Short Chain Chlorinated Paraffins in Mollusks from Coastal Waters in the Chinese Bohai Sea

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ABSTRACT: As an extremely complex group of persistent organic pollutants (POPs) candidates in the Stockholm Convention, short chain chlorinated paraffins (SCCPs) have been of extensive concern in recent years. In this study, nine bivalve and two gastropod species were collected in 2009 to evaluate the spatial distributions and potential factors influencing the bioaccumulation of SCCPs in mollusks in the Chinese Bohai Sea. The concentrations of ΣSCCPs in the mollusks were in the range 64.9−5510 ng/g (dry weight) with an average chlorine content of 61.1%. C10 and C11 were the predominant homologue groups of SCCPs, which accounted for about 29.7% and 34.9% of ΣSCCPs, respectively. Six and seven chlorinated substituents were the main congener groups. Mya arenaria (Mya), Macrura veneriformis (Mac), and Crassostrea talienwhanensis (Oyster, Ost) had higher average concentrations of SCCPs than other species, implying that these bivalves could be used as sentinels to indicate SCCPs contamination in this coastal region. A significant positive linear relationship was found between SCCP concentrations and lipid content of the mollusks, whereas the lipid-normalized SCCP concentrations were negatively linear-related to the trophic levels (TL), which implied that SCCPs did not show biomagnification in mollusks in this region.

INTRODUCTION

Chlorinated paraffins (CPs) belong to a class of chlorinated derivatives of n-alkanes containing 10−30 carbon atoms and are extensively used in metal-working fluids, paints, sealants, leather treatment chemicals, flame retardants, plasticizers in rubbers, and polymers such as polyvinylchloride (PVC).1 CPs are classified according to the carbon chain length into short chain CPs (C10−C13, SCCPs), medium chain CPs (C14−C17, MCCPs), and long chain CPs (C18−C30, LCCPs)2,3 or categorized based on the chlorination degree such as 40−50%, 50−60%, and 60−70%.4 SCCPs are particularly of concern due to their higher bioaccumulation potential and greater toxicity to aquatic animals and mammals than the other CP groups.4 The presence of SCCPs has been reported in various environmental matrices from different countries and regions such as air,5,6 water,7,8 sediment,9,10 soils,11,12 and biota.13−15 The Persistent Organic Pollutants (POPs) Review Committee of the Stockholm Convention (SC) has listed SCCPs as a POPs candidate and evaluated SCCPs against the criteria of Annex E in 2008.16

CPs production in China began at the end of 1950s and has grown rapidly in recent years. Most of the manufacturing plants are distributed around the central and northeastern parts of China. The distribution and contamination characteristics of SCCPs in the Chinese environment are very scarce although China is the largest CPs producer in the world.17 Our recent study found that high concentrations of SCCPs were present in soil samples collected from an e-waste dismantling area in China.18 Another study on the trophic transfer of SCCPs in an aquatic ecosystem near a municipal sewage treatment plant revealed that SCCPs can be bioaccumulated/biomagnified in the aquatic food chain.19

Mollusks have long been used to elucidate the distribution of contaminants such as heavy metals, organometallic compounds, and POPs because of their abundance in marine ecosystems and high accumulation capacity of some pollutants.20,21 In the Asia−Pacific Mussel Watch Program (APMW), bivalves were used to indicate POPs pollutions in the marine environment, due to their slow elimination of persistent pollutants, unique sessile lifestyle, and that they are water-respiring filter feeders.22

As one of the sampling regions of APMW, the Chinese Bohai Sea Rim economic zone shares a quarter of the total gross domestic product in China, while the ecosystem has also been seriously polluted with the rapid economic development. Mollusks collected from this region have previously been used as potential bioindicators/biomonitors of various POPs.23−25 In our previous work, we found that Mytilus edulis (Blue mussel, Myt) and Crassostrea talienwhanensis (Oyster, Ost) could be selected as useful bioindicators to evaluate the pollution of organochlorine pesticides (OCPs), polychlorinated...
biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in the Chinese Bohai Sea.26

In the present work, we provide a comprehensive investigation on the concentrations of SCCPs in different mollusk species collected from coastal waters of the Chinese Bohai Sea in 2009. The aims are to evaluate concentrations and distribution trends of SCCPs in mollusks and to evaluate whether mollusks are suitable as a biological indicator species of SCCPs in this region. Congener group patterns and possible factors influencing the bioaccumulation of SCCPs in mollusks are also discussed.

