Quantification of Short-Chain Chlorinated Paraffins by Deuterodechlorination Combined with Gas Chromatography−Mass Spectrometry
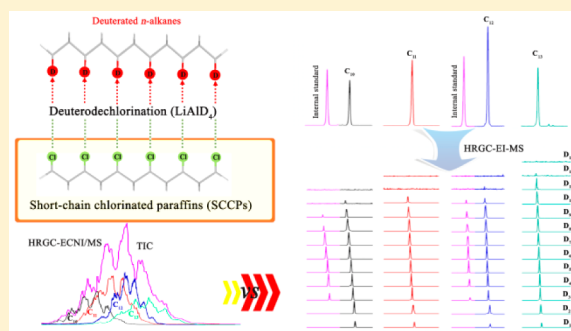
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Ⓢ Supporting Information

ABSTRACT: Analysis of short-chain chlorinated paraffins (SCCPs) is extremely difficult because of their complex compositions with thousands of isomers and homologues. A novel analytical method, deuterodechlorination combined with high resolution gas chromatography−high resolution mass spectrometry (HRGC−HRMS), was developed. A protocol is applied in the deuterodechlorination of SCCPs with LiAlD$_4$, and the formed deuterated n-alkanes of different alkane chains can be distinguished readily from each other on the basis of their retention time and fragment mass ([M]$^+$) by HRGC−HRMS. An internal standard quantification of individual SCCP congeners was achieved, in which branched C$_{10}$-CPs and branched C$_{12}$-CPs were used as the extraction and reaction internal standards, respectively. A maximum factor of 1.26 of the target SCCP concentrations were determined by this method, and the relative standard deviations for quantification of total SCCPs were within 10%. This method was applied to determine the congener compositions of SCCPs in commercial chlorinated paraffins and environmental and biota samples after method validation. Low-chlorinated SCCP congeners (Cl$_{1−4}$) were found to account for 32.4%−62.4% of the total SCCPs. The present method provides an attractive perspective for further studies on the toxicological and environmental characteristics of SCCPs.

INTRODUCTION

Short-chain chlorinated paraffins (SCCPs) are synthetic mixtures of chlorinated n-alkanes ranging from C$_{10}$ to C$_{13}$ with a chlorine content of 30%−70% (mass weight). As a constituent part of chlorinated paraffins (CPs), SCCPs are extensively used in metal-working fluids, paints, sealants, adhesives, and leather finishing agents as well as flame retardants and plasticizers in rubbers and polymers.[1] SCCPs are high production volume chemicals with total world production of approximately 150 kt per year.[1,2] Most of the SCCPs are used by blending with medium (C$_{14}$−C$_{17}$) and long (>C$_{17}$) chain CP components.[2] Because of their potential for long-range transport, persistence in the environment and high toxicity to aquatic organisms, SCCPs have been listed as "candidate" persistent organic pollutants (POPs) by the Convention's POP Review Committee (POPRC).[3,4]

SCCPs have thousands of isomers via free radical chlorination with respect to C$_{10−13}$ chains bonding with various numbers of chlorine atoms and different chlorine positions, and thus it is very difficult to completely separate SCCP congeners from each other on a GC capillary column. Comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOF/MS) or microelectron capture detection (μECD) can remarkably improve the separation of SCCP congeners in the GC section.[5,6] Nevertheless, complete chromatographic separation of individual congeners with GC remains impossible.[7] Therefore, SCCPs

have been described as "the most challenging group of substances to analyze and quantify".[8]

High resolution gas chromatography (HRGC) coupled with high/low resolution mass spectrometry (HRMS or LRMS) in electron capture negative ionization (ECNI) mode has been most commonly applied for the quantitative analysis of SCCPs in environmental matrices and biota.[5,9−14] However, large quantification errors of 65%−940% can occur if the chlorine content of the standard was changed or did not fit to the sample,[15,16] which has mainly resulted from the strong dependence of the ECNI/MS response factors of SCCP congeners on their chlorine atom numbers.[7,17] The highly chlorinated SCCP congeners are prone to have the higher ECNI/MS response factors, while the low chlorinated congeners (Cl$_{1−4}$) are not usually detected by ECNI/MS. Many efforts have been made to decrease or offset the influence of chlorination contents on the quantification of SCCPs. Zencak et al.[10] used a mixture of methane and dichloromethane (DCM) (80:20) as reagent gas for negative ion chemical ionization in combination with GC−LRMS. Similar response factors were obtained for SCCP congeners with different chlorine contents, opening a possibility of the detection of low

chlorinated congeners (Cl_{3-5}). However, the methane/DCM reagent mixture can lead to a rapid deterioration of the ion source due to the formation of black carbon residues that affect the performance of the MS system.[18] Reth et al.[15] developed a linear relationship between the total response factors and the chlorine contents, which improved the precision of the results and has been used most frequently. The linearity range is usually limited to the chlorine contents of 50%−70%, and thus it is not suitable for the quantitative analysis of SCCPs with a high proportion of low chlorinated congeners (Cl_{1-4}). Harada et al.[19] developed a semiquantitative method for individual SCCP congeners with Cl_{5-9}, in which the individual SCCP congeners in samples were calculated using a linear regression between the peak areas determined by HRGC−ECNI/HRMS and the concentrations determined by HRGC−electron impact ionization (EI)/TOFMS. In addition, based on the analysis of GC−ECNI/MS, Geiβ et al.[11] developed a multiple linear regression procedure for the quantification of total SCCPs with a chlorine content of 49%−67% in water.

