Bioaccumulation and biomagnification of short and medium chain polychlorinated paraffins in different species of fish from Liaodong Bay, North China

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Chlorinated paraffins (CPs) are highly complex technical mixtures, and the short chain chlorinated paraffins (SCCPs) are classed as persistent and have been included in the Stockholm Convention. However, there have been few studies of SCCPs and medium chain chlorinated paraffins (MCCPs) and their bioaccumulation and biomagnification in different species of fish. The present study investigated the levels, congener group profiles, bioaccumulation, and biomagnification of SCCPs and MCCPs in different species of fish from Liaodong Bay, North China. The ranges for the ΣSCCP and ΣMCCP concentrations were 376.3–8596 ng/g lipid weight (lw) and 22.37–5097 ng/g lw, respectively. The logarithms of bioaccumulation factors of ΣSCCPs ranged from 4.69 to 6.05, implying that SCCPs bioaccumulated in the fish. The trophic magnification factor of ΣSCCPs was 2.57, indicating that SCCPs could biomagnify in fish. Carbon chain length, the numbers of chlorine atoms, and octanol/water partition coefficients of the SCCPs and MCCPs might be important factors affecting the bioaccumulation of these chemicals in fish. The risk posed to human health by consumption of fish containing SCCPs was low. New SCCPs with nine carbons (C9) were detected in fish in this study.

Chlorinated paraffins (CPs) are polychlorinated n-alkanes with low volatility that have flame retardant and good electrical insulation properties. They are also inexpensive and widely used as flame retardants and plasticizers, and added to products such as paints, coatings, metal working fluids, and sealants1–3. Depending on their carbon chain length, CPs are classified into the following three categories: short chain chlorinated paraffins (SCCPs, C10–13), medium chain chlorinated paraffins (MCCPs, C14–17), and long chain chlorinated paraffins (LCCPs, C18–30)4, 5. The degree of chlorination of CPs is usually between 30 and 70% by weight4. Because of their toxicity6–8, persistence9–13, and potential to undergo long-range transport14, 15 and bioaccumulate16, 17, SCCPs have been included on the list of persistent organic pollutants in the Stockholm Convention.

China began to produce CPs in the late 1950s, and the total yield has continuously increased since then. In 2003, the annual production of SCCPs in China was approximately 150 kilotonnes18. This increased to 600,000 tons in 200719, and 1000,000 tons in 200920. China has become the main producer, user, and exporter of CPs in the world. Release of CPs can occur during their production, storage, transportation, and use, and during disposal of CPs and products that contain them21. SCCPs and MCCPs are found in all environmental matrices in China, including air22, water23, sediments24, soil25, biota26, terrestrial bird species27, mollusks28, and marine mammals29. Fish are known to accumulate hydrophobic organochlorine pollutants in the environment29. Because food, and especially fish, is an important route of uptake of CP contaminants27, it is necessary to assess the levels of pollution in fish. However, limited data are available on SCCP and MCCP concentrations in fish28–32. Zhou et al. recently investigated the total CP concentrations for one fish species from the Yangtze River Delta, but did not study CP homologue group patterns33. Saborido Basconcillo et al. discussed the atmospheric sources or urban/
Among the fish species, lipid normalized SCCP concentrations were the highest in bastard halibut (8596 ng/g lw) and MCCP concentrations (5097 ± 2242 ng/g lw). Bass also showed higher concentrations (22.37 ± 2.06 ng/g lw) than the MCCP concentrations. Turbot could have different absorption rates of SCCPs and MCCPs compared to other fish species. Ma et al., found that C10 and C11 (82.3 ± 7.7%) were the most abundant groups in organisms (zooplankton, invertebrates, and fishes) from Liaodong Bay, China. C10 and C11 homologue groups have been identified in eels from rice fields in the Yangtze River Delta, China. This comparison of results clearly shows that the CP concentrations measured to date in fish have been higher in China than in any other country in the world, and this emphasizes the importance of further studies of CPs in the environment in China.

To study the bioaccumulation and biomagnification of SCCPs and MCCPs in fish, different species of fish from Liaodong Bay, North China were collected. SCCPs and MCCPs were analyzed using comprehensive two-dimensional gas chromatography-electron-capture negative ionization-high resolution time-of-flight mass spectrometry (GC × GC-ECNI-HRTOF-MS). The three major objectives of the present study were as follows: (1) to investigate the levels and congener group profiles of SCCPs and MCCPs in different species of fish in this area; (2) to study bioaccumulation and biomagnification of SCCPs and MCCPs in the fish; and (3) to assess the human health risk of SCCPs and MCCPs in the fish.

