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Method validation and comparison of quantification strategies for analysis of chlorinated paraffins in indoor dust by liquid chromatography and high-resolution mass spectrometry

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Abstract

This paper describes the validation of a method for the simultaneous analysis of short-, medium-, and long-chained chlorinated paraffins (SCCPs, MCCPs, and LCCPs, respectively) in indoor dust by ultrasonic extraction and liquid chromatography quadrupole time-of-flight high-resolution mass spectrometry (LC-QTOF-HRMS). A series of spike and recovery experiments (n = 54) were conducted using CPs with varying carbon-chain lengths, chlorination degree, and concentrations. Technical standard mixtures of the SCCPs, MCCPs, and LCCPs were used to quantify spiking experiments by two commonly used calibration procedures: pattern deconvolution and chlorine-content calibration. The results quantified by pattern deconvolution meet the acceptability limits of the European Union Reference Laboratory (EURL) for all tests with trueness ranging from 72% to 141% and good precision represented by coefficients of variation (CVs) less than 15% in all experiments. The chlorine-content calibration also performed well overall, but on average overestimated concentrations for SCCPs and MCCPs by 32% and 25%, respectively, and did not meet the EURL's trueness limits in all cases. CVs were below 18% for all results derived from the chlorine-content quantification. The final method was successfully applied to indoor dust samples from offices (n = 4), homes (n = 3), and a vehicle (n = 1) from Melbourne, Australia, with SCCPs (C_{10-13}), MCCPs (C_{14-17}), and LCCPs (C_{18-20}) detected in all samples, up to 100, 240 and 190 μg/g, respectively. A preliminary human exposure assessment suggested that CP intake via dust may constitute a major pathway of exposure for populations in

Melbourne, Australia.

Keywords: Chlorinated paraffins, liquid chromatography, high-resolution mass spectrometry, indoor dust

INTRODUCTION

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated n-alkanes with the general chemical formula $C_xH_{2x+2-y}Cl_y$ and whose chlorination degree can vary between 30% and 70% by weight (wt %). They are typically divided according to their carbon chain length into short chain CPs (SCCPs, C_{10-13}), medium chain CPs (MCCPs, C_{14-17}), and long chain CPs (LCCPs, $C_{>17}$)[1]. CPs have been produced and used since the 1930s for a wide range of industrial applications including as additives in metalworking fluids and as flame retardants and plasticizers in polymers, paints, sealants and adhesives, textiles, and consumer products[2]. According to an investigation by Glüge *et al.*[3], CPs are produced in high volumes with an annual global production volume of more than 1 million tons since 2012, of which the worldwide production volume of SCCPs alone was estimated to be 165,000 tons per year.

CPs have been detected in a variety of environmental media[2,4-6] as well as human milk and serum[7,8]. Recent studies have shown that unintentional ingestion and inhalation of dust[5,6,9] and dietary intake[10,11] are among the most prominent pathways for human exposure to CPs. Due to their persistence, bioaccumulation potential, and toxic effects[12], international conventions and regulatory agencies have classified SCCPs as environmentally hazardous compounds and globally restricted their production[5,13]. The ban of SCCPs has thus caused a shift towards the production of MCCPs and LCCPs, whose safety is however also questioned[14,15].

CPs as applied within materials and products are composed of very complex mixtures of thousands of isomers differing by carbon chain length and chlorination degrees, which may also contain other hydrocarbon impurities[16]. Several analytical methods have been utilized, applying either gas or liquid chromatography (GC and LC, respectively) coupled with a range of mass spectrometric (MS) detectors. Among the most broadly applied methods has been GC-MS operated in single ion monitoring mode using electron capture negative ionization (ECNI)[16]. GC-ECNI/MS offers sensitive detection of $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ ions for individual SCCP and MCCP homologs using relatively simple instrumentation but suffers from partial isotopic overlap between homologs which cannot be resolved by low resolution (LR)MS or fully separated chromatographically[17,18]. Investigations into alternative GC applications such as electron impact ionization with triple-quadrupole MS or the use of an electron capture detector have also shown these methods to be highly sensitive but lacking in analyte information such as the homolog patterns generated by GC-ECNI/MS[18,19]. Recently, methods applying LC coupled to triple-quadrupole or high-resolution (HR)MS have become popular due to a number of advantages offered by the technologies. While GC analysis is generally restricted to SCCP and MCCPs due to the lower volatility of LCCPs, all three CP groups can be analyzed simultaneously by LC[10,18,20]. LC systems also offer the ability to introduce analytes directly to the MS without column separation for rapid sample analysis such as the direct injection HRMS method described by Bogdal *et al.*[21].