HRMS or LRMS in atmospheric pressure chemical ionization (APCI) or chloride-enhanced APCI mode have been also developed for the analysis of chlorinated paraffins.[20,21] The response factors were less dependent on the chlorination content than in ECNI/MS methods. Nevertheless, the low chlorinated SCCPs (Cl_{1-4}) are still not detected. Another interesting analysis approach of carbon skeleton reaction gas chromatography was achieved by detecting the corresponding *n*-alkanes generated from the catalytic hydrodechlorination of CPs on a Pd catalyst.[22−24] However, the information on the chlorination content is lost.

Accurate quantification of the individual SCCP congeners, especially the low chlorinated SCCPs, remains challenging, although interlaboratory studies since 2010 showed improving data agreements.[7,25] The environmental fates and toxicities of SCCPs have not yet characterized well due to the limitation in an analysis method. Reliable measurement data regarding SCCPs are strongly desired in view of the ongoing global regulatory actions and deliberations by all countries and regulatory bodies.[4,7,17] In this study, we report a novel approach for the analysis of SCCPs based on deuterodechlorination combined with HRGC−EI/HRMS, through which the concentrations of individual SCCP congeners with different carbon and chlorine numbers can be determined. Reduction of SCCPs with lithium aluminum deuteride ($LiAlD_4$) was conducted in ethylene glycol diethyl ether (EGDE), which generated the corresponding deuterated *n*-alkanes. Deuterated *n*-alkanes were then completely separated on a GC capillary column in according with their carbon chain length, rather than the coelution profile of the original SCCPs. The molecular ions ($[M]^+$) of the deuterated *n*-alkanes, reflecting the chlorine distribution of SCCPs, were thus monitored. This method enabled us to quantify the individual SCCP congeners even with less than 5 chlorine atoms.

■ EXPERIMENTAL SECTION

Synthesis of the Calibration Standards for Method Development. A series of SCCP calibration standards with fixed carbon chain lengths, C_{10}-CPs, C_{11}-CPs, C_{12}-CPs, and C_{13}-CPs, were synthesized by the substitution reaction of individual *n*-alkanes with sulfuryl chloride (SO_2Cl_2). The information on the carbon chain lengths and chlorine contents of the synthetic calibration standards are shown in Table S1. Chlorinated 2-methylnonane (branched C_{10}-CPs) and chlori-

nated 2-methylundecane (branched C_{12}-CPs) were also synthesized by the substitution reaction of 2-methylnonane and 2-methylundecane with SO_2Cl_2, respectively.

Under argon atmosphere, about 1 mL of alkane was treated with SO_2Cl_2 (9 mL) in DCM (30 mL). The reaction mixture was irradiated with UV-light (6 W mercury vapor lamp) and refluxed at 70 °C. The reaction was continued for different times in order to obtain SCCP calibration standards with different chlorine contents (Table S1). At the end of the reaction, the reaction flask was placed into an ice bath and bubbled with a gentle stream of argon for 10 min. The solvent and excess SO_2Cl_2 were removed by distillation at 50−100 °C under atmospheric pressure. The residue was cooled to ambient temperature after an ice bath, resolved with DCM (40 mL), and then washed with saturated NH_4Cl (40 mL), saturated $NaHCO_3$ (40 mL), and purified water (40 mL). The organic phase was dried over anhydrous Na_2SO_4 and filtered. All the volatiles were removed under reduced pressure at 35 °C, and the products were dried in vacuum. The chlorine content of the prepared SCCP calibration standards was calculated by the weight difference between the substrate alkane and the generated chlorinated alkane (details in the Supporting Information). The details on all purchased chemicals and reagents are also shown in the Supporting Information.

Procedure for the Deuterodechlorination of SCCPs. First, the saturated solution of $LiAlD_4$ in EGDE was prepared. Prior to use, EGDE was distilled by rectifying the column with sodium reflux. Approximately 0.05 g of $LiAlD_4$ was added into 1.0 mL of the distilled EGDE in a reaction vial and then heated at 110 °C for about 8 h until the upper solution became pellucid. The reaction vial was centrifuged at 3000 rpm for 10 min. The clear supernatant, i.e., the saturated solution of $LiAlD_4$, was used for the deuterodechlorination of SCCPs.

Sample collection, extraction, and cleanup of environmental samples were described in the Supporting Information. Prior to extraction, 5 μL of branched C_{10}-CPs (1 μg/μL nonane) was spiked into the sample as the extraction internal standard. Alkanes and other organochlorine compounds in environmental samples have been found to interfere with the instrumental analysis and the deuterodechlorination reaction, respectively. Gel permeation chromatography (GPC) and a successive silica gel and basic alumina columns have been used for the removal of lipids and other organochlorine compounds in samples.[26] The SCCP-containing extract was concentrated to about 1 mL and then transferred to a microreaction vial. Five μL of branched C_{12}-CPs (1 μg/μL nonane) was spiked into the microreaction vial as the reaction internal standard and then concentrated to near dryness by a gentle stream of N_2. The deuterodechlorination of SCCPs was conducted in a glovebox with the moisture content of <1 ppm. 100 μL of the saturated solution of $LiAlD_4$ in EGDE was added into the microreaction vial containing SCCPs and then heated at 90 °C for 72 h under nitrogen atmosphere. After reaction, the solution was transferred to a screw thread GC vial. The microreaction vial was further rinsed by 100 μL of DCM for three times, and then the rinse solution was combined with the reaction solution. The reaction was quenched by adding 100 μL of the deionized water to the solution dropwise, while the deionized water was further absorbed by about 0.3 g of anhydrous Na_2SO_4. The supernatant liquid was transferred to another GC vial after centrifugation and concentrated to near dryness by a gentle stream of N_2. 50 μL of *n*-hexane containing phenanthrene as