### Table 1. SCCP and MCCP concentrations in different species of fish.

| Fish species       | SCCP concentrations (ng/g ww) | SCCP concentrations (ng/g lw) | MCCP concentrations (ng/g ww) | MCCP concentrations (ng/g lw) |
|--------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Bastard halibut    | 1831 ± 586*                   | 8596 ± 2751                   | 150.5 ± 51.1                  | 706.5 ± 240.2                |
| Turbot             | 808.8 ± 347.8                 | 4035 ± 1735                   | 1022 ± 449                    | 5097 ± 2242                  |
| Ray                | 166.1 ± 69.8                  | 2233 ± 938                    | 8.11 ± 3.32                   | 109.0 ± 44.6                 |
| Neodovan septentrionalis | 390.0 ± 136.5              | 1750 ± 612                    | 83.76 ± 26.80                 | 375.9 ± 120.2                |
| Yellow croaker     | 328.1 ± 134.5                 | 1383 ± 567                    | 13.09 ± 5.62                  | 55.19 ± 23.73                |
| Bass               | 195.3 ± 85.9                  | 974.5 ± 428.7                 | 4.92 ± 2.06                   | 24.57 ± 10.31                |
| Capelin            | 231.3 ± 83.3                  | 863.0 ± 310.6                 | 8.11 ± 3.08                   | 30.26 ± 11.49                |
| Spanish mackerel   | 155.7 ± 60.7                  | 660.2 ± 257.4                 | 12.72 ± 5.34                  | 53.92 ± 22.64                |
| Abalone            | 103.7 ± 43.6                  | 440.2 ± 184.8                 | 14.96 ± 5.83                  | 63.48 ± 24.75                |
| Cod                | 67.80 ± 30.51                 | 376.3 ± 169.3                 | 4.03 ± 1.65                   | 22.37 ± 9.17                 |

Values shown are mean ± standard deviation.
species in this study (turbot, *Navodon septentrionalis*, and capelin), an almost equal abundance of SCCP homologue groups was observed and C10 and C11 accounted for 53.9% of the total SCCPs (Fig. 2(a)). This is similar to results for terrestrial bird species inhabiting an e-waste recycling site in Guangdong province, South China3. In the present study, the predominant chlorinated homologue group pattern for SCCPs in all the fish species was Cl6, Cl7, and Cl8. In total they added up to 93.6% of all SCCPs (Fig. 2(c)). Zeng *et al.* also found that C11–12 groups with 6–8 chlorines were the dominant congeners in fish from Gaobeidian Lake, China35. Although the dominant carbon chain lengths found by Zeng *et al.* (C11–12) were different from those in the present study (C10–11), the primary homologue group patterns (Cl6–8) were the same. This comparison result might be because of the different pollution sources34. In the present study, congener patterns varied widely among the different species, and this could be caused by differences in transport and distribution in the environment as well as bioaccumulation and metabolism34. The most abundant homologue groups of SCCPs in the present study were generally C10Cl6 and C10Cl7 in all the fish species.

Congener distributions of MCCPs showed that C14 was the dominating homologue group in all the fish species, accounting for 60.7–96.5% of total MCCPs (Fig. 2(b)). C15 was the second most abundant group (6.7–24.0%), followed by C16 and C17. The distribution of the homologue groups of MCCPs in present study was consistent with that in biota from the European Arctic31, and in top predatory fish from nine freshwater bodies across Canada34. MCCPs with between seven and nine chlorines (total contribution 90.1%) predominated in all fish samples (Fig. 2(d)). With C7Cl7 and C8Cl8 were the most abundant groups. A similar profile was observed in top predatory fish from Lake Huron, Lake Ontario, and the Saint Lawrence River34.

**Bioaccumulation.** Bioaccumulation factors (BAFs) are derived from concentration data collected in the environment, and used to determine whether it is possible for a chemical to bioaccumulate38. If the BAF of the chemical is greater than 5000, it is considered bioaccumulative. In the present study, BAFs were calculated in the fish species from Liaodong Bay based on SCCP values measured in fish and water samples. The details for the calculation method and results are shown in the SI (Table S1). The log

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**Figure 1.** Correlation between ∑SCCPs and ∑MCCPs in the fish.