HRMS systems such as quadrupole time-of-flight (QTOF) and Orbitrap-MS have also gained popularity for CP analysis due to their ability to provide congener group level separation of CPs via accurate mass identification not afforded by LRMS instruments[20,22,23]. While neither GC nor LC can fully separate congener groups chromatographically, robust data on CP patterns in samples may be generated by HRMS[18]. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) modes have

both been applied effectively for CP analysis by monitoring $[M-H]^-$, $[M+Cl]^-$, or other ions[10,24,25]. The post-column addition of dichloromethane to the mobile phase has been shown to favor the formation of $[M+Cl]^-$ ions to enhance sensitivity and selectivity[21,26], and the same effect was achieved more recently by Zheng *et al.*[27] using a 0.05 mM NH_4Cl buffered mobile phase. While dichloromethane addition has become widespread for LC-HRMS analysis, the use of NH_4Cl as a mobile phase modifier[27] has not yet been widely applied for CP measurements.

Another important challenge in achieving reliable CP quantification is a lack of commercially available, well-characterized standards suitable for analysis of each of the SCCP, MCCP, and LCCP groups. While several single compounds and single carbon-chain length standards are available, technical mixture standards are often used to cover the broad range of CP compositions possible in samples[28,29]. Standards for LCCPs are particularly limited. Several innovative quantification procedures have been proposed to allow quantification of CPs at the ∑SCCP, ∑MCCP, and ∑LCCP level, as well as chain-length and congener group levels[20]. Among the most commonly applied quantification strategies are pattern deconvolution, as first described by Bogdal *et al.*[21], and the chlorine-content calibration procedure introduced by Reth *et al.*[30], which both attempt to compensate for the variations in total CP response factors according to overall chlorine content. Pattern deconvolution achieves this by algorithmically matching homolog patterns in samples to those in a combination of CP mixture standards to determine appropriate response factors for quantification[21,25], while chlorine-content calibration utilizes the correlation between the measured chlorine content and the summed response of CP homologs to determine suitable response factors[30]. Specific challenges relating to the availability of suitable standards or particular behavior of CP responses are inherent to all proposed quantification procedures such that no one method has predominated[18].

The objective of this study was to validate a method for the analysis of SCCPs, MCCPs, and LCCPs in indoor dust by ultrasonic extraction and LC-QTOF-HRMS. Series of fortification experiments encompassing varying congener group compositions and total concentrations were quantified using two of the most commonly applied quantification procedures, namely pattern deconvolution and chlorine-content calibration, to assess the respective performance of these methods in terms of trueness and precision. Both calibration methods were applied to indoor dust from Melbourne, Australia, to assess the applicability of the overall procedure and illustrate the CP congener group profiles present in real samples.

EXPERIMENTAL

Standards and reagents

Technical mixture standards of SCCP 51.5%Cl (Lot: G976504CY), SCCP 55.5%Cl (Lot: G986911CY), SCCP 63%Cl (Lot: G986931CY), MCCP 42%Cl (Lot: 417125CY), MCCP 52%Cl (Lot: G162888CY), MCCP 57%Cl (Lot: G991196CY) LCCP (C18-20) 36%Cl (Lot: 114017CY), and LCCP (C18-20) 49%Cl (Lot: 855677CY) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and individual standards of β-1,2,5,6,9,10-hexabromo[$^{13}C_{12}$]cyclododecane (^{13}C-β-HBCD) and d18-γ-1,2,5,6,9,10-hexabromocyclododecane (d18-γ-HBCD) were obtained from Wellington Laboratories (Guelph, Canada). Dichloromethane was purchased from Sigma Aldrich (Darmstadt, Germany), n-hexane from Acros Organics (Geel, Belgium), and methanol from Biosolve (Valgenswaard, Netherlands). Concentrated sulfuric acid (H_2SO_4, 98%) was obtained from Sigma Aldrich and silica gel (SiO_2, 70-230 mesh) was from VWR (Leuven, Belgium). Bond Elut silica cartridges (500 mg, 3 mL) were purchased from Agilent Technologies (Machelen, Belgium). All solvents were of pesticide analysis grade except for acetone, which was of technical grade for glassware cleaning.

Sampling and sample extraction

Indoor dust was sampled from offices (n = 4), homes (n = 3), and a vehicle (n = 1), denoted as O, H, and V, respectively, in Melbourne, Australia during 2016, as described by McGrath *et al.*[31]. Briefly, nylon socks of pore size 25 μm were inserted into the inlet nozzle of a domestic vacuum cleaner and used to sample dust from 1 m^2 of floor space in carpeted rooms and 4 m^2 for rooms with hard floors. Dust was sampled with the vacuum cleaner from the front interior seats, doors, and dashboard surfaces of the vehicle. Samples were sieved to a < 500 μm fraction and stored at 4 °C in darkness prior to analysis.