the injection internal standard was added, and the vial was mixed by vortexing prior to instrumental analysis.
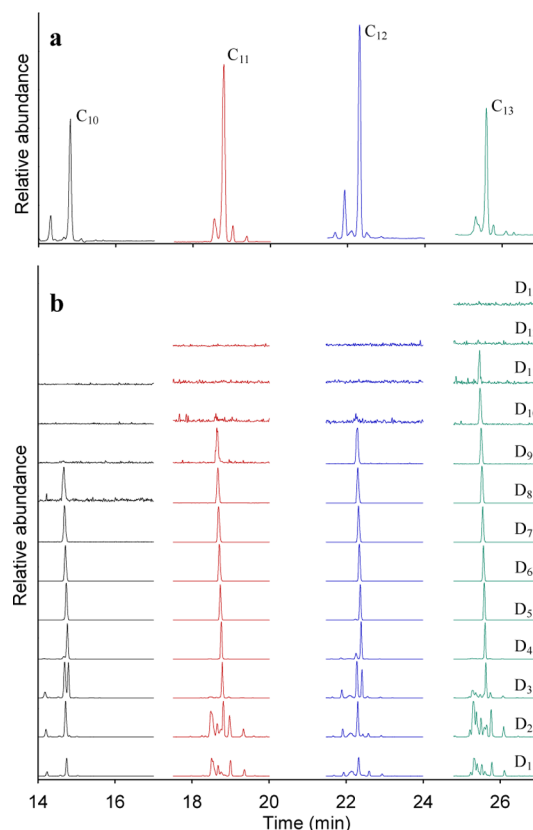
Instrumental Analysis. The generated deuterated alkanes were analyzed using an Autospec Ultima high resolution mass spectrometer (Micromass, UK) interfaced with a Hewlett–Packard (Palo Alto, CA, USA) 6890 Plus gas chromatograph. A capillary DB-5 column (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, USA) was used. A sample volume of 1 μL was injected in the splitless mode with an injector temperature of 280 °C, which was made by a CTC A200SE autosampler under data system control. The oven temperature program was as follows: 50 °C, isothermal for 0.5 min, then 20 °C/min to 80 °C, keeping 8 min, then 5 °C/min to 280 °C, and final isothermal for 20 min. The ion source was operated at 260 °C, and the mass spectrometer was tuned to a mass resolution of 5000 under positive EI conditions. The molecular ion clusters, $[M]^+$, of the formed deuterated alkanes, were monitored in SIM mode, and four windows during the scanning were divided to improve the efficiency. The m/z values of the monitored ions for deuterated alkanes with different carbon chain lengths and deuterium atom numbers are shown in Table S2.

In order to develop and validate the deuterodechlorination combined with the HRGC–EI/HRMS method for SCCP analysis, SCCPs in some samples were also analyzed by the traditional HRGC–ECNI/LRMS method, carbon skeleton reaction GC with the flame ionization detection (FID) method, and the deuterodechlorination combined with the HRGC–EI/LRMS method. The detailed conditions for instrumental analysis are described in the Supporting Information.

■ RESULTS AND DISCUSSION

Optimization for the Deuterodechlorination Reaction of SCCPs. In our case, LiAlD$_4$ was selected as the deuterium sources. The corresponding deuterated n-alkanes were formed as the major products (Figure S1). Preliminary experiments were carried out to improve the deuterodechlorination rates of SCCPs, which was calculated as the percentage of SCCPs reacted to the corresponding deuterated n-alkanes (detailed equations see the Supporting Information). The influences of reaction temperature and reaction time on deuterodechlorination rates of SCCPs were investigated. Two kinds of SCCP mixture stock standard solutions (51% Cl and 63% Cl, respectively, purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany) were tested, and the generated deuterated n-alkanes were analyzed using the HRGC–EI/LRMS method. The deuterodechlorination rates exhibited an increasing tendency with the increase of the reaction time, whereas higher temperature depressed the generation of the corresponding deuterated n-alkanes (Tables S3 and S4). The highest deuterodechlorination rates for the reduction of SCCPs was obtained at 90 °C for 96 h. Under this condition, 56.4% and 47.6% of SCCPs in two tested SCCP mixture standards (51% Cl and 63% Cl) have been deuterodechlorinated to the corresponding deuterated n-alkanes, respectively. During the deuterodechlorination reaction of SCCPs with LiAlD$_4$, deuterated n-alkenes were generated (Figure S2). The relative abundance of the deuterated n-alkenes decreased with the decreasing of the reaction temperature. A small quantity of low chlorinated deuterated n-alkanes was also formed during the deuterodechlorination reaction (Figure S3), which was discussed in detail in the Supporting Information.

