volume water bodies, e.g., oceans and lakes, a pond is a small-enclosed water body that can minimize interruption from activities such as the migration of organisms, which may make interpretation of food web magnification of chemicals problematic. In our previous study, a food web model from a pond contaminated by e-waste in South China has been created and successfully used to characterize trophic transfer of PCBs, PBDEs, and other flame-retardants (Wu et al., 2010a, 2009, 2010b). In the present study, aquatic organisms, including fish and invertebrates, water, and sediment were collected from the same pond as in the above previous studies. The aims of the present study were to investigate the bioaccumulation, tissue distribution, and trophic dynamic behavior of SCCPs in aquatic organisms.

2. Materials and methods

2.1. Sample collection

Wild fish and invertebrates were caught by net and electric fishing in an enclosed natural freshwater pond located in Longtang Town, Qingyuan County, Guangdong province, South China (23.60° N, 113.08° E) in December 2014. The pond covers 5000 square meters and reaches a depth of 2 m in summer. The discarded e-wastes were stacked at the bottom of the pond, which resulted in high e-waste related pollutant exposure for organisms inhabiting in this pond (Chen et al., 2011). Detailed information regarding this pond can be found in a previous publication (Wu et al., 2008). The species collected included oriental river prawn (*Macrobrachium nipponense*, 50 individuals divided up into 5 pooled samples), Chinese mitten crab (*Eriocheir sinensis,* 16 individuals divided up into 5 pooled samples), crucian carp (*Carassius auratus*, 16 individuals divided up into 5 pooled samples), mud carp (*Cirrhinus molitorella,* $n = 5$ for large group and 60 individuals divided up into 5 pooled samples for small group), catfish (*Clarias batrachus*, $n = 2$), and snakehead (*Ophiocephalus argus,* $n = 5$). Three water samples and four surface sediment samples were also collected. Mud carp was divided into two groups according to body size (large group: average body length and weight were 49 ± 3.0 cm and 1800 ± 230 g, respectively, and small group: average body length and weight were 8.2 ± 1.1 cm and 5.3 ± 1.5 g, respectively). The large group was used for the investigation of tissue distribution and the small group was used for the study of trophic transfer. The muscles of fish and invertebrates were sampled. Skin, gill, liver, kidney, and muscle were taken from the large group of mud carp and snakehead to perform the tissue distribution study.

2.2. Sample preparation and instrumental analysis

The extraction and cleanup method for SCCPs in organisms were the same as that used in a previous study (Sun et al., 2016). Briefly, after being spiked with a surrogate standard (10 ng of epsilon-hexachlorocyclohexane, ε-HCH), the lyophilized samples were Soxhlet extracted with 200 mL of *n*-hexane/dichloromethane (1:1, v:v) for 48 h. An aliquot of the extract (1/10) was used for the gravimetric determination of the lipid content. The remainder of the extract was purified with concentrated sulfuric acid and further cleaned on a complex column packed with Florisil and silica gel. The column was eluted with 80 mL of *n*-hexane (first fraction, containing PCBs, PBDEs, and most of dichlorodiphenyltrichloroethane and its metabolites) followed by 60 mL of dichloromethane (second fraction, containing short -chain and medium-chain CPs, HCHs, and a few of dichlorodiphenyltrichloroethane and its metabolites) (Fig. S1 in the Supporting Information, SI). The second fraction was concentrated to near dryness under a gentle nitrogen flow, and solvent exchanged with isooctane to a final volume of 200 μL. A total of 10 ng of $^{13}C_{10}$-trans-chlordane was added as a recovery standard for gas chromatography − mass spectrometry analysis.

The water filtered through glass fiber filter (Whatman 0.7 μm pore size, 47 mm diameter), and then dissolved SCCPs in water were liquid-liquid extracted three times using dichloromethane. Sediment samples were freeze-dried, passed through an 80-mesh sieve, and was extracted with 200 mL of *n*-hexane/dichloromethane (1:1, v:v) for 48 h. The extracts for water and sediment were cleaned on the same complex column as that for the organism analysis.

SCCP congeners having 10–13 carbon atoms and 5–10 chlorine atoms were analyzed by a Shimadzu model 2010 gas chromatograph coupled with a model QP-2010 mass spectrometer

(Shimadzu, Japan) with electron capture negative ionization in the selective ion-monitoring mode. Details of the gas chromatograph conditions were published in our previous study (Chen et al., 2011). In brief, the separation was achieved with a DB-5HT capillary column (15 m × 250 μm i.d. × 0.10 μm film thickness, J & W Scientific, USA), and the temperature program was as follows: initially isothermal 100 °C for 2 min, then 40 °C/min to 280 °C, keeping for 2 min, and finally 70 °C/min to 320 °C, held the final temperature for 6 min. Helium was used as the carrier gas with a constant flow rate of 1.3 mL/min, and methane was used as the reagent gas at a flow rate of 2 mL/min. The temperatures of the injector, interface and ion sources were kept at 250, 280 and 200 °C, respectively.

To enhance instrument sensitivity and to minimize the interference of medium-chain chlorinated paraffins (MCCP, $C_{14\text{-}17}$) congeners, SCCP and MCCP congeners with 5–10 chlorine atoms were simultaneously detected at each injection. All monitored SCCP and MCCP ions were divided into four groups (C_{10} and C_{15}, C_{11} and C_{16}, C_{12} and C_{17}, and C_{13} and C_{14}) by mutual combination, and were analyzed by four individual injections for each sample, based on a previous reported method (Zeng et al., 2011a). The mass-to-charge ratios used for quantification and confirmation have been published elsewhere (Tomy et al., 1997). The highest and second-highest abundant isotope ions of $[M-Cl]^-$ for each congener group were utilized for quantification and confirmation, respectively. Total SCCPs were quantified using the procedure described by Reth et al. (2005). Congener group abundance profiles in the standards and samples were established from the actual relative integrated signals corrected using isotopic abundance and response factors (Tomy et al., 1997).

Strict quality assurance and quality control protocol (QA/QC) was implemented by the adding of surrogate standard and analysis of procedural blanks, spiked blanks, spiked matrices, and triplicate samples to accurate quantification of the target compounds. The surrogate (ε-HCH) recoveries of all samples were 80–95%. The recoveries of SCCP standards (51.5%, 55.5%, and 63.0% chlorine content) were 89–101% in the spiked blanks and 87–95% the matrix-spiked samples, respectively. The relative standard deviations (RSDs) were less than 15% for all the target chemicals. The method detection limits (MDLs, defined as a signal-to-noise ratio of 3) of organisms, water, and sediment were estimated at about 40 $ng \cdot g^{-1}$ dry weight (dw), 10 $ng \cdot L^{-1}$, and 60 $ng \cdot g^{-1}$ dw for total SCCPs, respectively.

The stable carbon and nitrogen isotope of muscle was analyzed by a Flash EA 112 series elemental analyzer coupled to a Finnigan MAT ConFlo III isotope ratio mass spectrometer. Total organic carbon (TOC) in sediment was determined by an elemental analyzer (Vario EL III Elementar, Germany) after removal of carbonates treated with 10% hydrochloric acid. Detailed information on the sample pretreatment, instrumental analysis, and the calculation method of several bioaccumulation parameters (Bioaccumulation factor, BAF; Biota-sediment accumulation factor, BSAF; and trophic magnification factor, TMF) are provided in the SI.