Dust samples (50 mg) were weighed into 15 mL pre-cleaned glass vials, spiked with 20 ng of internal standard (IS) ^{13}C-β-HBCD and extracted in 5 mL of a 3:1 mixture of n-hexane:dichloromethane using 1 min of vortexing and 10 min of ultrasonication. Extracts were centrifuged at 2000 rpm for 3 min, the supernatant transferred to clean vials, and the extraction repeated once more with fresh solvent. Extracts were purified by addition of 2 g of acidified silica (44% w/w), and then evaporated under nitrogen flow to incipient dryness and reconstituted in 0.5 mL n-hexane. The concentrated extracts were then loaded onto Agilent Bond Elut silica cartridges pre-conditioned by 6 mL of dichloromethane, positive pressure drying, and then 6 mL of n-hexane. Extracts were eluted from the cartridges using 6 mL of hexane (waste fraction) and 12 mL of dichloromethane (analysis fraction). The analysis fraction was evaporated under nitrogen and reconstituted to a final volume of 100 μL of methanol containing 20 ng of external standard (ES) d18-γ-HBCD. Procedural blanks (n = 4) and fortified procedural blanks (n = 54) were performed to the same specifications as dust samples without the presence of the dust matrix. Field blanks (n = 2) were collected with samples by "sampling" precleaned Na_2SO_4 from a piece of aluminum foil by the same method as dust collection. Field blanks were stored and analyzed in the same manner as dust samples.

Instrumental analysis

All analyses were performed on an Agilent 1290 Infinity II LC coupled to an Agilent 6560 QTOF-MS operated in electrospray ionization negative mode (ESI-). Injections of 5 μL were delivered onto an Agilent Zorbax Eclipse Plus C18 Rapid Resolution HD column (2.1 mm × 50 mm, 1.8 μm pore size) fitted with a matching guard column of 5 mm length. The mobile phase consisted of (A) Milli-Q water and (B) methanol containing 0.025 mM NH_4Cl, which was applied using a gradient program that evolved linearly from 10% B to 95% B from 0 to 3 min, held at 95% B from 3 to 9 min, returned linearly to 10% B from 9 to 10 min, and remained at 10% B for 2 min. The flow rate was 0.2 mL/min, and the column temperature was maintained at 40 °C. MS acquisition was performed in full scan mode from 100 to 1700 m/z using real-time calibration of mass accuracy via reference standards of purine (119.0360 m/z) and hexakis phosphazine (966.0007 m/z) delivered to the MS during analysis. The MS gas temperature was 300 °C, the drying gas flow was 8 L/min, the nebulizer pressure was 40 psi, the sheath gas temperature was 300 °C, and the sheath gas flow rate was 6.5 L/min. The fragmentor and nozzle voltages were set to 300 and 100 V, respectively.

Analytes were identified and integrated using Agilent MassHunter Quantitative Analysis Version 10.0. The first and second most abundant isotopes for $[M+Cl]^-$ ions representing CP homologs ranging from C_{10} to C_{25} and Cl_4 to Cl_{12} were extracted with a mass accuracy tolerance of 20 ppm [Supplementary Table 1 and 2]. Analytes were considered detected when the retention time of peaks matched those of technical mixture standards within ±5%, isotope ratios were within ±20% of theoretical values, and the signal to noise ratio exceeded 3.

Quantification

Two separate quantification strategies were applied to the acquired sample data to assess the applicability of the methods. The pattern deconvolution quantification procedure was first described by Bogdal *et al.*[21] and was applied in this study using the open-source programming code provided by Perkons *et al.*[24] in RStudio

software Version 1.2.1335. Six-point calibrations at total concentrations ranging from 0.125 to 4.0 μg/mL were prepared separately for each of the eight CP technical standard mixtures, each containing the IS and ES at 200 ng/mL. Relative response factors were determined for each of the analyzed CP homolog groups from the eight CP calibration curves according to the total CP concentration in each calibrant. The pattern deconvolution algorithm provided by Perkons *et al.*[24] was then used to determine the linear combination of the prepared reference technical mixture standards that best approximated the homolog relative response distribution measured in samples. ∑SCCP, ∑MCCP, and ∑LCCP quantification was then performed using the supplied algorithm to apply a combination of relative response factors which corresponds to the proportions of individual CP technical mixtures utilized for the pattern reconstruction. A detailed description of the pattern deconvolution procedure was described by Perkons *et al.*[24], and the R code applied is available in GitHub (https://github.com/ingusperkons/CP-Crawler).