Fractionation and MS Detection. The formed deuterated n-alkanes were eluted from a DB-5 column, in which the deuterated n-alkanes were completely separated according to the number of carbon atoms (Figure 1a). The chromatography
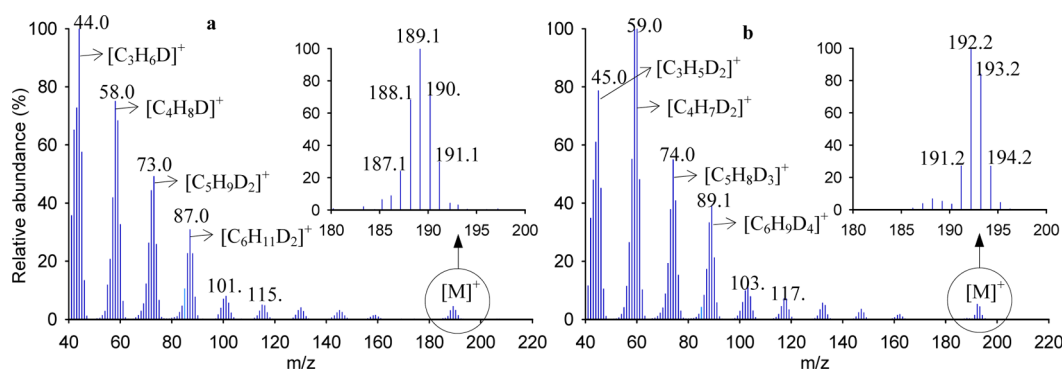

Figure 1. HRGC–EI/HRMS TIC (a) and SIM (b) chromatograms of deuterated n-decane (C$_{10}$), deuterated n-undecane (C$_{11}$), deuterated n-dodecane (C$_{12}$), and deuterated n-tridecane (C$_{13}$), respectively, produced from the deuterodechlorination of the SCCP mixture standard (C$_{10-13}$, 51% Cl). D$_1$ to D$_{13}$ represent the number of deuterium atom substitution in the corresponding deuterated n-alkanes.

profiles of the formed deuterated n-alkanes were similar to the n-alkane distribution patterns determined by carbon skeleton reaction with GC/FID (Figure S4).

The EI mode was used to ionize the individual deuterated n-alkanes. Figure 2 illustrated the EI mass spectra of the deuterated n-tridecane generated from the deuterodechlorination of the SCCP mixture standard (C$_{10-13}$, 51% Cl) and the synthesized SCCP calibration standard (C$_{13}$, 65.3% Cl), respectively. The resultant mass spectra were dominated by the fragment ion clusters with low mass-to-charge ratios (m/z), such as $[C_3H_6D]^+$, $[C_3H_5D_2]^+$, $[C_4H_8D]^+$, $[C_4H_7D_2]^+$, $[C_5H_9D_2]^+$ $[C_5H_8D_3]^+$, $[C_6H_{11}D_2]^+$, and $[C_6H_9D_4]^+$. Meanwhile, the molecular ion clusters, $[M]^+$, of the deuterated n-alkanes with different deuterium atom numbers, reflecting the chlorine distributions of SCCPs, were also detected (enlarged images in Figure 2). Because of the low abundances of the molecular ion clusters, HRMS was used to improve the selectivity and minimize matrix interference as well as interferences from deuterated n-alkenes, low-chlorinated deuterated n-alkanes, and ^{13}C isotope (details in the Supporting Information). To achieve simultaneous quantification of individual SCCP congeners with different carbon and chlorine

Figure 2. LRMS spectrum of the yielding deuterated *n*-tridecane from the deuterodechlorination of the SCCP mixture standard (C$_{10-13}$, 51% Cl) (a) and the synthesized SCCP calibration standard (C$_{13}$, 65.3% Cl) (b) in full scan mode. [M]$^+$ represents the molecular ion clusters.

numbers, the SIM mode was adopted to monitor the molecular ion clusters of the formed deuterated *n*-alkanes. The SIM chromatograms of the deuterated *n*-alkanes generated from the deuterodechlorination of the SCCP mixture standard (C$_{10-13}$, 51% Cl) is shown in Figure 1b. The influence of the deuterium atom numbers on the response factors of the deuterated *n*-alkanes was investigated. As compared with that of *n*-octane, the relative response factor of the molecular ion [M]$^+$ of *n*-octane-d$_{18}$ (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) only increased by 0.16-fold (Figure S5). This result reveals that the deuteration degree does not exhibit an obvious impact on the signal intensities of the individual deuterated *n*-alkanes generated from SCCPs usually containing 3−10 chlorine atoms. Therefore, the chlorine distribution of SCCPs can be calculated according to the deuteration degree of the formed deuterated *n*-alkanes with different deuterium atom numbers. The calculated chlorine contents in two tested SCCP mixture standards (51% Cl and 63% Cl) were 50.0% and 60.9%, respectively. The chlorine distribution on the fixed carbon chain is nearly conformed to a Gaussian curve (Figure S6).