**Figure 2.** Congener group abundance profiles of SCCPs and MCCPs in the fish.
BAFs of ΣSCCPs for the different fish species ranged from 4.69 to 6.05 with an average value of 5.24, indicating that SCCPs bioaccumulated in the fish. The log BAFs were slightly higher than those found in fish from Liaodong Bay, China16 (range 4.7–5.6, mean 5.08), but were slightly lower than those reported in trout from Lake Ontario17 (range 5.2–6.4, mean 6.1). The highest and lowest log BAFs in the present study were found in bastard halibut and cod, respectively. The lipid content and trophic level (TL) of bastard halibut were 1.94% and 3.81, respectively. The lipid content and TL of cod were 0.71% and 3.56, respectively. Wan et al. found that lipid content and TL were dominant factors determining accumulation of ΣPCBs in fish39. Based on this, and the fact that SCCPs and PCBs have similar properties, it is likely that lipid content and TL are important factors that determine the accumulation of SCCPs. Therefore, TL and lipid content might influence the accumulation of SCCPs in fish.

The log BAFs of 48 SCCP congeners ranged from 2.14 to 7.43 (mean 4.95), with the highest value for C13Cl8 in bastard halibut and the lowest value for C12Cl5 in Navodon septentrionalis. The ranges of log BAFs were similar to those in an earlier study16, where the log BAFs of SCCP congener groups for all organisms varied from 4.1 to 6.7 (average 5.1). In addition, they were similar to the range (4.1–7.5) in Lake Ontario for SCCP congeners that were detected in water and aquatic organisms17. Based on the average log BAFs for SCCP congener groups in the different fish species, three SCCP congeners (C11Cl5, C12Cl5, and C13Cl5) might not bioaccumulate in the fish. The log BAFs of C11Cl5, C12Cl5, and C13Cl5 were 3.03, 2.83, and 3.26, respectively. The low BAFs could be attributed to the low chlorination of these SCCPs, which would mean they would be easy to metabolize and eliminate compared with SCCPs with higher chlorination32, 40.

The log BAF values of the SCCP congener groups increased with increasing carbon chain length (Fig. 3(a)), although there was no significant linear relationship between them. This result is consistent with the conclusion of an earlier study of dietary exposure of juvenile rainbow trout, which found that the bioaccumulation potential of SCCP congeners generally increased with carbon chain length12. Another earlier study found a slight increasing trend for log BAFs with the number of carbon atoms (p > 0.05) in different fish species35. In addition, Ma et al. found a significant increasing trend between BAF values of SCCP congener groups and carbon chain length16. Therefore, as the carbon chain length increases, the bioaccumulation potential of SCCP congeners will increase.

A parabolic correlation was observed between log BAFs and the number of chlorine atoms (R2 = 0.90, p < 0.001), with the maximum value occurring at approximately eight chlorine atoms16. Similar results were observed for the log BAF and chlorine contents (R2 = 0.56, p < 0.001) (Fig. 3(c)). These results are similar to those of Zhu et al.40 and Wang et al.41, who found a parabolic correlation between log BCFs and the number of chlorine atoms for PCB congeners. By contrast, Ma et al. and Zeng et al. observed a significant or non-significant linear relationship between log BAFs and the number of chlorine atoms46, 35. In the present study, the upward trend in the initial part of the parabolic curve could be attributed to the following: (1) a significant linear relationship between log Kow and the number of chlorine atoms (R2 = 0.48, p < 0.001) (Fig. S1), and (2) for small molecules, the bioaccumulation potential (log BAF) increased as the hydrophobicity increased (log Kow). The downward trend in the latter part of the parabolic curve when the number of chlorine atoms was greater than

Figure 3. Relationships between log BAF of the SCCP congener groups and the number of carbon atoms, chlorine atoms, chlorine contents, and log Kow.
eight could be attributed to the following: (1) the difficulty for highly chlorinated SCCP congeners (large molecules) to migrate across membranes, and (2) the relatively fast metabolic degradation of higher chlorinated SCCPs in fish compared with lower chlorinated congeners. The BAF values of the SCCP congener groups showed a significant linear increasing trend with increasing $K_{ow}$ (Fig. 3d), indicating that $K_{ow}$ might be a major factor governing congener specific bioaccumulation. Similar results have been reported by Zeng et al. and Ma et al. To a certain extent, the above results imply that carbon chain length, number of chlorine atoms, $K_{ow}$ values, lipid content, TL, fish habit, and metabolism might be important factors determining the bioaccumulation of SCCP congeners in fish.