3. Results and discussions

3.1. Stable isotopic composition of C and N among aquatic species

Stable isotope analysis is the critical mean for examining the structure and dynamics of food webs. The ratio of nitrogen isotope ($\delta^{15}N$) exhibits stepwise enrichment with trophic transfers, and provided measurement of trophic level in wildlife. Ratios of carbon isotopes ($\delta^{13}C$) vary substantially among primary producers with different photosynthetic pathways (e.g. C3 versus C4 photosynthetic pathways in plants), but change little with trophic transfers, and thus can be used to identify original sources of dietary carbon. The $\delta^{15}N$ values of the aquatic organisms in the present study generally followed the expected trend with $\delta^{15}N$ increasing in the order crab, shrimp, omnivorous fish, and carnivorous fish (Fig. 1). The mud carp, however, exhibited size dependence. The $\delta^{15}N$ values in the large mud carp were significantly lower than those in the small mud carp ($p < 0.001$), although both mud carp size groups have the similar $\delta^{13}C$ values. The similar $\delta^{13}C$ values between the large and small mud carp may indicate they have the same original sources of dietary carbon. The $\delta^{13}C$ values of benthic carnivorous fish (snakehead and catfish) were closer to those of prawn than those of other samples, indicating prawn might be the main food item of the two benthic carnivorous fish. Meanwhile, the small mud carp (6–9 cm of approximate body length) appeared frequently in the stomach contents of snakehead at the dissection (Fig. S2), which indicated small carp is also an important food item of snakehead. The larger mud carp could not be the prey of the predators (catfish and snakehead) in the investigated food web owing to their large body size. Hence, the larger mud carp group was not used for the study of trophic transfer of SCCPs in the present study.

3.2. Concentrations and homologue profiles of SCCPs

The concentrations of SCCPs in the muscle tissues of the different organisms studied ranged from 1700 to 95,000 ng/g lipid weight (lw). The highest and lowest mean concentrations were obtained in the Chinese mitten crab and the large mud carp group (44,000 ng/g lw and 2100 ng/g lw, respectively) (Table 1). The SCCP levels in the large mud carp group were significantly lower than those in the small mud carp group (mean of 3400 ng/g lw, $p < 0.01$). Similarly, the large catfish (body weight of 1340 g) showed lower SCCP concentration (2600 ng/g lw) than the small catfish (body weight of 177 g with SCCP level of 13,000 ng/g lw). These results indicate a dilution of SCCPs in fish with growth and associated increases in body size, which is consistent with the observation on SCCPs in marine organisms from the Pearl River Estuary (Sun et al., 2016).

The levels of SCCPs in the present study were similar to those in fish collected in Gaobeidian Lake in China (11,000 ng/g lw in catfish and 25,000 ng/g lw in crucian carp). The water in the Gaobeidian

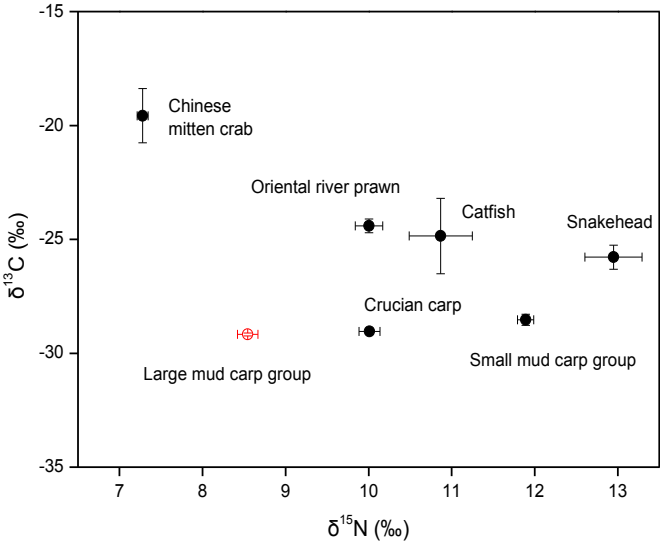

Fig. 1. Stable isotope ratios of carbon and nitrogen in muscle of aquatic organisms from the pond polluted by electronic waste in South China. The cross-hatches on the graph represents the average isotopic ratios and the standard deviation (SD).

Table 1
Biological parameters and SCCP concentrations (average ± standard deviation) in the aquatic species (ng/g lipid weight), water (ng/l), and sediments (ng/g dry weight).

Sample	N [a]	Body length (cm)	Body mass (g)	Lipid or TOC (%)	SCCPs [b]
Snakehead	5	35 ± 3.4	359 ± 32	0.74 ± 0.37	2700 ± 900
Catfish	2	39 ± 16	759 ± 822	2.8 ± 3.3	7900 ± 7500
Mud carp (Large)	5	49 ± 3.0	1818 ± 253	2.7 ± 1.2	2100 ± 160
Mud carp (Small)	5 (60)	8.2 ± 1.1	5.3 ± 1.5	3.4 ± 0.54	3400 ± 1100
Crucian carp	5 (16)	12 ± 0.58	28 ± 4.8	2.2 ± 0.29	9800 ± 8200
Oriental river prawn	5 (50)	2.0 ± 0.21	1.2 ± 0.14	1.9 ± 0.15	12,000 ± 2400
Chinese mitten crab	5 (16)	2.7 ± 1.1	2.3 ± 0.26	1.3 ± 0.26	44,000 ± 40,000
Water	3	–	–	–	61 ± 5.5
Sediment	4	–	–	15 ± 16	100,000 ± 170,000

[a] Numbers of pooling samples (individual samples).
[b] Sum of the 24 SCCP congeners analyzed ($C_{10}H_{17}Cl_5$, $C_{10}H_{16}Cl_6$, $C_{10}H_{15}Cl_7$, $C_{10}H_{14}Cl_8$, $C_{10}H_{13}Cl_9$, $C_{10}H_{12}Cl_{10}$, $C_{11}H_{19}Cl_5$, $C_{11}H_{18}Cl_6$, $C_{11}H_{17}Cl_7$, $C_{11}H_{16}Cl_8$, $C_{11}H_{15}Cl_9$, $C_{11}H_{14}Cl_{10}$, $C_{12}H_{21}Cl_5$, $C_{12}H_{20}Cl_6$, $C_{12}H_{19}Cl_7$, $C_{12}H_{18}Cl_8$, $C_{12}H_{17}Cl_9$, $C_{12}H_{16}Cl_{10}$, $C_{13}H_{23}Cl_5$, $C_{13}H_{22}Cl_6$, $C_{13}H_{21}Cl_7$, $C_{13}H_{20}Cl_8$, $C_{13}H_{19}Cl_9$, $C_{13}H_{18}Cl_{10}$).

mainly comes from the Gaobeidian sewage treatment plant (about 30% of the effluent is directly discharged into Gaobeidian Lake), the largest sewage treatment plant in Beijing (Zeng et al., 2011b). SCCP concentrations detected in fish in this study were also comparable to the SCCP concentrations obtained in marine fish in Liaodong Bay (9700–33,000 ng/g lw), China (Ma et al., 2014b). However, the levels of SCCPs in the present study were higher than those obtained from fish in Lake Ontario and Lake Michigan (4.6–34 ng/g lw) (Houde et al., 2008) and levels in the top predatory fish across Canada (12–290 ng/g lw) (Basconcillo et al., 2015). Differences in the concentration of SCCPs in different areas are associated with their production and usage. China has been the largest producer and consumer of CPs worldwide since 2007, and the extensive production and use of commercial formulations may cause elevated SCCP levels in China. This finding indicates that it is urgent for China to understand occurrences, behavior, fate, and potential risk of SCCP in the environment.