Quantitative Analysis of SCCPs in Samples. An internal standard calibration method for SCCP quantification was developed. Branched C$_{10}$-CPs and branched C$_{12}$-CPs were used as the extraction and the reaction internal standards, respectively. They have not been detected in commercial chlorinated paraffins, environmental samples, or biota samples (see the Supporting Information). Similar behaviors of the extraction internal standard were found in sample extraction and column cleanup procedures with SCCPs. The average recoveries of branched C$_{10}$-CPs were calculated to be 92.5% (see the Supporting Information). The deuterodechlorination rates of branched C$_{10}$-CPs and branched C$_{12}$-CPs were also evaluated, which were calculated to be 50.7% and 55.1%, respectively, under the optimized condition. Their deuterodechlorination products, deuterated 2-methylnonane and deuterated 2-methylundecane, can be completely separated from the deuterated *n*-alkanes on the DB-5 column. The molecular ion clusters of the deuterated branched-chain alkanes exhibited relative lower signal intensities due to the isomeric effects. By comparing the standard mass spectra from NIST database, the relative signal intensity of 2-methylnonane was found to be about 20% of the value of *n*-decane. In this study, the extraction internal standard and straight-chain C$_{10}$-CPs have similar deuterodechlorination rates; however, the relative signal intensity of the generated deuterated 2-methylnonane

was only about 15% of the value of the generated deuterated *n*-decane.

To quantify the individual SCCP congeners, the relative correction factor (RCF$_i$) of individual deuterated *n*-alkane with fixed carbon chain length (*i*) generated from the SCCP calibration standard compared to the deuterated 2-methylnonane generated from the extraction internal standard was calculated according to eq 1

$$RCF_i = \frac{Q_{\text{d-IS}}}{Q_{\text{d-CS},i}} \times \frac{TA_{\text{d-CS},i}}{TA_{\text{d-IS}}} \quad (1)$$

where $Q_{\text{d-IS}}$ and $Q_{\text{d-CS},i}$ are the theoretical masses of deuterated 2-methylnonane and deuterated *n*-alkanes generated from the extraction internal standard and the SCCP calibration standard, respectively; $TA_{\text{d-IS}}$ and $TA_{\text{d-CS},i}$ are the actually measured total peak areas of deuterated 2-methylnonane and deuterated *n*-alkanes generated from the extraction internal standard and the SCCP calibration standard, respectively. The total peak area was the sum of the peak areas of individual deuterated alkanes with different deuterium atom numbers monitored in SIM mode. Three replicates were applied for each SCCP calibration standard. The relative standard deviations of RCF$_i$ values for each SCCP calibration standard with fixed carbon chain length and different chlorine contents were within 30% (Table S5).
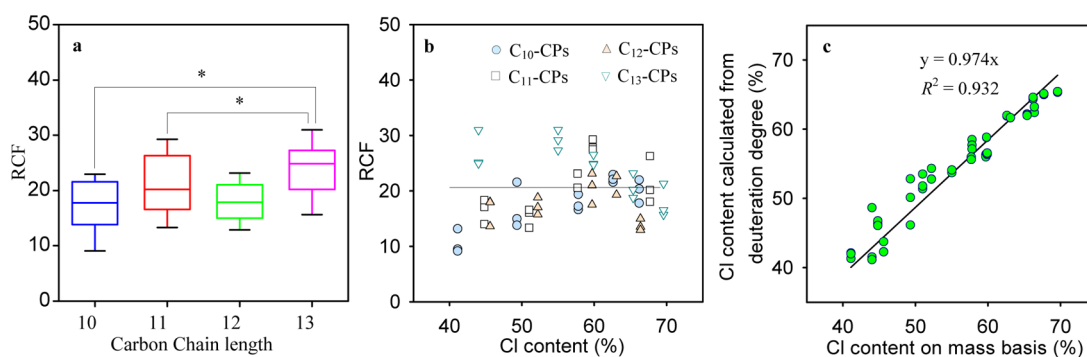
The theoretical masses of deuterated 2-methylnonane and deuterated *n*-alkanes, *i.e.* $Q_{\text{d-IS}}$ and $Q_{\text{d-CS},i}$, can be calculated according to eqs 2 and 3, respectively

$$Q_{\text{d-IS}} = Q_{\text{IS}} \times \left(1 - \frac{M_{\text{Cl}} - M_{\text{D}}}{M_{\text{Cl}}} \times K_{\text{IS}}\right) \quad (2)$$

$$Q_{\text{d-CS},i} = Q_{\text{CS},i} \times \left(1 - \frac{M_{\text{Cl}} - M_{\text{D}}}{M_{\text{Cl}}} \times K_{\text{CS},i}\right) \quad (3)$$

where Q_{IS} and $Q_{\text{CS},i}$ are the masses of the extraction internal standard and the SCCP calibration standard, respectively; M_{Cl} and M_{D} are the molecular weights of the Cl atom (average: 35.5) and the D atom, respectively; and K_{IS} and $K_{\text{CS},i}$ are the chlorine contents (mass percentage) of the extraction internal standard and the SCCP calibration standard, respectively.

Combining eqs 1−3, the RCF$_i$ values of SCCP homologues with fixed carbon chain lengths can be obtained. According to the calculation procedure described above, the obtained RCF$_i$ generally reflects the deviation in the deuterodechlorination rates, sample preparation, and instrumental response between deuterated *n*-alkanes and deuterated branched-chain alkanes. As

Figure 3. Variation of RCF values with carbon chain lengths (a) and chlorine contents (b) of SCCPs as well as the correlation between the chlorine contents calculated from the deuteration degree and those calculated from the weight differences (c). A total of 20 synthetic SCCP calibration standards with fixed carbon chain lengths (C_{10}, C_{11}, C_{12}, and C_{13}) were adopted, each of which consisted of 5 chlorine contents (41.1%−69.6%). Three replicates were applied for each SCCP calibration standard. * represents the significant differences of RCF values at the 0.05 level.

shown in Figure 3a, there are no statistically significant differences in RCF_i values among C_{10}-CPs, C_{11}-CPs, and C_{12}-CPs; whereas C_{13}-CPs showed higher RCF_i values compared with C_{10}-CPs and C_{12}-CPs. Meanwhile, for the different SCCP homologues with fixed carbon chain lengths, the RCF_i values did not present a consistent variation with the change of the chlorine content (Figure 3b). As a whole, the RCF_i values seemed to be independent of the chlorine contents. Therefore, the RCF_i values of individual SCCP homologues with fixed carbon chain lengths and different chlorine contents could be considered as constants.