■ MATERIALS AND METHODS

Sampling. For each sample and sampling site, about 300−500 g of wet mollusk muscles were composited into one sample. A total of 91 composite mollusk samples were collected from nine coastal cities along the Bohai Sea, including Beidaihe (BDH), Dalian (DL), Huludao (HLD), Penglai (PL), Shouguang (SG), Tianjin (TJ), Weihai (WH), Yantai (YT), and Yingkou (YK) in August, 2009 (Figure 1). Nine of the species were bivalves, including Amusium (Amu), Chlamys Farreri (Chl), Cyclina sinensis (Cyc), Crassostrea talienwhanensis (Oyster, Ost), Mactra veneriformis (Mac), Meretrix meretrix (Mer), Mya arenaria (Mya), Mytilus edulis (Blue mussel, Myt), and Scapharca subcrenata (Sca). The other two species were gastropods, Neverita didyma (Nev) and Rapana venosa (Rap).

Sample Pretreatment. Pesticide grade acetone, cyclohexane, dichloromethane (DCM), n-hexane (n-Hex), and toluene were purchased from Fisher (Hampton, NH). Silica gel (180−280 mesh) and Florisil (60−100 mesh) were obtained from Merck (Whitehouse Station, NJ). Silica gel was activated at 550 °C for 12 h, Florisil at 140 °C overnight, and anhydrous sodium sulfate at 660 °C for 6 h before use. Reference SCCPs (chlorine contents of 51.5%, 55.5%, and 63.0%) and MCCPs (chlorine contents of 42.0%, 52.0%, and 57.0%) with concentrations of 100 ng/μL were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 13C10-trans-chlordane (100 ng/μL solution in n-nonane, purity 99%) was purchased from Cambridge Isotope Laboratories (Andover, MA).

An amount of 0.5−1.0 g of freeze-dried and homogenized mollusk sample was mixed with 15 g of anhydrous Na2SO4 spiked with 1 ng of 13C10-trans-chlordane, and extracted by dichloromethane and n-hexane (1:1) using a Dionex 350 accelerated solvent extractor (150 °C and 1500 psi). The thermal equilibration time was 10 min, and the static extractions were performed within three cycles (8 min/cycle). The cell was purged with gaseous nitrogen for 100 s. After extraction, about 8 g of acid silica gel (44% concentrated sulfuric acid, w/w) was added to the extract to remove lipid and other interferences and then filtered through approximately 5 g of anhydrous sodium sulfate. The extract was then cleaned by a multilayered column consisting, from bottom to top, of 3 g of activated Florisil, 2 g of activated silica gel, 5 g of acid silica gel (30%, w/w), and 4 g of anhydrous sodium sulfate. The detailed cleanup procedures can be found in our previous published work.18

Instrumental Analysis. The instrument analysis was performed on a 7000A triple quadrupole mass spectrometer combined with a 7890A GC in ECNI mode (Agilent, CA). Samples were injected by a 7683B series injector into a DB-5MS column (30 m length, 0.25 mm i.d., 0.25 μm film thickness; Agilent, CA). Instrumental parameters and the monitored fragmentation ions of the GC-ECNI-LRMS method have been described in our previous work.12 The initial oven temperature was isothermal at 100 °C and held for 1 min, increased at 30 °C/min to 160 °C for 5 min, then increased to 310 °C at 30 °C/min and held for 22 min. Helium was the carrier gas at a constant flow rate of 2.00 mL/min, and methane was used as the moderating gas at a constant flow rate of 0.40 mL/min. The injector temperature was 280 °C. The ion source and transfer line temperatures were 200 and 280 °C, respectively. The target SCCP congener groups included...
chlorine numbers from 5 to 10, and a total number of 24 congener groups were monitored. The two most abundant isotopes of the \( [M - Cl]^{+} \) ion were selected for the quantitative and qualitative ions.