Total SCCP levels were 61 ± 5.5 ng/L in water, while in sediments, the levels ranged over several orders of magnitude (82–350,000 ng/g dry weight, dw) (Table 1). When the TOC content of sediment was measured, two sediments with extremely high SCCP levels (50,000 and 350,000 ng/g dw) also had extraordinary high TOC content (13% and 40%). These sediments were mainly composited with discarded e-waste particles, and using these data will likely result in underestimation of biota-sediment accumulation factors (BSAFs). Thus, it would be more appropriate to refer to the SCCP levels (82 and 420 ng/g dw) obtained from the other two sediment samples that had general TOC values (1.5% and 3.4%) to calculate BSAF values.

As expected, water samples contained more short carbon chain and low-chlorinated congeners, while the sediment had more long carbon and high-chlorinated congeners (Fig. S3). Because the longer carbon and higher chlorinated SCCPs with higher log K_{OW} (octanol/water partition coefficient) values are more inclined to be partitioned into the sediment than the water. The homologue profiles of SCCPs varied among aquatic organisms (Fig. 2). Crucian carp and mud carp shared similar homologue profiles with very low abundance of C_{10} homologues (<7%). In contrast, prawns and crabs exhibited similar homologue profiles to each other with relatively high abundances of C_{10}. C_{11} homologues were the most abundant chemicals in both catfish and snakehead. The homologue profile of the snakehead was almost the same as the oriental river prawn. Differences in feeding habit and habitat could contribute to these observed homologue profile differences, which will be discussed in detail in the following section 3.3–3.5.

3.3. Bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs)

BAF is calculated as the ratio of the average SCCP congener concentrations on wet weight basis in the muscle of the organism to average dissolved SCCP concentration in the water. The BSAF was defined as the ratio of the average SCCP congener lipid-normalized concentration in the organism and average organic carbon normalized concentration in the sediment. The log BAFs for snakehead, catfish, small mud carp group, and crucian carp group were 2.46, 3.19, 3.25, and 3.45, respectively, which were lower than log BAFs for PCBs (4.50, 4.78, and 4.61 for snakehead, mud carp, and crucian carp, respectively) and PBDEs (4.30, 4.53, and 4.11 for snakehead, mud carp, and crucian carp, respectively) in the same pond, indicating lower bioaccumulation potential of SCCPs than PCBs and PBDEs (Wu et al., 2008).

The correlations between log BAF and log K_{OW} of SCCPs were species specific (Table S1, Fig. 3). For the two benthopelagic omnivorous fish species (mud carp and crucian carp), in the present study, a parabolic model can be used to describe the observed relationships. This parabolic model has been identified and explained in previous studies (Bintein et al., 1993; Fisk et al., 1998; Kannan et al., 1998; Meylan et al., 1999, Wang et al., 2007; Wu et al., 2008). The break point from positive to negative correlation was typically observed to be approximately log K_{OW} 7, and the decline in BAF was generally thought to be caused by the low bioavailability of high log K_{OW} chemicals in water. In fact, the results may be different in situ accumulation of chemicals due to complex environmental conditions (e.g., dissolved organic matter). In a separate study, the influence of dissolved organic matter on the correlation between log BAF and log K_{OW} of PBDEs was analyzed for freshwater fish, and this showed that linear positive correlation remained even when log K_{OW} > 10 after correction of the effect of dissolved organic carbon (Luo et al., 2016). In addition, chemicals with different physicochemical properties may influence the curvilinear relationship. Previous research results are concentrated in PCBs and PBDEs whose physicochemical properties are different from SCCPs, but there is no systematic overall report on SCCPs. Hence, this correlation suggests that conventional bioconcentration theory, i.e. the parabolic model can be applied to explain the bioaccumulation of SCCPs in mud carp and crucian carp species.

The log BAF decreased with increasing log K_{OW} for both oriental river prawns and Chinese mitten crabs, which is in contrast to predictions based on the bioconcentration theory. Thus, absorption of SCCPs from the water might not be the main pathway whereby prawns and crabs accumulate SCCPs. An explanation for this is that prawns and crabs are benthos, i.e., they live and are active in bottom sediments, which means they are more likely to absorb

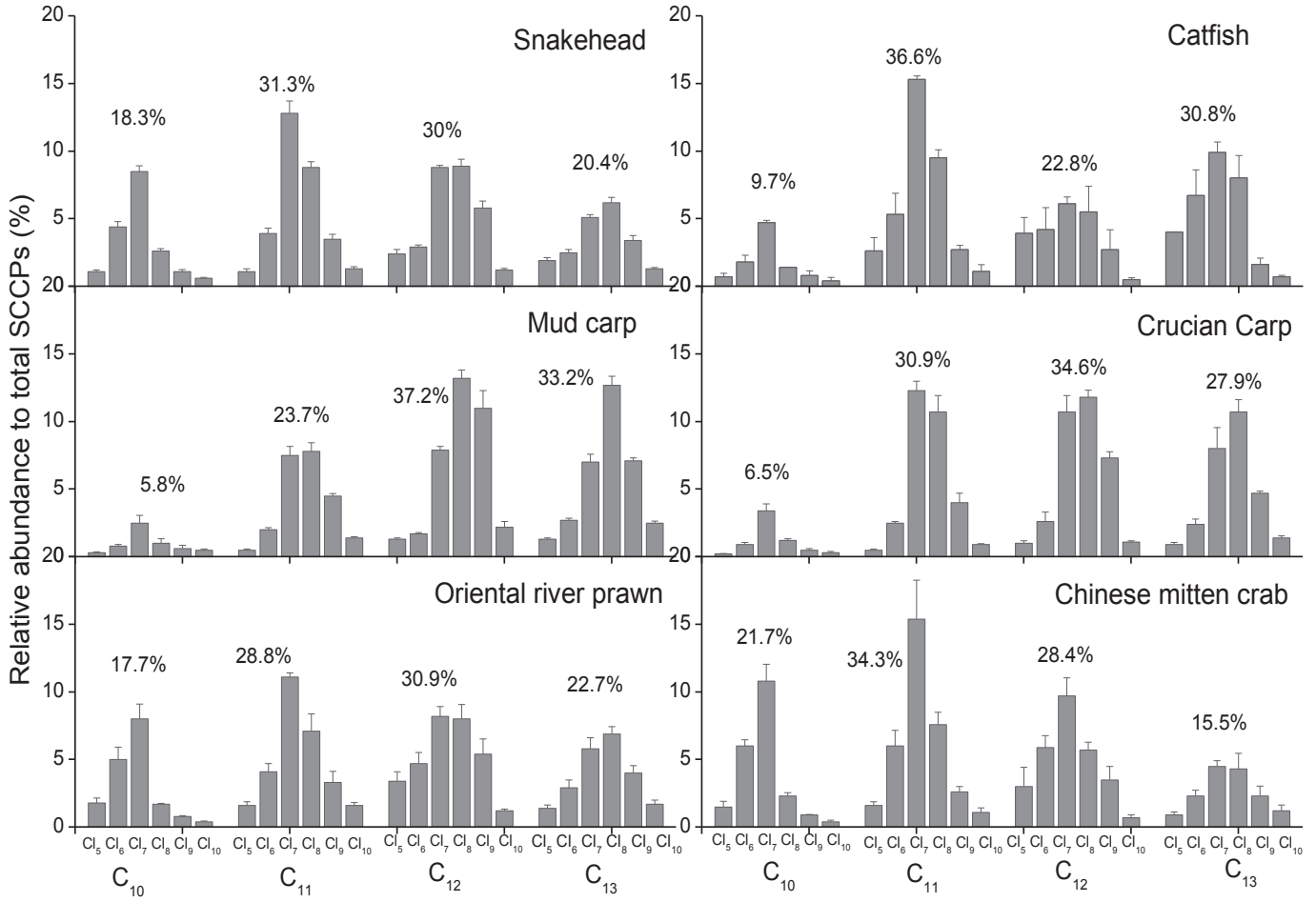

Fig. 2. Composition profiles of SCCP congeners in aquatic organisms from the pond polluted by electronic waste in South China. The error bars represent standard deviation.

contaminants from the sediment rather than from the surrounding water. Additionally, their hard exoskeletons (shells) can prevent contaminants from diffusing into their body. Thus, BAF is not a good indicator to reflect the bioaccumulation of SCCPs in these two species.