In view of the similar deuterodechlorination probability of individual SCCP congeners, the abundances of deuterated n-alkanes with different deuterium atom numbers can reflect the quantities of the individual SCCP congeners. Therefore, the chlorine content of SCCPs with fixed carbon chain length (K_i, %) can be calculated from the deuteration degree of the formed deuterated n-alkanes and their relative abundances according to eq 4

$$K_i = \sum_{j}^{1-n} R_{i,j} \times \frac{j \times M_{Cl}}{M_{formula}} \tag{4}$$

where j is the number of deuterium atoms, and n is the maximum number of deuterium atoms. $M_{formula}$ is the molecular weight of the individual SCCP congener, which can be calculated by the molecular formula. $R_{i,j}$ is the relative abundance (%) of each deuterated n-alkane with fixed carbon chain length (i) and a different number of deuterium atoms (j) compared to the total amount of the individual deuterated n-alkane with fixed carbon chain length (i), which can be calculated according to eq 5

$$R_{i,j} = \frac{A_{d-CS,i,j}}{TA_{d-CS,i}} \times 100\% \tag{5}$$

where $A_{d-CS,i,j}$ is the peak area of the deuterated n-alkane with fixed carbon chain length and fixed deuterium atom number generated from the SCCP calibration standard. It was found that the calculated chlorine contents in SCCP calibration standards were very close to those values measured by the weight difference, with a linear slope factor of 0.97 (Figure 3c).

In view of the fact that individual SCCP homologues with fixed carbon chain length and different chlorine contents had similar RCF values, an average RCF_i value ($\overline{RCF_i}$) was used for the quantification of individual SCCP homologues in actual samples. According to eq 1, the following equation can be obtained

$$\overline{RCF_i} = \frac{Q_{d-IS}}{Q_{d-sample,i}} \times \frac{TA_{d-sample,i}}{TA_{d-IS}} \tag{6}$$

where $Q_{d-sample,i}$ and $TA_{d-sample,i}$ are the actually determined mass and total peak area of the deuterated n-alkanes with fixed carbon chain length (i) generated from SCCPs in the sample, respectively. The total peak area was the sum of the peak areas of individual deuterated n-alkanes with fixed carbon chain length and different deuterium atom numbers monitored in SIM mode.

According to eq 3, $Q_{d-sample,i}$ can be expressed by the following equation

$$Q_{d-sample,i} = Q_{sample,i} \times \left(1 - \frac{M_{Cl} - M_D}{M_{Cl}} \times K_{sample,i} \right) \tag{7}$$

where $Q_{sample,i}$ and $K_{sample,i}$ are the mass and chlorine content of the individual SCCP homologue with fixed carbon chain length (i) in the sample, respectively. $Q_{sample,i}$ can be expressed by eq 8 after combining eqs 6 and 7
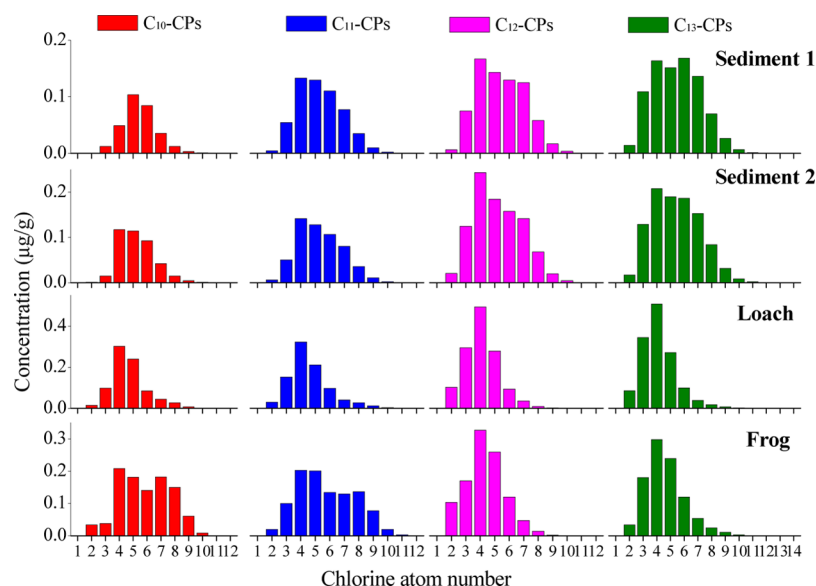
$$Q_{sample,i} = \frac{Q_{d-IS}}{\overline{RCF_i}} \times \frac{TA_{d-sample,i}}{TA_{d-IS}} \times \frac{M_{Cl}}{M_{Cl} - (M_{Cl} - M_D) \times K_{sample,i}} \tag{8}$$