In order to minimize the possible interferences of MCCPs in environmental samples, the retention time range of each SCCP or MCCP congener group was carefully determined. The integration range of the quantitative ion was set by the comparison of the peak cluster shape between quantitative ion and qualitative ion and must be in the range of the corresponding retention time of the standards. The ratios of the quantitative ion and qualitative ion were calculated based on the chromatographic and mass spectrometric results of SCCP and MCCP standards and then corrected for in the samples. The ratios of each pair of monitored ions were compared between the standards and real samples. If the ratio in the sample fell into the range of the MCCP standard, the end point of the integration range would be modified according to the starting retention time of the corresponding MCCP congener.

**Stable Nitrogen Isotope Analysis and Trophic Level Calculations.** \( \delta^{15}\text{N} \) was determined in mollusk (dry samples) using a Thermo DELTA V Advantage isotope ratio mass spectrometer interfaced to a Flash EA1112 HT elemental analyzer (Thermo Fisher, Waltham, MA), and atmospheric nitrogen was used as a standard. The delta (\( \delta \)) unit is given in parts per thousand according to

\[
\delta^{15}\text{N} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\% 
\]

where \( R \) is the corresponding ratio \( ^{15}\text{N}/^{14}\text{N} \) and the analytical precision was \( \pm 0.2\% \).

Based on the measured nitrogen isotope ratios in mollusks, TLs of mollusks in this study were calculated to indicate the trophic position of the species using the following formula:

\[
\text{TL} = \left( \delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{zooplankton}} \right) / 3.8 + 2
\]

**Quality Assurance/Quality Control.** All glassware was thoroughly rinsed with dichloromethane and heated at 400 °C overnight before use. The ASE cell was ultrasonicated twice and rinsed with dichloromethane twice by the ASE instrument at 150 °C prior to use. Method blank samples were included in each batch of eight samples to monitor potential contamination. In general, levels of SCCPs in blanks were close to or below the limit of detection (LOD), which was estimated at 0.1 ng. The recovery of \( ^{13}\text{C}_{10} \)-trans-chlordane was between 88% and 101%.

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### RESULTS AND DISCUSSION

**Comparisons among Sampling Sites.** Table 1 summarizes the concentrations of total SCCPs (\( \sum \text{SCCPs} \)) based on dry weight (d.w.) in mollusks. The concentration of \( \sum \text{SCCPs} \) in the mollusks ranged from 64.9 (Sca, YK) to 5510 ng/g (Mya, TJ) with an average of 1410 ng/g. The average converted wet weight concentration (201 ng/g) was lower than 1205 ng/g (wet weight) found in zebra mussels reported by Tomy et al. Figure 1 shows SCCP concentrations (ng/g d.w.) in mollusks from different sampling sites. In Tianjin (TJ), the largest city in the Bohai Sea region and an important chemical base in China, the collected mollusks showed the highest \( \sum \text{SCCPs} \), with an average value of 2830 ng/g d.w. \( \sum \text{SCCPs} \) in mollusks from Yingkou (YK) and Penglai (PL) were the lowest with average concentrations of 293 and 708 ng/g d.w., respectively.

Figure 2 illustrates the carbon chain length and chlorine number patterns in mollusk samples from the sampling locations. Overall, contributions from \( C_{10} \) and \( C_{11} \) congeners were relatively high in mollusks of the Chinese Bohai Sea, which accounted for 29.7% and 34.9% of \( \sum \text{SCCPs} \), respectively. Most congers were \( C_{6} \) and \( C_{7} \) substituents, which accounted for about 43.7% and 33.5% of \( \sum \text{SCCPs} \), respectively. The SCCP congener group abundance patterns in mollusk samples were consistent with those reported for sediments in the same region.

Figure 2 also illustrates the percent relative contributions of SCCP homologues in regards to individual carbon chain groups and chlorine number groups. Samples from YK and PL exhibited the lowest concentrations of \( \sum \text{SCCPs} \), and the percent relative contributions of \( C_{15} \) congeners were significantly lower than those in other locations, which might be due to the relatively lower amount of the usage of MCCPs commercial products in these sampling areas.