No correlations were obtained between log BAF and log K_{OW} for the two carnivorous fish species (catfish and snakehead) ($p > 0.05$, Fig. 3). These two fish species are strictly benthic fish that can rest on the sediments of the pond, and their major food sources are aquatic insects, mollusks, crustaceans, and fishes. For these species, obtaining contaminants from their diet or from the sediment may be more important than obtaining contaminants directly from the water. The homologue profiles of SCCPs in snakeheads were the same as in prawns, implying that prawns could be a major food item of snakeheads in the study pond. In addition, the similar $\delta^{13}C$ values between the snakehead and the prawn provide further evidence of the relationship between predator and prey (Fig. 1).

The BSAFs of total SCCPs varied from 0.28 in snakeheads to 4.53 in Chinese mitten crabs. Only the BSAFs in the two invertebrates (1.26 for prawns and 4.53 for crabs) were greater than 1. The BSAFs of each SCCP homologue group ranged from 0.37 to 33 for crab, 0.2 to 12 for prawn, and 0.03 to 15.2 for four fish species (Fig. 4). Generally, C_{12} and C_{13} homologues had lower BSAFs than C_{10} and C_{11} homologues, and the BSAFs significantly decreased with increasing chlorinated content in a given homologue ($p < 0.05$); indicating homologues with low log K_{OW} prefer to accumulate in organisms rather than in sediments. The main reason for this may be high log K_{OW} SCCPs having higher affinity to organic matters in sediments than to lipids in organisms. This was the opposite trend observed in BAFs, implying the bioaccumulation of chemicals from water is different than their sorption to sediment. Exponential decay with increasing log K_{OW} was obtained for BSAFs in both invertebrates (Fig. 4). Similar exponential decay correlations were also obtained in the snakehead and catfish. However, a linear decrease with increasing log K_{OW} was obtained for BSAFs in mud carp and crucian carp. The consistency in the trend of BSAFs between benthic carnivorous fish species and invertebrates confirms that the two benthic carnivorous fish species accumulate greater amounts of SCCPs using the organism-sediment system or feeding on benthic crustaceans than by means of the organism-water system or feeding on benthopelagic fish in the studied pond. Ma et al. (2014b) also reported a decreasing tendency of BSAF of individual SCCP formula groups with the increase of log K_{OW} for benthic crab and shrimp, which was obviously opposite to the trend of BAFs.

3.4. Tissue distribution

The tissue distribution of total SCCPs in two fish species (snakehead and mud carp) followed the same order when concentrations were expressed on a wet weight basis, i.e., liver > gill > kidney > skin > muscle (Table S2). The wet weight SCCP levels were shown to correlate positively with the content of lipids in tissues for both fish species ($p < 0.001$, Fig. S4). Thus, lipids play an important role in deposition of SCCPs in tissues. The same

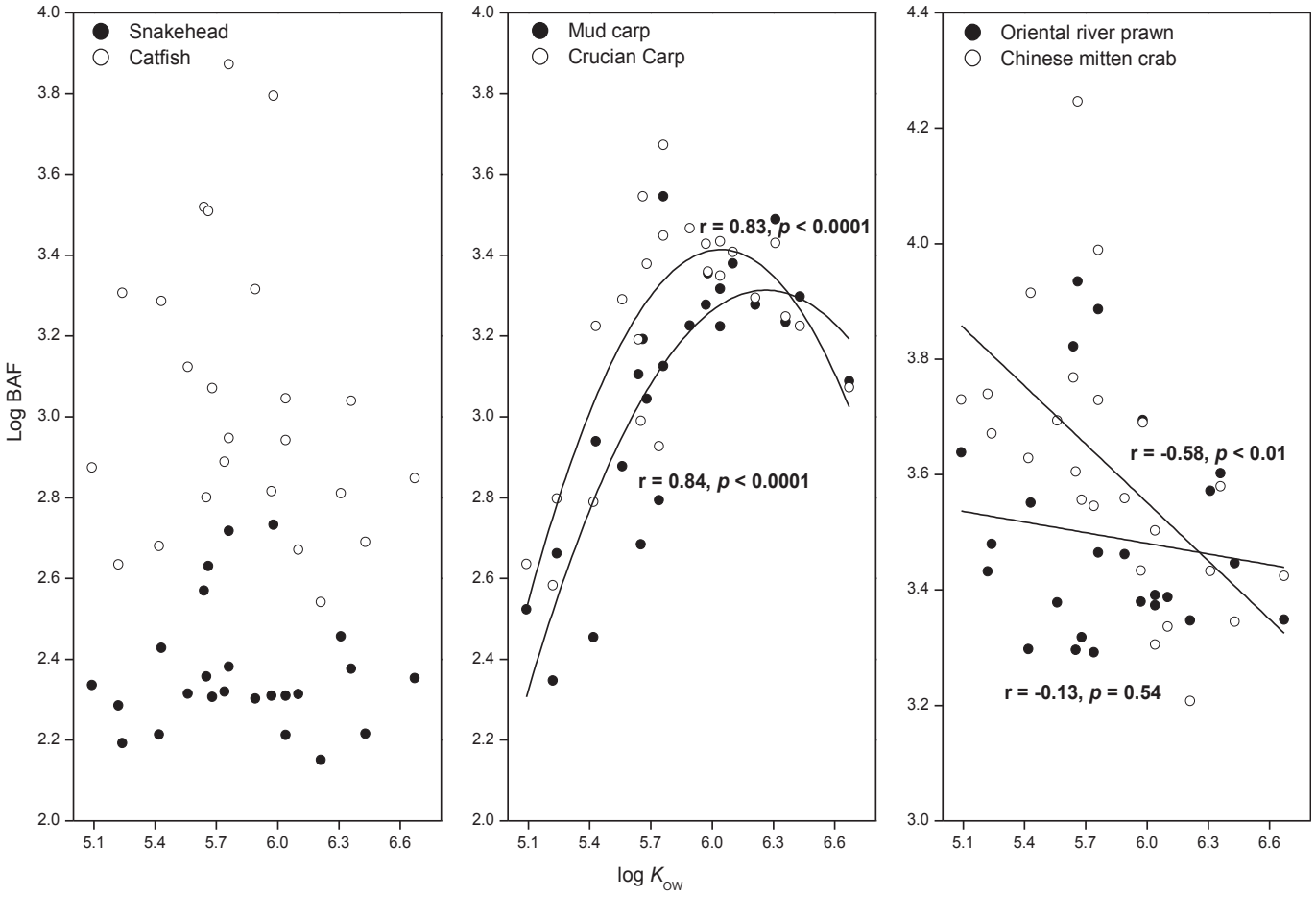

Fig. 3. Correlation between log BAF and log K_{OW} of SCCP congeners. Each point in the figure is the average value of samples.