The bioaccumulation potential of MCCPs in the fish could be evaluated using BAF and $K_{ow}$ values. In the present study, the MCCP concentrations in the water from Liaodong Bay were not available, and BAF values could not be calculated for the MCCPs. The current international protocol for persistent organic pollutants and management policies in Canada consider chemicals with a $K_{ow}$ > 5 as bioaccumulative. Reported $K_{ow}$ values for MCCPs were in the range 6.83–8.96. Therefore, MCCPs are considered as bioaccumulative, and this has been shown in other study.

**Biomagnification.** To investigate biomagnification, we selected a number of aquatic species across multiple trophic levels and with predator–prey relationships. Samples were collected of invertebrates (jellyfish, Conch neptunia, clams, and Patinopecten yessoensis and mantis shrimp) and fish (bastard halibut, ray, Navodon septen-trionalis, bass, and abalone). Stable isotopes of nitrogen are useful for assessing the TL of a marine species. In the present study, TLs were determined based on stable nitrogen isotope ratios to investigate if biomagnification of SCCPs and MCCPs occurred in the organisms. The results (Fig. S2) showed that the TLs of the selected aquatic species ranged from 2.31 to 3.81. Trophic magnification factors (TMFs) were calculated as 10 to the power of the slope of the linear regression line between the logarithms of the concentrations (lw) of the CPs and the TLs (e.g. TMF = $10^b$ where $b$ = the slope). The TMFs were used to estimate the magnitude of biomagnification of CPs in the organisms. The above ten species organism were included in the TMF determinations (see Table S2).

The calculated TMFs ranged from 0.39 to 11.47 for the SCCP congeners (24 congeners analyzed individually, Table S3). The TMFs of SCCP congener groups in this study were similar to or slightly higher than those (1.45–5.65) of SCCP congeners in the marine web in Liaodong Bay, and in Lake Ontario (0.47–1.5), and in Lake Michigan (0.41–2.4). The TMFs of $C_{10}Cl_2$ and $C_{10}Cl_6$ were 4.80 and 6.91, respectively (Table S3, $p < 0.05$). The TMFs of $C_{17}Cl_2$, $C_{17}Cl_4$, and $C_{17}Cl_6$ were 3.96, 10.33, 11.47 and 8.32, respectively (Table S3, $p < 0.05$). The above TMFs were all greater than one and indicated biomagnification occurred in the organism. The specific TMFs for other homologue groups were not evaluated because of their weak linear relationships ($p > 0.05$). For the predominant carbon chains, the mean TMFs were 3.69 for $C_{10}$ ($p = 0.06$), and 8.39 for $C_{14}$ ($p < 0.05$), showing biomagnification of these compounds occurred. The TMF of $\Sigma$SCCPs was 2.57, indicating that biomagnification of SCCPs could occur in the fish.

The calculated TMFs of MCCP congeners ranged from 0.23 to 2.92 (Table S3). The TMFs in the present study were higher than those (0.06–0.36) found for MCCP congeners in a food web in Lake Ontario. For the predominant carbon chain length ($C_{10}$), the mean TMF was 3.69 ($p > 0.05$). The TMF of the $\Sigma$MCCPs was 0.71 ($R^2 = 0.02$, $p > 0.05$). Linear relationships (Table S3) between the logarithms of the concentrations of $\Sigma$MCCPs (lw) in the organisms and TLs were weak, with almost all the $r^2$ values smaller than 0.1 and all $p$ values greater than 0.05. Therefore, MCCP biomagnification in the fish did not occur.