The chlorine-content calibration procedure was modified from the methods first described by Reth *et al.*[30] for quantification of SCCPs and MCCPs from GC-ECNI/MS analysis data. In the present study, technical CP standards were used to prepare calibration curves at five degrees of chlorination separately for each of the SCCP, MCCP, and LCCP groups at fixed ∑SCCP, ∑MCCP, and ∑LCCP concentrations of 4.0 μg/mL each, respectively. Each calibrant contained the IS and ES at 200 ng/mL to correspond with concentrations added to samples. Relative response factors were derived from the summed relative responses of CP homologs measured in calibrants, plotted against calculated chlorine-contents in respective standards, and fitted with exponential regression curves. The chlorine-contents and total relative response measured in samples were then used to derive ∑SCCP, ∑MCCP, and ∑LCCP concentrations using the respective calibration equations. Full details of the chlorine-content calibration procedure are given by McGrath *et al.*[17] and in Section S1 of the Supplementary Information, and calibration curves are shown in Supplementary Figure 1.

Response of the ^{13}C-β-HBCD IS was used to derive relative responses for individual CP homolog groups for both applied quantification procedures. While suitable isotopically labeled CP standards remain commercially unavailable[28], ^{13}C-β-HBCD was selected for use as an IS as it behaves similarly to CPs during sample preparation (e.g., it is found in the same fraction as the CPs during the silica fractionation step) and has been found to perform well in correcting for extraction losses and analytical variability[10].

Statistics

Statistical calculations were performed using Microsoft Excel 16. Percentage trueness was defined in fortified blank tests as the measured concentration in extracts divided by the known fortification concentration multiplied by 100. Precision was expressed as the coefficient of variation (CV) between measurements in triplicated fortified blank tests, i.e., the standard deviation between measurements divided by the average of measurements multiplied by 100. For assessment of the difference in values in dust samples as calculated by pattern deconvolution and chlorine-content calibration procedures, percentage differences were determined as the absolute difference between the two values divided by the average of the values multiplied by 100.

Estimates of CP intake via ingestion of indoor dust were calculated by the procedure described by McGrath *et al.*[31], as detailed in Section S2.

RESULTS AND DISCUSSION

Technical standard impurities and implications for method validation

A common and practical way to assess the accuracy and precision of an analytical procedure is to fortify a representative sample matrix with known levels of a given analyte and compare the measurement with the known, spiked concentration. Assessment of methods for CP analysis is, however, complicated by the lack of availability of well-defined standards. The individual technical standards used for calibration in this study were found to contain homologs not consistent with the defined SCCP, MCCP, and LCCP carbon-chain groupings. For example, on the basis of summed relative response, SCCPs only accounted for 71% of the CPs measured in the SCCP 55.5%Cl standard, with MCCPs and LCCPs constituting 17% and 12% of the mixture, respectively. The MCCP 52%Cl standard contained traces of SCCPs and LCCPs, each < 1%, while the LCCP 49%Cl standard mixture was found to contain the MCCP C_{17} homolog at a proportion of 29% of summed relative response. Furthermore, despite the LCCP standards having stated carbon-chain length compositions of only C_{18-20} homologs, congener groups of C_{21} and C_{22} were determined at low levels of each < 1% in the LCCP 49%Cl standard and homologs up to C_{25} were present in the LCCP 36%Cl standard.

Similar findings in technical standards from the same manufacturer, Dr. Ehrenstorfer, have been evidenced in other studies[27,32,33]. Zheng *et al.*[27] reported proportions of MCCPs at rates of up to 10.5% in SCCP technical mixtures and ranging from 11.2% to 29.3% in the LCCP standards. Separate analyses by Li *et al.*[33] and Huang *et al.*[32] each determined substantial levels of the MCCP C_{17} homologs in LCCP mixtures at proportions of 7%-21% and 13.5%-30.6%, respectively, while C_{21-27} homologs were also detected in the LCCP (C_{18-20}) standards at estimated proportions up to 34% of total response.