The detection frequency (Supporting Information, Table S1-1) of all congeners with 6–8 chlorine atoms (\( C_{n}C_{l}\text{Cl}_{x}x = 10–13 \) ) were above 70% in the samples, while the others (\( C_{12}C_{l}\text{Cl}_{x} \) ) were below 50%. \( C_{10}C_{12}\text{Cl}_{6} \) and \( C_{11}C_{12}\text{Cl}_{7} \) could be detected in over 90% of the samples and accounted for 14.5%, 13.5%, and 10.4% of \( \sum \text{SCCPs} \), respectively. Among all samples, \( C_{13}H_{25}\text{Cl}_{6} \) was only found in one sample from TJ (Mac), and \( C_{12}H_{25}\text{Cl}_{3} \) was found only in Ost from PL and Sca from WH.

**Comparisons among Mollusks Species.** It was interesting to find that bivalves generally had higher concentrations of SCCPs than gastropods in most of the sampling sites although they are positioned lower in the food chain than gastropods. The average concentrations of SCCPs in Mya was 3100 ng/g.
between 25th and 75th percentiles. The whiskers extending from the box show the median value, and the lower and upper edges of the box mark the 25th and 75th percentiles. The horizontal black line in the box represents the average concentration of SCCPs in mollusks (Supporting Information, Figure SI-1A). The average percentage of C12 (18.2%) and C13 congeners (18.6%) in bivalves (Figure 3A) were higher than those in bivalves.

An exception among the bivalve samples was Mya (Figure 3D), where Cl8 congeners were the second most abundant chlorine homologue and the percentage exceeded that of the Cl6 group. The taxonomic order Pterioidea, including Amu and Chl, had the second lowest levels of \( \sum \) SCCPs and showed different SCCPs homologue patterns compared to other bivalve species and gastropods. No congeners with Cl10 were detected in Chl (Figure 3C) and were only detected in a few samples of Amu. C10H16Cl6 and C11H18Cl6 were the main congener groups in Amu and Chl, which was similar to Rap and Nev. The characteristic SCCP patterns in these species might indicate their different bioaccumulation and elimination potential of SCCPs.

Relevance of Lipid Content. SCCPs are hydrophobic and tend to be accumulated in lipids. Previous works reported that the octanol–water partition coefficient (log \( K_{ow} \)) values of a series of commercial and synthesized SCCPs ranged from 4.01 to 8.67, which indicated high lipid solubility and strong bioaccumulation potential in aquatic organisms. In this work, the lipid content of selected mollusks was in the ranges 1.98–18.8% (d.w.) or 0.53–2.96% on a wet weight basis. The highest lipid contents were found in Ost (15.7 ± 2.22% d.w.). The two gastropods, Nev and Rap, that had the lowest levels of \( \sum \) SCCPs, also contained the lowest lipid content.

Significant linear relationship was found between the lipid content of mollusks and \( \sum \) SCCPs (\( P < 0.05 \), Figure 4A). Lipid normalized concentrations of SCCPs in mollusks were linear-related to the length of the carbon chain (\( R = 0.41 \), \( P < 0.05 \)), and a significant but relatively weak parabolic correlation was found between the concentrations and the number of chlorines (\( R = 0.55 \), \( P < 0.05 \)). In order to evaluate the bioaccumulation of an individual congener in the marine species, we also analyzed the relationships among the concentrations of individual congeners (d.w.) and lipid contents. In general, positive linear relationships were found between the concentrations of an individual SCCP congener and the lipid content, although the correlations of some congeners (Supporting Information, Table SI-2) were not significant.

The slope of the linear regression can be considered as an indication of the bioaccumulation capacity of SCCP congener groups in mollusks. A steeper slope implies that the corresponding congener is more influenced by the lipid content. Based on the results of the regression analysis shown in Table SI-2 of the Supporting Information, the relationships among the slopes and carbon and chlorine numbers were analyzed. A relatively weak but significant positive linear correlation was found between carbon number and the concentration–lipid slope (Figure 4B), which indicated that longer carbon chain congeners could be more affected by the lipid contents of the species. Log \( K_{ow} \) is the principal factor describing the liposolubility of POPs. The trend in Figure 4B is consistent with the experimental results obtained by Hilger et al. where the log \( K_{ow} \) values of SCCPs are linear dependent on the alkane chain length (at chlorination degrees of 45–70%) and also similar to the work by Drouillard et al., which reported decreasing aquatic solubility of SCCPs when the carbon numbers increased from 10 to 12.