dependence on the lipid content was also observed in tissues of chicken (Ueberschär and Matthes, 2004; Ueberschär et al., 2007). One-way analysis of variance (ANOVA) indicated that no significant differences in the total SCCP levels based on lipid weight among tissues in mud carp ($p > 0.05$) (Table S2). However, the total SCCP concentrations based on lipid weight varied largely among different tissues in snakeheads, and the descending levels followed the order of kidney, skin, gill, muscle, and liver. (Table S2). SCCP concentrations in the liver were significantly lower than those in other tissues ($p < 0.05$), except for the muscles in snakeheads, meaning other factors (e.g. bioaccumulation pathway, metabolism) besides the lipid content are affecting the SCCP deposition in different organs (Table S2).

To investigate the distribution of SCCP homologues between liver and other tissues, a ratio of lipid-normalized concentrations in other tissue (i.e. kidney, gill, skin, or muscles) apart from the liver ($C_{other}/(C_{other} + C_{Liver})$) was calculated (Fig. 5). If the ratio deviated significantly from 0.5, then this implied a significant difference between the liver and other tissues. One sample *t*-test results indicated that none of ratios of total SCCPs differed significantly from 0.5 in the mud carp ($p > 0.05$), indicating no significant differences in total SCCP loading between the liver and other tissues. Similar to the results of the one-way ANOVA, the ratios of gill to liver, kidney to liver, and skin to liver were all significantly greater than 0.5 for snakehead ($p < 0.05$), implying the concentrations were significantly lower in the liver than in these other tissues. The liver is the most important detoxification organ in the body, and the lower concentrations in the liver could be related to the metabolism of SCCPs in the liver. The metabolism of SCCPs is primarily regulated by cytochrome P-450 enzymes (Darnerud, 1984), and the metabolites in the bile have been proven to be the corresponding conjugates with *N*-acetylcysteine and glutathione (Åhlman et al., 1986). The ratios in the snakehead were all larger than those in the mud carp. This may be caused by the higher metabolism capacity of snakeheads than mud carps, owing to the former organism occupying a higher trophic level.

The ratios varied among different homologue groups, although no significant differences were shown in the ratios of total SCCPs in the liver versus other tissues in mud carps ($p > 0.05$). A simple linear regression analysis between the ratio and the physicochemical factor (such as log K_{OW}) of chemicals revealed that the ratio significantly and negatively related to the log K_{OW} for four organs (kidney, gills, muscles, and skin) in mud carps ($p < 0.05$). Similar correlations were also obtained in the kidneys and muscles for snakeheads but were not present in the gills and skin. In a previous study of the distribution of halogen organic chemicals including PBDEs, decabromodiphenyl ethane, 1,2-bis(2,4,6-tribromophenoxy) ethane, polybrominated biphenyls, and dechlorane plus between the liver and muscle in neonate chicks, the ratio of muscle to liver also showed significant negative correlation with log K_{OW} of chemicals (Zheng et al., 2014). These significant negative correlation between log K_{OW} and the ratio ($C_{other}/(C_{other} + C_{Liver})$) of chemicals could be attributed to the liver preferentially accumulating high lipophilic chemicals compared to other tissues. This correlation was obtained in the gills and skin in the mud carp but was not shown in the snakehead, which could be

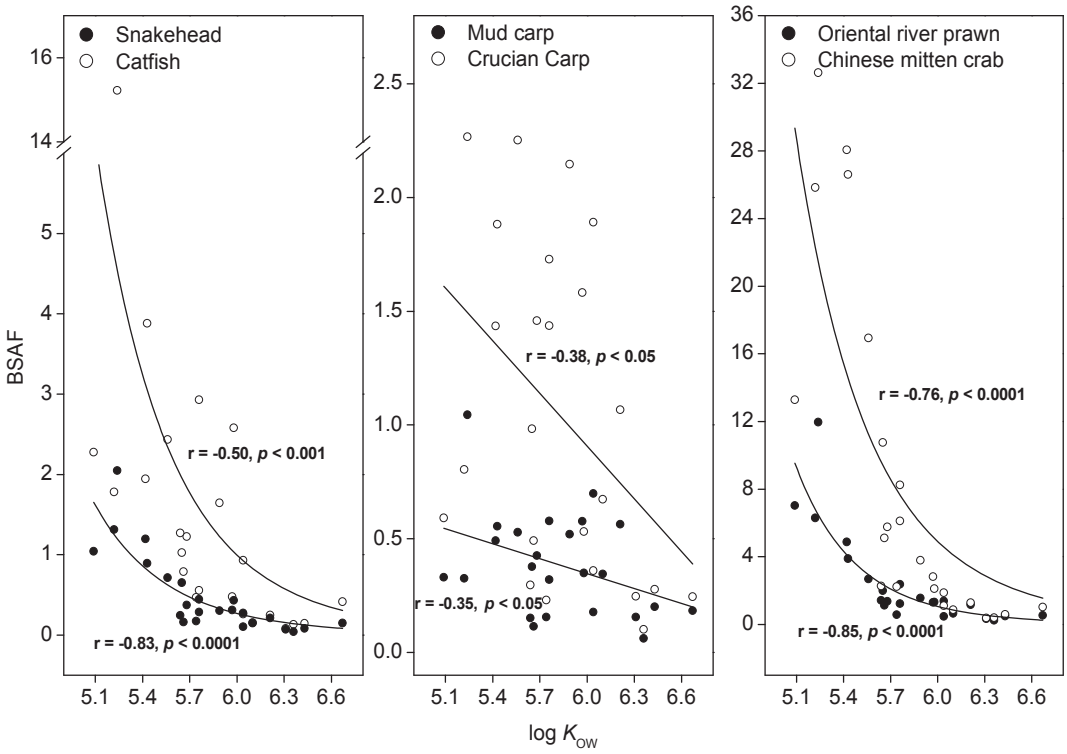

Fig. 4. Correlation between BSAF and log K_{OW} of SCCP congeners. Each point in the figure is the average value of samples.

related to differences in exposure to SCCPs between the mud carp and the snakehead. Gills and skin are two organs that have direct contact with water, and it is expected that bioconcentration plays an important role in the determination of the concentration of chemicals in these two tissues. As discussed above, absorption of SCCPs directly from the water was the main source of SCCPs in mud carp and crucian carp, with no difference seen between the inner organs or surface organs. However, SCCPs in the inner organs (muscle, liver, and kidneys) of the snakehead might derive from their diet or the sediment, while SCCPs in the surface organs (gills and skin) might come predominately from the water. Significant positive correlations between log BAF calculated based on gills and skin in the snakehead and log K_{OW} support the above hypothesis ($p < 0.01$, Fig. S5). The bioaccumulation potential by an organism-water system was positively related to log K_{OW}, which could offset the negative correlation between the ratio of gills or skin to liver and log K_{OW} in the snakehead. This result indicates that the source of chemicals in different tissues or organs for aquatic organisms might differ, especially for high trophic level organisms where obtaining chemicals from diet could play more of an important role than in lower level organisms.