Since the exact proportion of CP impurities in the technical standards cannot be determined, spike and recovery tests must necessarily be performed from separate spikes of the SCCP, MCCP, and LCCP groups so the fortification level of any individual group can be known. In the present study, validation experiments were designed to assess the trueness and precision of the analytical method quantified by both pattern deconvolution and chlorine-content calibration procedures in a series of fortified procedural blank extractions. Due to the ubiquitously high levels of CPs measured in indoor dust, a "clean" dust matrix with which to conduct fortification testing was not available[34]. Nine individual CP spike mixtures, denoted A-I, were prepared to represent three different chlorine compositions for each of the SCCP, MCCP, and LCCP homolog groups, as detailed in Supplementary Table 3. Fortified blank extraction tests were performed for each of the A-I mixtures in triplicate at two concentration levels to total 54 individual extraction and analysis experiments. Low spike tests contained 100 ng (equivalent to a concentration of 2.0 μg/g dust based on a 50 mg dust extraction) and a high spike of 400 ng (equivalent to 8.0 μg/g dust) of ∑SCCPs, ∑MCCPs, or ∑LCCPs, respectively.

Trueness and precision

The results of the fortified blank assessment are shown in Figure 1. For SCCPs, individual measurements as quantified by the pattern deconvolution procedure ranged from 72% to 117% trueness among tests at both high and low spike levels. Trueness values were each within the acceptability limits of 50%-150% as applied in recent proficiency testing of CP analysis in food by the European Union Reference Laboratory (EURL)[35,36]. Average SCCP measurements determined by the chlorine-content procedure were higher than those of the pattern deconvolution method for each of the fortification scenarios with trueness among the individual samples ranging from 93% to 174% (4 of 18 measurements outside the EURL criteria). High precision was observed in the results obtained by both quantification procedures for SCCPs with CVs ≤ 15% in all cases. Similar results were observed for the MCCP assessment, with trueness derived from pattern deconvolution again meeting the EURL criteria in all instances with a range of 85%-141% among individual measurements. MCCP values quantified by the chlorine-content quantification typically overestimated