The parabola was open downward for the regression between chlorine number and concentration–lipid slopes of the
individual congener (Figure 4C, $R = 0.55$, $P < 0.05$). The vertex can be found at $\#\text{Cl} = 7.43$, which could imply that bioaccumulation of congeners with $\text{Cl}_{7,8}$ in mollusks are most influenced by lipid contents. The chlorination degree of SCCPs was in the range from 49.8% ($C_{13}H_{23}Cl_{5}$) to 72.9% ($C_{10}H_{12}Cl_{10}$). If taking chlorination degree (Cl%) of the congener groups as the $x$ axis, similar trends were also found with significant correlations (Supporting Information, Figure SI-2, $R = 0.55$, $P < 0.05$). The second-order polynomial correlation was also found in the study by Hilger et al. The main difference was that the vertex value in our work was found at Cl% = 60.6% whereas the calculated vertex was at about 50%
in their report. If assuming lipid content is the only factor dominating the accumulation of SCCPs in mollusks, the trend of concentration−lipid slope should be similar as with the log \( K_{ow} \) of the congener groups, which would be increasing with the increase of Cl%. However, the results deduced in this work deviated from the theoretical trend.

A parabolic relationship between \( K_{ow} \) and the total number of carbon and chlorine atoms (\( N_{tot} \)) of SCCPs has been found by Sijm and Sinnige.\(^{32} \) They pointed out that \( K_{ow} \) increased with \( N_{tot} \) and began to decrease when \( N_{tot} \) exceeded 23.1 (in this study, the maximum \( N_{tot} \) is 23 for \( C_{13}H_{18}Cl_{10} \)). However, the maximum slope was at \( N_{tot} = 20.3 \) in the parabolic curve of concentration−lipid slope and \( N_{tot} \) (Supporting Information, Figure SI-4, the parabolic relationship were not signifi- cant). The shift between the maximum value of \( N_{tot} \) and the deviation of the relationship between \( K_{ow} \) and Cl% in this work from the references studies indicated that some other factors could affect the bioaccumulation of SCCPs.

It is notable that, in most cases, the \( \sum \) SCCPs of Mya and Mac were generally higher than other species, although the average lipid contents were only 11.3 ± 0.89% d.w. and 7.93 ± 0.64% d.w., respectively. On the contrary, the \( \sum \) SCCPs in Chl samples was as low as gastropod samples, and no Cl\(_{5}\) or Cl\(_{10}\) congeners were detected in spite of the high lipid content (14.0 ± 2.55% d.w.).

**Relevance of Trophic Level (TL).** Nitrogen isotope ratios can indicate the trophic position of a marine species and have been widely used to study the bioaccumulation/biomagnification of organic contaminants through the food chain.\(^{27,33} \) Mollusks are water-respiring filter feeders and generally considered at the second lowest level in the marine food web. The main sources of contaminants are via food intake and water. The TL for all species was in the range 2.12−4.00 (Table 1), which is consistent with previous work.\(^{34} \) Among the 11 species, the predatory gastropods Nev and Rap were at a higher TL than other species (Nev: 3.30 ± 0.15; and Rap: 3.32 ± 0.22), although \( \sum \) SCCPs in Nev and Rap were lower than in bivalves. Mya had the highest average concentrations of SCCPs in spite of the lowest TL (2.64 ± 0.27).

Investigation on the correlations between the lipid-normalized \( \sum \) SCCPs and TL showed a significant negative linear relationship between \( \sum \) SCCPs and TL (\( p < 0.05 \), Figure SA), which was similar to the trends of higher chlorinated polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and PCBs in food chains of the Chinese Bohai Sea.\(^{26,35} \) No significant linear relationship was found between carbon number and the concentration−TL slope although the trend was slightly increasing (Table SI-2 of the Supporting Information and Figure 5B). The correlation between chlorine number and the concentration (l.w.)−to−TL slopes of an individual congener was parabolic (\( R = 0.83, P < 0.05 \)). If the \( x \) axis was changed from the number of chlorine to Cl% (Supporting Information, Figure SI-3), the parabolic correlation was significant as well (\( R = 0.67, P < 0.05 \)), and the vertex was found at Cl% = 61.5%.