3.5. Trophic transfer

The concentrations of SCCPs in the muscles of fish were used to calculate the TMFs, taking into consideration that the chemicals deposited in this organ accounted for the largest proportion of chemicals in the body. TMFs are defined as the antilog of the slope with base e ($TMF = e^{slope}$) of the linear regression of natural logarithm SCCP lipid normalized concentration versus trophic level of freshwater species (Wu et al., 2009), and the calculation of TMF in detail is given in the SI.

As shown in Fig. 6, a significant linear negative correlation ($p < 0.001$) between the trophic level (TL) and logarithmic transformed total SCCP concentration was observed, and the TMF value was calculated to be 0.17. This result indicates that SCCPs underwent trophic dilution rather than trophic magnification in this aquatic food web, which is completely different from other halogen organic contaminants (such as PCBs and PBDEs). Trophic magnification (TMF > 1) for PCBs, PBDEs, and dechlorane plus has been reported in aquatic food webs collected in the same pond in previous studies (Wu et al., 2009, 2010b). However, it is unclear what caused the differences among different chemicals.

In previous studies, trophic magnification of SCCPs has been observed in aquatic food webs. For example, the TMF for a zooplankton-shrimp-fish food web in Liaodong Bay, north China was 2.38 (Ma et al., 2014b), and for an aquatic food chain sampled in Gaobeidian Lake in northern China was 1.6 (Zeng et al., 2011b). Trophic magnification (TMF = 1.2) was also discovered in the food web in Lake Michigan (Houde et al., 2008). The explanation for the difference observed between the present study and previous studies is complex. The structure of food chains, size of organisms collected, food habits, biotransformation of SCCPs in organisms, treatment method for any outliers, and even environmental parameters such as water temperature, dissolved organic matter, and suspended particles might all contribute to the differences obtained. In the study of trophic transfer of SCCP in Liaodong Bay, Ma et al. (2014b) excluded shelled benthic species when conducting their regression analysis, because the bivalve species acutely distorted the general relationship. In fact, these bivalves have lower trophic levels but higher SCCP concentrations and a negative correlation can be observed among these species. Zeng et al. (2011b) collected several similar fish species in their study of SCCP trophic transfer in the aquatic food chain from Gaobeidian Lake as those used in the present study (catfish and crucian carp). The catfish showed lower SCCP concentration than the crucian carp in the present study and the study of Zeng et al. (2011b). In contrast, the catfish exhibited higher trophic level than did crucian carp in

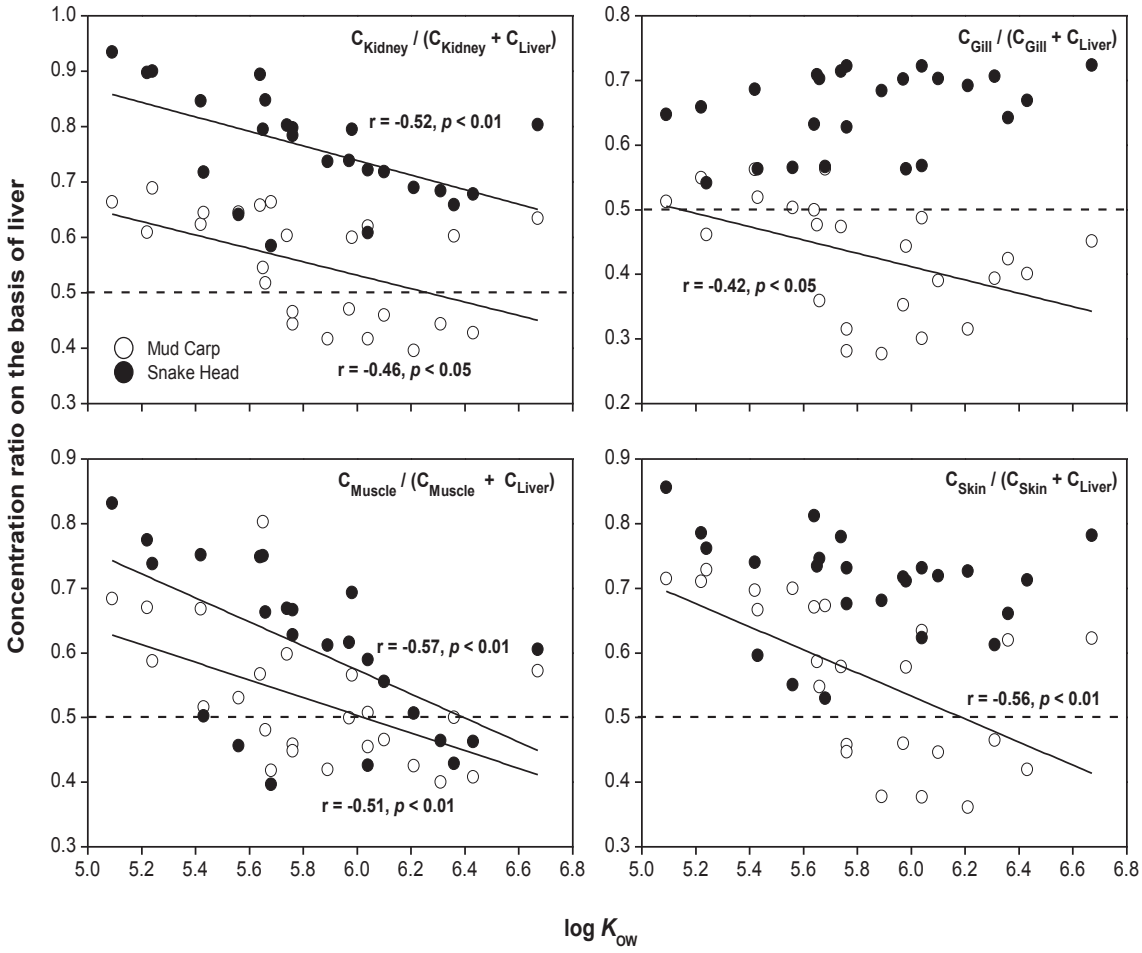

Fig. 5. Correlation between ratio of other tissue to liver and log K_{OW}. Each point in the figure is the average value of samples.

our study, which is inconsistent with Zeng et al. (2011b). As shown in the present study, the size of fish can affect both trophic levels and SCCP levels. Thus, the size of the individual can also be a confounding factor that affects the calculated TMF. TMF less than 1 was also observed in the Lake Ontario food web, because the lake trout have lower SCCP concentrations than several of their prey on a lipid basis, and metabolism of SCCPs in lake trout was suggested as a possible reason for their low SCCP concentrations (Houde et al., 2008). As mentioned above, snakeheads had a higher metabolism potential for SCCPs than did mud carps. Thus, the difference in metabolism of SCCPs in different species can also result in the trophic dilutions observed in the present study. Additionally, a real equilibrium may not reach in the pond ecosystem, considering the existence of a continuous source of SCCPs (i.e. discarded e-wastes containing SCCPs stacked at the bottom of the pond). This may lead to underestimation of TMFs in the investigated food web. Further studies are required to evaluate the biomagnification of SCCPs.