**Evaluation of the risk to human health.** The risk evaluation for SCCPs was based on the following: (1) the World Health Organization (WHO) health guidelines for neoplastic effects (tumor formation) of 11 µg/kg bw/day; and (2) the International Programme on Chemical Safety (IPCS) tolerable daily intake for SCCPs of 100 µg/kg bw/day. The estimated daily intake (EDI, ng/kg bw/day) was used to represent the daily intake of SCCP via fish consumption per person per day and was calculated as follows:

$$\text{EDI} = \frac{C \times CV}{BW} \quad (1)$$

where $C$ is the average concentration of SCCPs in the fish (ng/g ww), $CV$ is the quantity of fish consumed per person per day (g/person/day), and $BW$ is the average mass of the consumer (set at 60 kg). In the Chinese population, the rate of fish consumption for the low consumption group was set at 11 g/person/day, and the rate of fish consumption for the high fish consumption group was set at 119 g/person/day. For the low and high consumption groups, the EDIs for consumption of all species of fish (Table 2) were lower than the WHO and IPCS guidelines. However, when the high consumption group ate bastard halibut, the EDI was 33% of the WHO health guideline (11 µg/kg bw/day), which means that the WHO guideline could be easily exceeded if a person consumes this kind of fish regularly. Therefore, consumers, especially those who eat fish regularly, should adjust their diet to reduce the risk of exceeding the WHO and IPCS guidelines. In addition, because of the similar physico-chemical properties and toxicity profiles of SCCPs and MCCPs, simultaneous exposure to SCCPs and MCCPs will increase the risk.

**Detection of new SCCPs with nine carbon atoms in the fish.** CPs are extremely complex mixtures because there are many possible positions for chlorine atom substitution. In these complex mixtures, many of the CP congeners have similar chromatographic retention characteristics and cannot be separated and identified using one-dimensional gas chromatography. The GC × GC-HRTOF-MS method used in this study has high resolution, high sensitivity, and high peak capacity, and could separate CPs in these complex mixtures. Previously, studies have focused on only the $C_{10-13}$ SCCPs, and SCCPs with nine carbons ($C_9$) have not been investigated. One study made reference to $C_9$ congeners because they have similar mass-to-charge ratios to $C_{14}$ congeners and
cannot be separated from them using low resolution mass spectrometry. Wyatt et al. pointed out that studies in rats and mice have shown SCCPs are potentially carcinogenic, while there is no evidence of carcinogenicity for MCCPs and LCCPs. In addition, some studies have reported that the toxicities of CP congeners generally increase as the carbon chain length decreases. Therefore, it is important to study C9 congeners. In the present study, standard SCCP (C10–13) and MCCP (C14–17) mixtures with different chlorine contents were used to establish linear calibration curves. Because these standards did not contain C9 compounds, a semi-quantitative method was to describe the relative amounts of C9 congeners as the percentage ratio of relative abundance of each homologue over the total relative abundance. Because most of the C9 congeners were detected at very low concentrations, only two C9 congeners (C9Cl6 and C9Cl7) were determined. The relative amounts of these C9 congeners in all the fish ranged from 0.92% to 8.38% (Table 3). The relative amounts of C9 congeners were more than those of C12 or C13 congeners in half of the fish species (ray, yellow croaker, bass, Spanish mackerel and cod). Because C9 and C10 have similar characteristics, the percentage ratio of relative abundance of C9 over that of C10 was calculated. The results from this ranged from 1.46% to 14.05%. Therefore, C9 is important in risk assessments and an accurate method needs to be developed for its quantification.

**Conclusion**

The SCCP and MCCP levels in the fish from Liaodong Bay are higher than or comparable to those in other studies. The C10 and Cl6–8 SCCPs and C14 and Cl7–9 MCCPs are the primary homologue groups in all of the fish species. The log BAFs of the SCCPs indicate bioaccumulation of SCCPs occurs in the fish, except for three SCCP congeners (C11Cl5, C12Cl5, and C13Cl5). The properties of the SCCP congeners (e.g. carbon chain length, number of chlorine atoms, and Kow), lipid content, trophic level and habit of the fish, and metabolization might be important factors affecting the bioaccumulation of SCCP congeners in the fish. Based on the Kow values of the MCCP congeners, the MCCPs are considered as bioaccumulative. For the predominant carbon chain, the mean TMFs are 3.69 for C10, and 8.39 for C11, showing biomagnification of these compounds occurs in the organism. The TMF of ΣSCCPs is 2.57, indicating that SCCPs also have biomagnification potential in fish. The results suggest the risk to humans posed by consumption of fish containing SCCPs is low. We detected new SCCPs (C9) in the fish samples. Further research is required for toxicology and risk assessments.