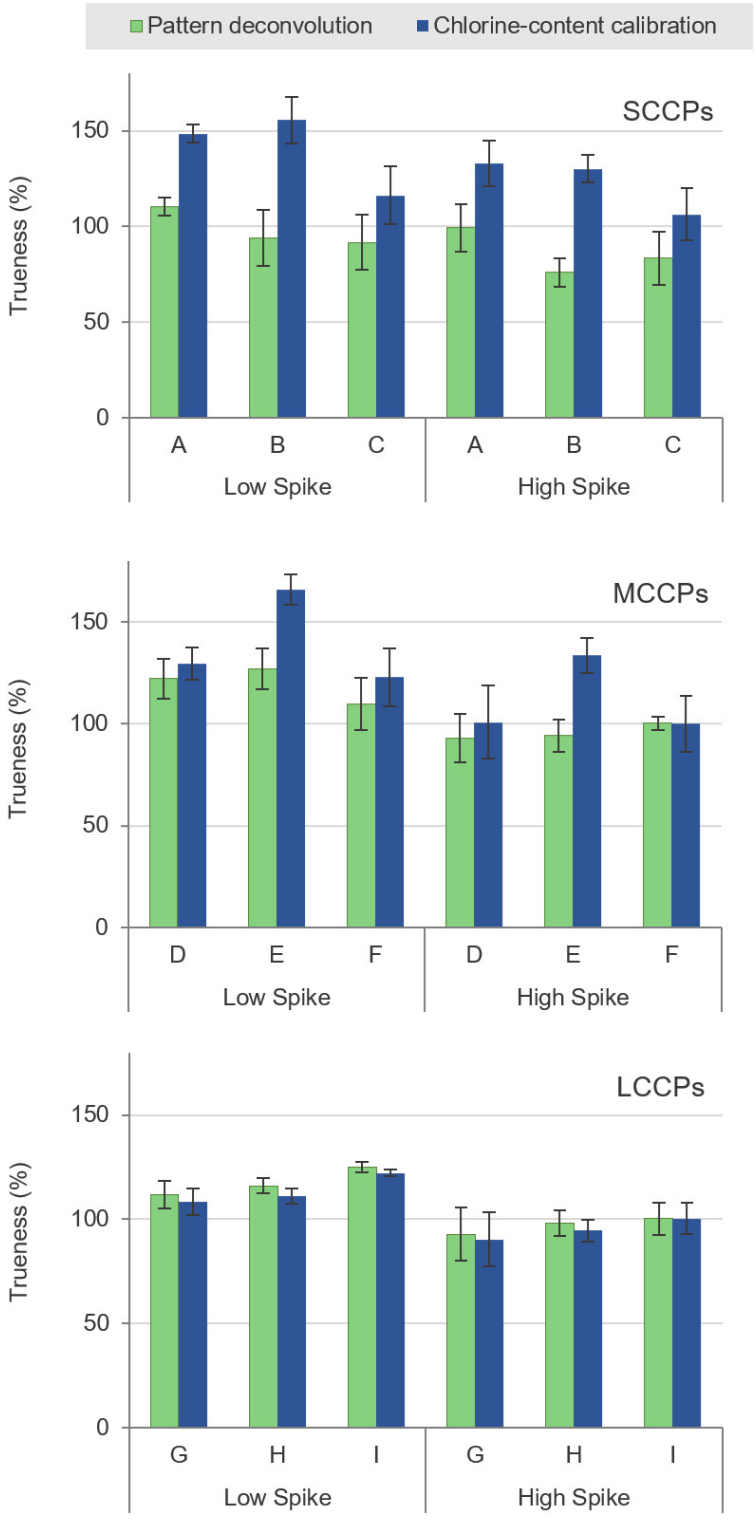

Figure 1. Trueness (%) of ∑SCCP, ∑MCCP, and ∑LCCP measurements of fortified blank extraction as quantified by pattern deconvolution or chlorine-content calibration. Low and high spike levels were 100 and 400 ng, respectively, of ∑SCCPs, ∑MCCPs, or ∑LCCPs prepared at total chlorination degrees denoted as A-I. Full fortification details are shown in Supplementary Table 3. Horizontal guidelines are included to highlight the target trueness value of 100% and the accuracy criteria range of 50%-150% as defined by the European Union Reference Laboratory (EURL)[35].

concentrations in the spike analyses, with trueness ranging 85%-178% and all but the low-level E spikes meeting the EURL criteria. Comparisons of MCCP CV values for each of the scenarios demonstrated marginally better precision in the pattern deconvolution results, all ≤ 13%, while CVs in the tests as computed by chlorine-content calibration were also acceptable with a maximum of 18%. The results for LCCP tests considering only the C_{18-20} homolog groups stated within standard concentrations are remarkably similar for both quantification strategies, with individual trueness ranging 80%-129% and 77%-124% for the pattern deconvolution and chlorine-content outputs, respectively. Precision was also similar between the two procedures and markedly better than that seen in the SCCP and MCCP analyses with the majority of CV values ≤ 8% and ≤ 13%, respectively.

Both the pattern deconvolution and chlorine-content approaches have been applied successfully for the analysis CPs in a wide range of matrices, although previous validation of these methods has not typically compared the calibration capabilities directly[17,23,24,37]. A range of 91%-123% trueness was recorded by Bogdal *et al.*[21] in SCCP, MCCP, and LCCP analyses of spiked sediment and fish by direct-injection APCI-QTOF-HRMS using the pattern deconvolution procedure, while Brandsma *et al.*[37] reported mean trueness ranging 86%-123% in similar analyses of spiked sewage sludge. Chlorine-content quantification of SCCPs in spiked fish and sediment samples analyzed by both GC-ECNI/MS and APCI-QTOF-HRMS resulted in low quantification error of 23% and 12%, respectively[23], although interlaboratory studies have shown application of this procedure to produce errors of up to 200%[38]. The results presented here indicate that the pattern deconvolution procedure performs significantly better than the chlorine-content calibration in terms of trueness across the multiple Cl% fortification scenarios for SCCPs and MCCP, while each of the methods demonstrated excellent accuracy for LCCP quantification.

Limits of detection and quantification

In this study, limits of detection (LODs) were determined as the lowest concentration of ∑SCCP, ∑MCCP, and ∑LCCP in which at least one congener group can be detected in both the lowest and highest Cl% technical standard mixtures. SCCP, MCCP, and LCCP LODs determined by these criteria were 10, 1.0, and 5.0 ng/mL, respectively, in calibrant solutions, corresponding to dust concentrations of 20, 2.0, and 10 ng/g [Table 1]. With regards to instrument sensitivity, these LODs are similar to those reported for other chlorine-enhanced LC-ESI-QTOF methods[27,39] and slightly higher than those determined for SCCP and MCCP analysis by GC/ECNI-Orbitrap-MS[40].

Limits of quantification (LOQs) were defined as the lowest ∑SCCP, ∑MCCP, and ∑LCCPs concentration at which at least half of the congeners detected in the highest concentration calibrants were also detected in both the lowest and highest Cl% standard mixtures. These criteria were selected following technical mixture standard dilution series analysis that revealed this to be the minimum level of congener group identification required for effective chlorine-content determination. Instrumental LOQs for SCCPs, MCCPs, and LCCPs were 100, 50, and 50 ng/mL, respectively, corresponding to concentrations of 200, 100, and 100 ng/g in dust, which were, again, similar to those of previous LC-ESI-QTOF analyses[27]. Lower LOQs were reported for SCCPs and MCCPs using GC-ECNI-Orbitrap[40] and SCCPs and LCCPs using LC-APCI-HRMS[1], but marginally higher ones were reported for GC-ECNI-LRMS[17].