The TMFs for each SCCP formula group were also calculated to investigate congener specific biomagnification (Fig. S6). Significant negative correlations were obtained between the trophic levels and logarithmic transformed SCCP concentrations for all SCCP formula groups ($p < 0.05$), except for $C_{10}H_{17}Cl_5$, $C_{12}H_{21}Cl_5$, $C_{13}H_{13}Cl_5$, $C_{12}H_{16}Cl_{10}$, and $C_{13}H_{19}Cl_9$ (Table S3). A significant positive correlation between TMF and log K_{OW} was observed (Fig. S4), which is consistent with the study by Ma et al. (2014b). Additionally, a decreasing trend was obtained when log $K_{OW} > 6.3$, signifying that medium-chain chlorinated paraffins have no biomagnification

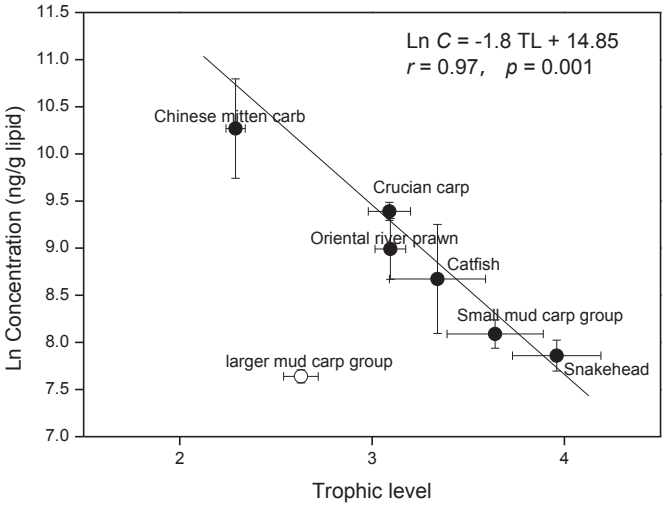

Fig. 6. Correlation between concentration (Ln transformed) and trophic level in organisms. The cross-hatches on the graph represents the average values and the standard deviation (SD). This negative correlation indicates that TMF value was below 1 and trophic dilution.

potential in aquatic food webs, because MCCPs exhibit higher log K_{OW} than do SCCPs. This result is similar to field observations of the trophic transfer of medium-chain chlorinated paraffins in aquatic

food webs (Houde et al., 2008; Thompson and Vaughan, 2014).

4. Conclusions

Our results demonstrate that SCCPs extensively accumulate in aquatic organisms (fish, crab, and shrimp) in a pond contaminated by e-waste, and the bioaccumulation of SCCPs exhibit species-specific profiles among the investigated aquatic species. SCCP congeners with higher log K_{OW} were preferentially distributed in the liver of the fish species, and the pathway (i.e. water or sediment) of bioaccumulation can affect the tissue distribution of SCCP congeners. TMF indicated that trophic dilutions of SCCPs occurred in the studied aquatic food webs. Further research is needed on the biotransformation and trophic transfer of SCCPs in ecosystems.

Acknowledgments

This work was jointly supported by the National Basic Research Program of China (2015CB453102), the National Nature Science Foundation of China (Nos. 41673100, 41273118, 41473102, and 41230639), the National Postdoctoral Program for Innovative Talents of China (BX201600166), and the Science and Technology Project of Guangdong Province, China (2014B030301060). This is contribution No. IS-2327 and SKLOGA201602 from GIGCAS.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.12.060.