**Methods**

**Sample collection and preparation.** Liaodong Bay is one of the three bays forming the Bohai Gulf, the innermost gulf of the Yellow Sea, in northeast China. And it borders Liaoning province. Ten species of fish and five species of invertebrates were collected from Liaodong Bay, North China in July 2014. All samples were wrapped...
in aluminum foil and transported to the laboratory. The fish samples were weighed and their lengths measured (Table 4). Details for the invertebrate samples are listed in Table S4. All samples were freeze-dried, ground, homogenized, and stored in amber glass bottles at −20°C until required for extraction. The mass differences before and after freeze-drying the samples were used to calculate their water contents (Table 4). A 2-g dry sample was spiked with surrogate standard (2.5 ng of 13C10-trans-chlordane), and then extracted with dichloromethane (DCM)/n-hexane (1:1, v/v) in an accelerated solvent extraction apparatus (ASE350; Dionex, Sunnyvale, CA, USA). The extraction conditions were as follows: three extraction cycles at 100 °C and 1.03 × 10^4 kPa, 5 min of heating, a 10 min static extraction, a flush volume of 60% and a N2 purge time of 60 s. The extract was evaporated to about 2 mL using a rotary evaporator (Heidolph, Schwabach, Germany). The lipid content was determined gravimetrically (Table 4), and the details for the calculation are given in the Supplementary information (SI).

The extracts were primarily cleaned up using gel permeation chromatography to remove sulfur containing compounds, lipids, and other interfering compounds (e.g. toxaphenes). The sample was added to the column, and then the column was cleaned with 70 mL of DCM/n-hexane (1:1 v/v), which was discarded. The sample was eluted with 130 mL of DCM/n-hexane (1:1 v/v), which was collected for further cleanup. The extract was then reduced to about 1 mL under reduced pressure. A multi-layer silica gel column was prepared by packing with 3 g of Florisil, 2 g of activated silica gel, 5 g of acidified silica gel (44% mass fraction sulfuric acid), and 5 g of anhydrous Na2SO4 from bottom to top. The multilayer column was rinsed with 50 mL of n-hexane before use. Then the sample was added and eluted with 40 mL of n-hexane, which was discarded. Afterwards, the column was eluted with 100 mL of DCM/n-hexane (1:1 v/v), which was collected for analysis of CPs. The eluate was concentrated to about 5 mL using a rotary evaporator. The fraction containing SCCPs and MCCPs was reduced to about 0.5 mL and transferred to a vial. The solution in the vial was further concentrated to near dryness under a gentle stream of N2. The solvent was replaced with 50 μL of cyclohexane. Before analysis, 2.5 ng of γ-hexachlorocyclohexane (γ-HCH) was added to the vial as an injection internal standard.

### Instrumentation and quantification.

The GC × GC-ECNI-HRTOF-MS analyses were conducted using an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) fitted with a ZX2004 loop cryogenic modulator (Zoex corporation, Houston, TX, USA) interfaced with a high resolution time-of-flight MS (Tofwerk, Thun, Switzerland) operated in ECNI mode. For all fish samples, SCCPs and MCCPs analyses were carried on a GC × GC-ECNI-HRTOF-MS instrument. The first-dimension column was an Agilent DB-5 (5% diphenyl, 95% dimethyl polysiloxane; 30 m × 0.25-mm inner diameter (i.d.), 0.25-μm film thickness). The second-dimension column was a SGE BPX-50 (50% diphenyl, 50% dimethyl arylene polysiloxane; 1 m × 0.10-mm i.d., 0.10-μm film thickness). The initial GC oven temperature was 140 °C for 1 min, and then increased to 10 °C/min to 200 °C, and finally increased at 1.5 °C/min to 310 °C, and maintained at 310 °C for 5 min.

Injections were performed in splitless mode with an injection volume of 1.0 μL and an inlet temperature of 280 °C. The carrier gas flow rate (helium, 99.999% pure) was constant at 1 mL/min. Methane was used as the ECNI ionization agent with a flow rate of 2 mL/min. The electron energy was 125 eV and the emission current was 0.1 mA. The ion source and transfer line temperatures were 200 °C and 280 °C, respectively. The modulation period was 8 s. The hot gas duration time was 300 ms. The modulator hot gas temperature was 350 °C. The data acquisition speed was 100 Hz. This instrument had a mass resolution of 5000 (full width at half maximum) and a mass precision of 5 ppm or 0.002 u, using perfluoroperhydrophenanthrene for mass calibration. GC × GC data were processed using GC Image® R2.8 Software (GC Image, Lincoln, NE, USA).