Procedural blanks extracted and analyzed with each batch of 20 samples contained low but consistent levels of some C_{14} congener groups of Cl_{5-7} and C_{15} of Cl_{6-7}, while SCCPs and LCCPs were not detected. The number of congener groups detected in procedural blanks was below the proportion required for reliable quantification, and relative responses were substantially lower than those in the described MCCP LOQs, thus no blank subtractions were deemed necessary. Analysis of field blanks revealed that the sampling

Table 1. Limits of detection and quantification (LODs and LOQs, respectively) for SCCPs, MCCPs, and LCCPs

	Instrument (ng/mL)		Dust (ng/g)	
	LOD	LOQ	LOD	LOQ
SCCP	10	100	20	200
MCCP	1.0	50	2.0	100
LCCP	5.0	50	10	100

LODs: Limits of detection; LOQs: limits of quantification; SCCPs: short-chained chlorinated paraffins; MCCPs: medium-chained chlorinated paraffins, LCCPs: long-chained chlorinated paraffins.

protocol did not contribute CP contamination to the analyses. The IS was well recovered from all procedural blanks, fortified procedural blanks, and dust samples with an average (± CV) of 101% ± 16%. Acceptable limits of IS recovery for the method were defined as 50%-130%, such that recovery was not factored into the LOQ determinations.

Analysis of real samples

The applicability of the analytical method was demonstrated via simultaneous measurements of SCCPs, MCCPs, and LCCPs in eight real dust samples from offices, homes, and a vehicle from Melbourne, Australia. Concentrations derived by the pattern deconvolution and chlorine-content quantification methods were similar, as shown in Figure 2. ∑SCCPs ranged from 7.7 to 100 μg/g (median, 38 μg/g) by pattern deconvolution and 4.6 to 130 μg/g (median, 55 μg/g) by chlorine-content calibration. ∑MCCP concentrations were the dominant homolog group in all samples ranging from 62 to 240 μg/g (median, 140 μg/g) in pattern deconvolution measurements and 84 to 250 μg/g (median, 120 μg/g) according to chorine-content calculations. ∑LCCP (C_{18-20}) levels in the dust samples were similar to those of ∑SCCPs, ranging from 3.4 to 190 μg/g (median, 50 μg/g) by pattern deconvolution and 3.5 to 120 μg/g (median, 41 μg/g) by chlorine-content quantification.

Overall, the percentage differences in ∑CP concentrations in dust between the two quantification procedures had a maximum of 22% (O-3), while most samples differed by ≤ 15%, and two samples varied only by 1% each (O-4 and V-1). No particular trends could be discerned for levels of SCCPs, MCCPs, or LCCPs among the three indoor environment types (office, home, and vehicle).

The levels of CPs measured in the current study spanned a similar range to those determined in 44 samples of indoor dust from houses, offices, and vehicles in Brisbane and Canberra, Australia[6]. MCCPs were also the dominant group in the analysis by He *et al.*[6], ranging from 5.1 to 540 μg/g with a median of 95 μg/g. SCCPs ranged from 0.29 to 58 μg/g and LCCPs were detected in 86% of samples up to a maximum concentration of 27 μg/g in the same study, which reported overall concentrations to be generally greatest in vehicles, followed by offices and then houses. Studies of indoor dust from Canada[9], Norway[1], South Africa[5,41], and China have each demonstrated similar predominance of MCCPs and ∑CP concentrations in the mid-microgram per gram range, suggesting that CPs are some of the most prevalent organohalogen contaminants in the indoor environment[34].

The carbon-chain and chlorine number distribution of congener groups based on relative response in all dust samples are shown in Figure 3, with full congener group patterns shown for selected samples in Figure 4. While appropriate analytical standards are not available to allow for congener group-specific quantification, a comparison of the relative responses among the groups provides an indication as to the distribution of CP homologs, although the difference in compound sensitivity cannot be accounted for in these comparisons. SCCPs were dominated by the $\sum C_{13}$ homologs, which accounted for approximately half