According to eq 8, the total mass of SCCPs in the sample (Q_{sample}) can be calculated by the following equation

$$Q_{sample} = \sum_{i}^{10-13} \left[\frac{Q_{d-IS}}{\overline{RCF_i}} \times \frac{TA_{d-sample,i}}{TA_{d-IS}} \times \frac{M_{Cl}}{M_{Cl} - (M_{Cl} - M_D) \times K_{sample,i}} \right] \tag{9}$$

Considering the chlorine atoms in SCCPs is one-to-one replaced by deuterium atoms, the mass of the individual SCCP congeners with fixed carbon chain length and fixed number of chlorine atoms ($Q_{sample-i,j}$) can be calculated based on the relative abundance ($R_{i,j}$) of the individual deuterated n-alkanes as follows

$$Q_{sample-i,j} = Q_{sample,i} \times R_{i,j} = \frac{Q_{d-IS}}{\overline{RCF_i}} \times \frac{TA_{d-sample,i}}{TA_{d-IS}}$$
$$\times \frac{M_{Cl}}{M_{Cl} - (M_{Cl} - M_D) \times K_{sample,i}} \times R_{i,j} \tag{10}$$

Figure 4. Congener profiles of SCCPs in sediment and biota samples determined by the method of deuterodechlorination combined with HRGC−EI/HRMS.

where i and j are the carbon chain length and number of chlorine atoms, respectively. Tables S6 and S7 listed the $R_{i,j}$ values for each deuterated n-alkane in actual samples. The sum of $R_{i,j}$ values for an individual homologue with a fixed carbon chain length (i) was 1.

Method Performance. Method performance of the whole analytical procedure was investigated according to EURA-CHEM and EPA guidelines.[27,28] Method selectivity and linearity, instrumental detection limit (IDL) of n-alkanes, and method detection limit (MDL) of SCCPs have been evaluated in the Supporting Information. There was a good linearity (R^2 > 0.99) in the range of 17−212 μg/L for the four kinds of n-alkanes with different carbon chain lengths, n-decane, n-undecane, n-dodecane, and n-tridecane n-alkane, respectively (Figure S7). The IDL value for n-alkanes (C_{10}−C_{13}) was estimated to be 1.5 pg at a signal-to-noise ratio (S/N) of 3:1, respectively. The blank sediment sample was prepared using Soxhlet extraction until no detectable quantities of SCCPs analyzed by the HRGC−ECNI/LRMS method. The MDL value for total SCCPs, calculated as 3-fold the standard deviation of SCCPs in blank sediment samples, was 33 ng/g (n = 8). The value was a little higher than that by the ECNI/LRMS method (14 ng/g)[3], which was undoubtedly sensitive for high chlorinated SCCPs. The average chlorine content of SCCPs in blank sendiment samples was calculated to be 55.0%.

Four kinds of SCCP homologue stock standard solutions (C_{10}-CPs, 60.09% Cl; C_{11}-CPs, 55.2% Cl; C_{12}-CPs, 65.08% Cl; C_{13}-CPs, 65.18% Cl; 10 ng/μL in cyclohexane) and a SCCP mixture stock standard solution (C_{10-13}; 51% Cl, 100 ng/μL in cyclohexane) were used to test the reliability of deuterodech-lorination combined with the HRGC−EI/HRMS method for the analysis of SCCPs. The determined mass and chlorine content of SCCPs were shown in Table S8. The relative errors between the expected and the determined values were within 21% for the quantification of total SCCPs and within 6% for the chlorine content. The relative standard deviations of the determined values were within 10% for the concentration of total SCCPs and within 1% for the chlorine content.

The method repeatability and precision for the analysis of SCCPs in environmental samples were evaluated using the blank sediment sample. Approximately 5 g of the blank sediment sample was spiked with the SCCP homologue stock standard solutions (C_{10}-CPs, 60.09% Cl; C_{11}-CPs, 55.2% Cl; C_{12}-CPs, 65.08% Cl; C_{13}-CPs, 65.18% Cl; 10 ng/μL in cyclohexane) and the internal standards, respectively. Sample pretreatment, deuterodechlorination reaction, and quantification of SCCPs were conducted. The relative errors between the expected and the determined values were within 26% for the quantification of total SCCPs and within 7% for the chlorine content (Table S9). The relative standard deviations of the determined values were within 10% for the concentration of total SCCPs and within 1% for the chlorine content.

The recoveries of the extraction standard (R_{ex}) and the reaction internal standard (R_{re}) have been used for the evaluation of the sample pretreatment and deuterodechlorina-tion reaction in samples, respectively. They were calculated as described in detail in the Supporting Information, taking the EPA 1613 method as a reference.[28] The recoveries of the extraction internal standard throughout the sample pretreat-ment were above 75% (Table S9), while the recoveries of the reaction internal standard were above 79% (Tables S8, S9).