The concentration (l.w.)−to−TL slope first slightly decreased and then increased with the increase of \( N_{tot} \) (Supporting Information, Figure SI-5). If this trend was described as parabolic, in spite that the relationship was not significant (\( R =

\[ \text{Figure 5. Relationships between lipid-weight-based } \sum \text{ SCCPs with TL (A: } \sum \text{ SCCPs ng/g l.w.} = 46000 - 10700 \times \text{TL, } R = -0.34, P < 0.05) \text{ and the concentration-to-TL slope (slope}_{\text{SCCP-TL}}) \text{ with the number of carbon atoms (B: slope}_{\text{SCCP-TL}} = -885 + 37.7 \times \text{number of carbon atoms, } R = 0.10, P = 0.66) \text{ and chlorine numbers (C: slope}_{\text{SCCP-TL}} = 6810 - 2090 \times \#\text{Cl} + 142.21 \times \#\text{Cl}_2, \#\text{Cl}: \text{chlorine numbers, } R = 0.83, P < 0.05, \#\text{Cl} = 7.35 \text{ when slope}_{\text{SCCP-TL}} \text{ was a maximum).} \]
0.41, \( P = 0.16 \), the vertex would be at \( N_{\text{crit}} = 18.4 \), which was lower than that in lipid content (\( N_{\text{crit}} = 20.3 \)).

Trophic magnification factors (TMFs) were also calculated as the following equations: \[ \text{log concentration}_{\text{lipid weight}} = b \times \text{TL} + \text{constant}; \quad \text{TMF} = 10^b. \] The mean TMF value of major congener groups (\( C_{10-13} \)) was 0.238 (Supporting Information, Table SI-3), and TMF for \( \sum \text{SCCPs} \) was 0.396 (Supporting Information, Figure SI-6), which indicated no trophic magnification of SCCPs in mollusks in the Chinese Bohai Sea.

Similar negative relationships between concentrations and TL were previously also found for other persistent semivolatile compounds such as PCBs, HCHs, PBDEs, and DDTs in mollusks in this region.26 However, biomagnification of SCCPs has also been observed in some aquatic food webs, such as plankton—Diporeia—Mysis—alewife—rainbow smelt—sculpin—lake trout and catfish—carp—turtle—tilapia aquatic food chain.19 The different biomagnification behaviors of SCCPs in different areas and food webs indicate the complex interplay of physical—chemical and biological processes on the bioaccumulation/biomagnification properties of SCCPs in organisms, such as depuration rate, growth dilution, respiratory elimination, and more.

Although not collected in all sampling sites, the bivalve Mya showed the highest average concentration of \( \sum \text{SCCPs} \) despite belonging to the lowest trophic level in this study. Mya are abundant in many aquatic and marine ecosystems, including Bering Strait, the west coast of North America, the northern coast of Japan and Korea, and China’s Yellow Sea and Bohai Sea. Our previous work found that Mya has a high ability to accumulate organotin compounds, in particular tributyltin (TBT), and could be used as bioindicators to monitor the pollution of organotin compounds in marine systems.37 Besides Mya, Ost also showed higher concentrations of \( \sum \text{SCCPs} \) than other species. In addition, we also found that Ost could be used as a potential bioindicator for pollutions of OCPs, PCBs, and PBDEs in this study region. These two bivalve species therefore have the prospect of being potential bioindicators of SCCPs contamination or POPs in general.

# ASSOCIATED CONTENT

## Supporting Information

Tables showing the detection frequency of individual SCCP congeners in each species collected from the Bohai Sea, the slopes from correlation of individual SCCP congener groups with lipid content and the trophic level and their p values, and the trophic magnification factors of major SCCP congener groups, and figures of the average percentage of SCCP homologues of individual carbon chain groups and chlorine groups, relationship between the concentration-to-lipid content slope and the chlorination degree, relationship between the lipid-weight-based \( \sum \text{SCCPs} \) to TL slope and the chlorination degree, relationship between the concentration-to-lipid content slope and the total number of carbon and chlorine atoms, relationship between the lipid-weight-based \( \sum \text{SCCPs} \) to TL slope and the total number of carbon and chlorine atoms, and the relationship between the logarithm of lipid-weight-based \( \sum \text{SCCPs} \) and trophic level. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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