The two most abundant [M-Cl]− ions were detected in full scan mode as quantitative and qualitative ions. The most abundant [M-Cl]− ion was used as a quantification ion and the next most abundant ion was used as a qualification ion. The quantification of SCCP congeners and MCCP congeners was conducted based on an established technique. The quantification method has been reported in another study and was mainly dependent on linear correlation between the total response factors for CP standard mixtures and their chlorine content. In total, 48 SCCP (C10–13Cl5–10) and MCCP (C14–17Cl5–10) congeners were analyzed in the samples in this study. Detailed information on the chemicals can be found in the SI.

| English names | Latin names | Number of samples | Weight (g) | Length (cm) | Trophic level | Water content (%) | Lipid content (%) |
|--------------|-------------|-------------------|------------|-------------|---------------|-----------------|-----------------|
| Capelin | mallotusvillosus | 23 | 12.10–15.60 | 9–12 | 3.33b | 70.48b | 9.19b |
| Yellow croaker | Larimichthys polyacis | 15 | 46.51–68.80 | 16–20 | 3.62 | 74.50 | 6.97 |
| Cod | Gadus | 3 | 319.1–487.0 | 41–47 | 3.56 | 81.85 | 0.71 |
| Turbot | Scophthalmus maximus | 1 | 891.3 | 35 | 3.87 | 79.77 | 0.91 |
| Bastard halibut | Cleothenes herzensteini | 5 | 101.1–118.1 | 23–25 | 3.81 | 78.28 | 1.94 |
| Navodon septentrionalis | Thynnacorus modestus | 3 | 199.9–378.2 | 24–31 | 3.41 | 77.60 | 0.54 |
| Bass | Cantharus | 2 | 589.5–1038 | 38–42 | 3.18 | 79.35 | 2.94 |
| Abalone | Abalone | 10 | 24.48–62.32 | 5–10 | 2.98 | 76.27 | 0.70 |
| Spanish mackerel | Spanish lacertus | 3 | 320.8–488.4 | 32–37 | 3.65 | 75.38 | 4.19 |
| Ray | Rajiformes | 8 | 102.3–123.5 | 30–33 | 3.53 | 92.50 | 0.84 |

Table 4. Details of fish samples collected from the Liaodong Bay, North China. a,b,c are arithmetic mean value.
Quality assurance and quality control. To eliminate background contamination, all glassware was heated to 200 °C, and thoroughly rinsed with methanol, acetone, and dichloromethane in succession. The results for three procedural blanks indicated that the concentrations of both SCCPs and MCCPs in the blanks were less than 5% of those found in the fish samples. Therefore, the final concentrations of SCCPs and MCCPs reported in this study were not blank corrected. The method detection limit (MDL), which was defined as the average CP contents in the blanks plus three times the standard deviation, was 9.4 ng/g for the SCCPs and 7.0 ng/g for the MCCPs in the fish. The recovery was calculated by dividing the ratio of the surrogate standard (13C10-trans-chlordane) and injection internal standard (ε-HCH) in each sample by the ratio of 13C10-trans-chlordane and ε-HCH in the appropriate standard solution. The surrogate recoveries of 13C10-trans-chlordane in all the fish samples ranged from 61.0% to 92.6%. Two of ten species of fish were randomly selected for parallel experiments. The relative standard deviation obtained after repeating the analysis of each sample seven times was less than 15%. Atmospheric nitrogen was used as δ15N standard. The laboratory working standard was STD-27 (δ15Nair = 7.0 ± 0.15‰). Replicate measurements of STD-27 gave a measurement error of 0.15% for stable nitrogen isotope measurements.

Ethic Statements. No experiment on live vertebrates and higher invertebrates was included in this study. The study was carried out in compliance with relevant laws, guidelines, and regulations of China and under a permit issued by the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

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Author Contributions
L.G. and H.H. designed the experiments; H.H. and L.Q. collected the samples; H.H. performed the experiments. H.H. and D.X. analysed the data; H.H. wrote the manuscript; L.G. and H.H. reviewed and commented on the manuscript.

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