References

Åhlman, M., Bergman, Å., Darnerud, P., Egestad, B., Sjövall, J., 1986. Chlorinated paraffins: formation of sulphur-containing metabolites of polychlorohexadecane in rats. Xenobiotica 16 (3), 225–232.
Basconcillo, L.S., Backus, S.M., McGoldrick, D.J., Zaruk, D., Sverko, E., Muir, D.C., 2015. Current status of short-and medium chain polychlorinated n-alkanes in top predatory fish across Canada. Chemosphere 127, 93–100.
Bintein, S., Devillers, J., Karcher, W., 1993. Nonlinear dependence of fish bioconcentration on n-octanol/water partition coefficient. Sar. QSAR. Environ. Res. 1 (1), 29–39.
Bytingsvik, J., 2015. Literature Survey of Emerging Contaminants in the Barents Sea and Climate Change Effects on Contaminant Fate, Akvaplanniva AS (Tromsø, Norway).
Chen, M.Y., Luo, X.J., Zhang, X.L., He, M.J., Chen, S.J., Mai, B.X., 2011. Chlorinated paraffins in sediments from the Pearl River Delta, South China: spatial and temporal distributions and implication for processes. Environ. Sci. Technol. 45 (23), 9936–9943.
Darnerud, P.O., 1984. Chlorinated paraffins: effect of some microsomal enzyme inducers and inhibitors on the degradation of 1-^{14}C-Chlorododecanes to $^{14}CO_2$ in mice. Acta. Pharmacol. Toxicol. 55 (2), 110–115.
Fiedler, H., 2010. Chlorinated Paraffins. Springer, pp. 1–40.
Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C., 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ. Toxicol. Chem. 17 (5), 951–961.
Geng, N., Zhang, H., Zhang, B., Wu, P., Wang, F., Yu, Z., Chen, J., 2015. Effects of short-chain chlorinated paraffins exposure on the viability and metabolism of human hepatoma HepG2 cells. Environ. Sci. Technol. 49 (5), 3076–3083.
Herzke, D., 2013. Perfluorinated Alkylated Substances, Brominated Flame Retardants and Chlorinated Paraffins in the Norwegian Environment - Screening 2013.
Houde, M., Muir, D.C., Tomy, G.T., Whittle, D.M., Teixeira, C., Moore, S., 2008. Bioaccumulation and trophic magnification of short-and medium-chain chlorinated paraffins in food webs from Lake Ontario and Lake Michigan. Environ. Sci. Technol. 42 (10), 3893–3899.
Huang, Y., Chen, L., Feng, Y., Ye, Z., He, Q., Feng, Q., Qing, X., Liu, M., Gao, B., 2016. Short-chain chlorinated paraffins in the soils of two different Chinese cities: occurrence, homologue patterns and vertical migration. Sci. Total Environ. 557–558, 644–651.
Kannan, K., Nakata, H., Stafford, R., Masson, G.R., Tanabe, S., Giesy, J.P., 1998. Bioaccumulation and toxic potential of extremely hydrophobic polychlorinated biphenyl congeners in biota collected at a superfund site contaminated with Aroclor 1268. Environ. Sci. Technol. 32 (9), 1214–1221.
Li, X., Gao, Y., Wang, Y., Pan, Y., 2014. Emerging persistent organic pollutants in Chinese Bohai Sea and its coastal regions. Sci. World J. 2014 (1), 149–168.
Luo, X.J., He, M.J., Zeng, Y.H., Wu, J.P., Chen, S.J., Mai, B.X., 2016. Influence of dissolved organic matter on bioaccumulation factor: a case study of bioaccumulation of polybrominated diphenyl ethers (PBDEs) in fish from Dongjiang River. Asian Journal of Ecotoxicology. Asian J. Ecotoxicol. 11 (2), 188–193.
Luo, X.J., Sun, Y.X., Wu, J.P., Chen, S.J., Mai, B.X., 2015. Short-chain chlorinated paraffins in terrestrial bird species inhabiting an e-waste recycling site in South China. Environ. Pollut. 198, 41–46.
Ma, X., Chen, C., Zhang, H., Gao, Y., Wang, Z., Yao, Z., Chen, J., Chen, J., 2014a. Congener-specific distribution and bioaccumulation of short-chain chlorinated paraffins in sediments and bivalves of the Bohai Sea, China. Mar. Pollut. Bull. 79 (1), 299–304.
Ma, X., Zhang, H., Wang, Z., Yao, Z., Chen, J., Chen, J., 2014b. Bioaccumulation and trophic transfer of short chain chlorinated paraffins in a marine food web from the liaodong bay, North China. Environ. Sci. Technol. 48, 5964–5971.
Ma, X., Zhang, H., Zhou, H., Na, G., Wang, Z., Chen, C., Chen, J., Chen, J., 2014c. Occurrence and gas/particle partitioning of short-and medium-chain chlorinated paraffins in the atmosphere of Fildes Peninsula of Antarctica. Atmos. Environ. 90, 10–15.
Meylan, W.M., Howard, P.H., Boethling, R.S., Aronson, D., Printup, H., Gouchie, S., 1999. Improved method for estimating bioconcentration/bioccumulation factor from octanol/water partition coefficient. Environ. Toxicol. Chem. 18 (4), 664–672.
Reth, M., Zencak, Z., Oehme, M., 2005. New quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionization mass spectrometry. J. Chromatogr. A 1081 (2), 225–231.
Strid, A., Bruhn, C., Sverko, E., Svavarsson, J., Tomy, G., Bergman, Å., 2013. Brominated and chlorinated flame retardants in liver of Greenland shark (Somniosus microcephalus). Chemosphere 91 (2), 222–228.
Sun, R., Luo, X., Tang, B., Li, Z., Huang, L., Wang, T., Mai, B., 2016. Short-chain chlorinated paraffins in marine organisms from the Pearl River Estuary in South China: residue levels and interspecies differences. Sci. Total Environ. 553, 196–203.
Sverko, E., Tomy, G.T., Märvin, C.H., Muir, D.C., 2012. Improving the quality of environmental measurements on short chain chlorinated paraffins to support global regulatory efforts. Environ. Sci. Technol. 46 (9), 4697–4698.
Tan, X.X., Luo, X.J., Zheng, X.B., Li, Z.R., Sun, R.X., Mai, B.X., 2016. Distribution of organophosphorus flame retardants in sediments from the Pearl River delta in South China. Sci. Total Environ. 544, 77–84.
Thompson, R., Vaughan, M., 2014. Medium-chain chlorinated paraffins (MCCPs): a review of bioaccumulation potential in the aquatic environment. Integr. Environ. Assess. Manage 10 (1), 78–86.
Tomy, G.T., Stern, G.A., Muir, D.C., Fisk, A.T., Cymbalisty, C.D., Westmore, J.B., 1997. Quantifying C10-C13 polychloroalkanes in environmental samples by high-resolution gas chromatography/electron capture negative ion high-resolution mass spectrometry. Anal. Chem. 69 (14), 2762–2771.
Ueberschär, K.H., Matthes, S., 2004. Dose–response feeding study of chlorinated paraffins in broiler chickens: effects on growth rate and tissue distribution. Food Addit. Contam. 21 (10), 943–948.
Ueberschär, K.H., Dänicke, S., Matthes, S., 2007. Dose–response feeding study of short chain chlorinated paraffins (SCCPs) in laying hens: effects on laying performance and tissue distribution, accumulation and elimination kinetics. Mol. Nutr. Food Res. 51 (2), 248–254.
UNEP (2015a) Report of the Persistent Organic Pollutants Review Committee UNEP/POPS/POPRC. 11/10. United Nations Environmental Programme Stockholm Convention on Persistent Organic Pollutants, Rome.
UNEP (2015b) Short-chained chlorinated paraffins: risk profile UNEP/POPS/POPRC.11/10/Add.2. United Nations Stockholm Convention on Persistent Organic Pollutants, Rome.
van Mourik, L.M., Gaus, C., Leonards, P.E., de Boer, J., 2016. Chlorinated paraffins in the environment: a review on their production, fate, levels and trends between 2010 and 2015. Chemosphere 155, 415–428.
Wang, Y., Li, J., Cheng, Z., Li, Q., Pan, X., Zhang, R., Liu, D., Luo, C., Liu, X., Katsoyiannis, A., 2013. Short-and medium-chain chlorinated paraffins in air and soil of subtropical terrestrial environment in the Pearl River Delta, South China: distribution, composition, atmospheric deposition fluxes, and environmental fate. Environ. Sci. Technol. 47 (6), 2679–2687.
Wang, Y., Li, X., Li, A., Wang, T., Zhang, Q., Wang, P., Fu, J., Jiang, G., 2007. Effect of municipal sewage treatment plant effluent on bioaccumulation of polychlorinated biphenyls and polybrominated diphenyl ethers in the recipient water. Environ. Sci. Technol. 41 (17), 6026–6032.
WCC, 2014. World Chlorine Council. International Chlorinated Alkanes Industry Association (ICAIA) newsletter.
Wu, J.P., Guan, Y.T., Zhang, Y., Luo, X.J., Zhi, H., Chen, S.J., Mai, B.X., 2010a. Trophodynamics of hexabromocyclododecanes and several other non-PBDE brominated flame retardants in a freshwater food web. Environ. Sci. Technol. 44 (14), 5490–5495.
Wu, J.P., Luo, X.J., Zhang, Y., Luo, Y., Chen, S.J., Mai, B.X., Yang, Z.Y., 2008. Bioaccumulation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in wild aquatic species from an electronic waste (e-waste) recycling site in South China. Environ. Int. 34 (8), 1109–1113.
Wu, J.P., Luo, X.J., Zhang, Y., Yu, M., Chen, S.J., Mai, B.X., Yang, Z.Y., 2009. Biomagnification of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls in a highly contaminated freshwater food web from South China.

Environ. Pollut. 157 (3), 904–909.
Wu, J.P., Zhang, Y., Luo, X.J., Wang, J., Chen, S.J., Guan, Y.T., Mai, B.X., 2010b. Isomer-specific bioaccumulation and trophic transfer of Dechlorane Plus in the freshwater food web from a highly contaminated site, South China. Environ. Sci. Technol. 44 (2), 606–611.
Yuan, B., Wang, T., Zhu, N., Zhang, K., Zeng, L., Fu, J., Wang, Y., Jiang, G., 2012. Short chain chlorinated paraffins in mollusks from coastal waters in the Chinese Bohai Sea. Environ. Sci. Technol. 46 (12), 6489–6496.
Zeng, L., Wang, T., Han, W., Yuan, B., Liu, Q., Wang, Y., Jiang, G., 2011a. Spatial and vertical distribution of short chain chlorinated paraffins in soils from wastewater irrigated farmlands. Environ. Sci. Technol. 45 (6), 2100–2106.
Zeng, L., Wang, T., Wang, P., Liu, Q., Han, S., Yuan, B., Zhu, N., Wang, Y., Jiang, G., 2011b. Distribution and trophic transfer of short-chain chlorinated paraffins in an aquatic ecosystem receiving effluents from a sewage treatment plant. Environ. Sci. Technol. 45 (13), 5529–5535.
Zhang, Q., Wang, J., Zhu, J., Liu, J., Zhang, J., Zhao, M., 2016. Assessment of the endocrine-disrupting effects of short-chain chlorinated paraffins in in vitro models. Environ. Int. 94, 43–50.
Zheng, X.B., Luo, X.J., Zeng, Y.H., Wu, J.P., Chen, S.J., Mai, B.X., 2014. Halogenated flame retardants during egg formation and chicken embryo development: maternal transfer, possible biotransformation, and tissue distribution. Environ. Toxicol. Chem. 33 (8), 1712–1719.