Analysis of SCCPs in Commercial CP Products and Environmental Samples. The concentrations and chlorine contents of SCCPs and their individual homologue groups in commercial CP products, sediment, and biota samples, respectively, were analyzed. The contents of SCCPs in the three commercial CP mixtures, CP-42 (CPs with a chlorine content of 42%), CP-52, and CP-70, were determined to be 3.1%, 40.2%, and 1.7%, respectively (Table S10). The corresponding chlorine contents of the SCCPs were determined to be 42.9%, 54.9%, and 65.2%, respectively. The contents of SCCPs in CP-42 and CP-70 products were relatively low (<5%), while high content of SCCPs was found in CP-52. The results were also in agreement with those values determined by HRGC−ECNI/LRMS.[12] Significant differences in SCCP homologue profiles have been found among the three commercial CP products (Figure S8). C_{10}-CPs predominated in the congener profiles in CP-42, while the amounts of the four homologues were comparable in CP-52 and CP-70 products. The chlorine profiles of the SCCP homologues

with fixed carbon chain length almost exhibited a Gaussian distribution. Similar chlorine contents of the individual SCCP homologues have been found. The most abundant chlorine congeners were Cl_{2-4} congeners in CP-42, Cl_{5-6} congeners in CP-52, and Cl_{8-10} congeners in CP-70, respectively.

The concentrations of total SCCPs in two sediment samples were determined to be 2.71 $\mu g/g$ dry weight (dw) and 3.28 $\mu g/g$ dw, with chlorine contents of 52.9% and 52.4%, respectively (Table S11). The concentrations of the total SCCPs in the loach and frog samples were determined to be 4.92 $\mu g/g$ dw and 4.49 $\mu g/g$ dw, respectively, corresponding to the chlorine contents of 47.6% and 52.0%, respectively (Table S11). A near Gaussian distribution was also found in the chlorine profile of the SCCP homologues with fixed carbon chain length in the sediment and biota samples (Figure 4). The longest chain C_{13}-CPs were the predominant congeners of SCCPs in sediment samples, followed by C_{12}-CPs, C_{11}-CPs, and C_{10}-CPs. The Cl_{4-6} congeners were the most abundant congeners in the sediment samples. Smaller differences among the concentrations of the four SCCP homologues were indicated in biota samples. The Cl_4 congeners showed the highest abundances in the loach sample, while more congeners have been detected in the frog sample. It was found that Cl_{4-8} congeners for C_{10}-CPs and C_{11}-CPs and Cl_{3-5} congeners for C_{12}-CPs and C_{13}-CPs predominated in the congener profile.

In addition, we analyzed the sediment and biota samples by the HRGC−ECNI/LRMS method (Table S12). As compared to the HRGC−ECNI/LRMS analysis, the results obtained by deuterodechlorination combined with HRGC−EI/HRMS showed higher concentrations of total SCCPs and lower chlorine content (Tables S11, S12). The concentrations and chlorine contents of SCCPs determined by the deuterodechlorination combined with HRGC−EI/HRMS were 1.7−13.6 times and 0.82−0.90 times the values determined by the HRGC−ECNI/LRMS method, respectively. This result should be partly attributed to the detection of a larger quantity of low chlorinated SCCP congeners (Cl_{1-4}), which are ignored by the HRGC−ECNI/LRMS analysis.[29] In our study, SCCPs with Cl_{1-4} accounted for 32.4%−62.4% of the total SCCPs in the tested sediment and biota samples. The large quantitive difference of total SCCPs between carbon skeleton analysis and GC−ECNI/LRMS analysis was also observed by Hussy et al., who found that the concentrations of SCCPs in sediments determined by carbon skleton reaction combined with GC/FID were 0.4−50 times the values determined by GC−ECNI/LRMS.[30]

In this work, we present a new analytical method for SCCPs, i.e. deuterodechlorination combined with HRGC−EI/HRMS. This analytical approach can achieve accurate quantification of individual SCCP congeners, and the method reliability can be ensured by internal standard calibration. This method overcomes some disadvantages of the previously reported ECNI/MS methods. SCCP congeners with Cl_{1-4} can be detected, and the calculated concentration of SCCPs does not considerably depend on the distribution profile of SCCP congeners in the selected analytical standards. The present method can simultaneously acquire both carbon and chlorine profiles of SCCPs, which guarantees a promising application in further studies on the toxicological and environmental characteristics of SCCPs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05115.

Detailed information on chemicals and reagents, sample collection and pretreatment, instrumental analysis, generation of byproducts and their interferences, method performance, calculation of the deuterodechlorination rates and recoveries; tables showing the calibration standards for method development (Table S1), m/z values of the molecular ions $[M]^+$ for the deuterated n-alkanes (Table S2), deuterodechlorination rates and calculated chlorine contents of SCCPs (Tables S3, S4), calculated RCF and $R_{i,j}$ values (Tables S5−S7), the quantitative results of SCCP congeners in different stock standard solutions, commercial CPs, sediment and biota samples (Tables S8−S11), and the concentrations and chlorine contents of SCCPs in sediment and biota samples analyzed by HRGC−ECNI/LRMS (Table S12); figures showing the reduction of SCCPs with $LiAlD_4$ (Figure S1), mass spectra and HRGC−EI/LRMS chromatogram for the products from the deuterodechlorination (Figures S2, S3), the SCCP homologue profiles determined by the deuterodechlorination combined with HRGC−EI/HRMS and carbon skeleton reaction with GC/FID (Figure S4), the dependence of the relative response factor on the deuteration degree of n-octane (Figure S5), congener profiles of SCCPs in standard mixtures and commercial CP mixtures (Figures S6, S8), and linear correlation between the n-alkane concentrations and its relative peak areas determined by HRGC−EI/HRMS (Figure S7) (PDF)

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Notes

The authors declare the following competing financial interest(s): The patent (CN104181266 A) is relevant to the deuterodechlorination reaction and quantification method principle of SCCPs and MCCPs. The data about SCCP quantification in this manuscript have not been published in the patent.

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