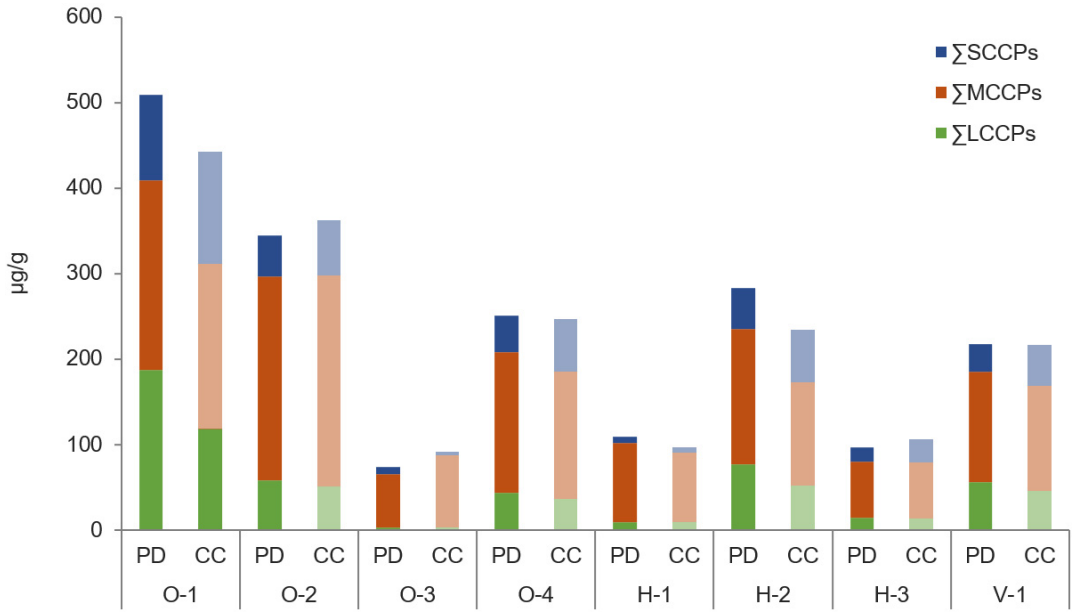

Figure 2. Concentrations of ∑SCCPs, ∑MCCPs, and ∑LCCPs determined by pattern deconvolution (PD) and chlorine-content calibration (CC) in office (O), home (H), and vehicle (V) dust samples from Melbourne, Australia.

of total relative response, with smaller levels of $\sum C_{12}$ and $\sum C_{11}$ and lower levels again of $\sum C_{10}$. The distribution of carbon-chain groups was more even for MCCPs with levels of $\sum C_{14}$ marginally higher than other groups, accounting for around 30%-45% of total relative response and the C_{15-17} congener groups present at similar levels. Of the LCCPs with stated concentration in the standards used for quantification, the $\sum C_{18}$, $\sum C_{19}$, and $\sum C_{20}$ relative response levels were approximately equal, while these congener groups alone only accounted for approximately 30%-60% of the total relative response among the C_{18-25} LCCPs measured. All C_{21-25} LCCP carbon-chain groupings were detected in each of the eight dust samples. While proportions differed by sample, summed relative responses for the $\sum C_{21}$ and $\sum C_{22}$ homologs were each only slightly lower than those of the $\sum C_{18-20}$ groups, with the $\sum C_{23}$, $\sum C_{24}$, and $\sum C_{25}$ groups slightly lower again. In at least one sample, O-1, the C_{21-25} groups not incorporated into ∑LCCP quantification dominated the overall relative response [Figure 4]. The distribution of chlorine number was mostly consistent between the dust samples for SCCPs and MCCPs, with a dominance of Cl_{7-8} observed in both groups. Greater variation in Cl distribution for LCCPs between dust samples was evident, but with Cl_{7-10} groups dominant overall.

The carbon-chain and chlorine distributions of CPs in dust have typically mirrored patterns observed in known commercial formulations of CPs[41] but may also be influenced by environmental processes affecting release rates from indoor sources and partitioning between dust and air[1]. The dominance of C_{13} homologs among SCCPs and C_{14} among the MCCPs measured in the Melbourne dust samples appears to correspond to a prevalence of these groups in CP-42 and CP-52 mixtures[42]. Various LCCP homolog patterns of C_{18-30} were observed among the three CP-52 and three CP-70 technical products analyzed by Li *et al.*[42], however, which may partly explain the more diverse LCCP patterns measured in dust. LCCPs are only now being analyzed more regularly in dust and other environmental samples with the advance of analytical technologies. Although the lack of appropriate analytical standards continues to prevent quantification of LCCPs of $C_{>20}$, homologs in this range have been indicated to represent up to 80% of the total LCCP response in some dust samples, with chain lengths up to C_{32} reported[1,5].

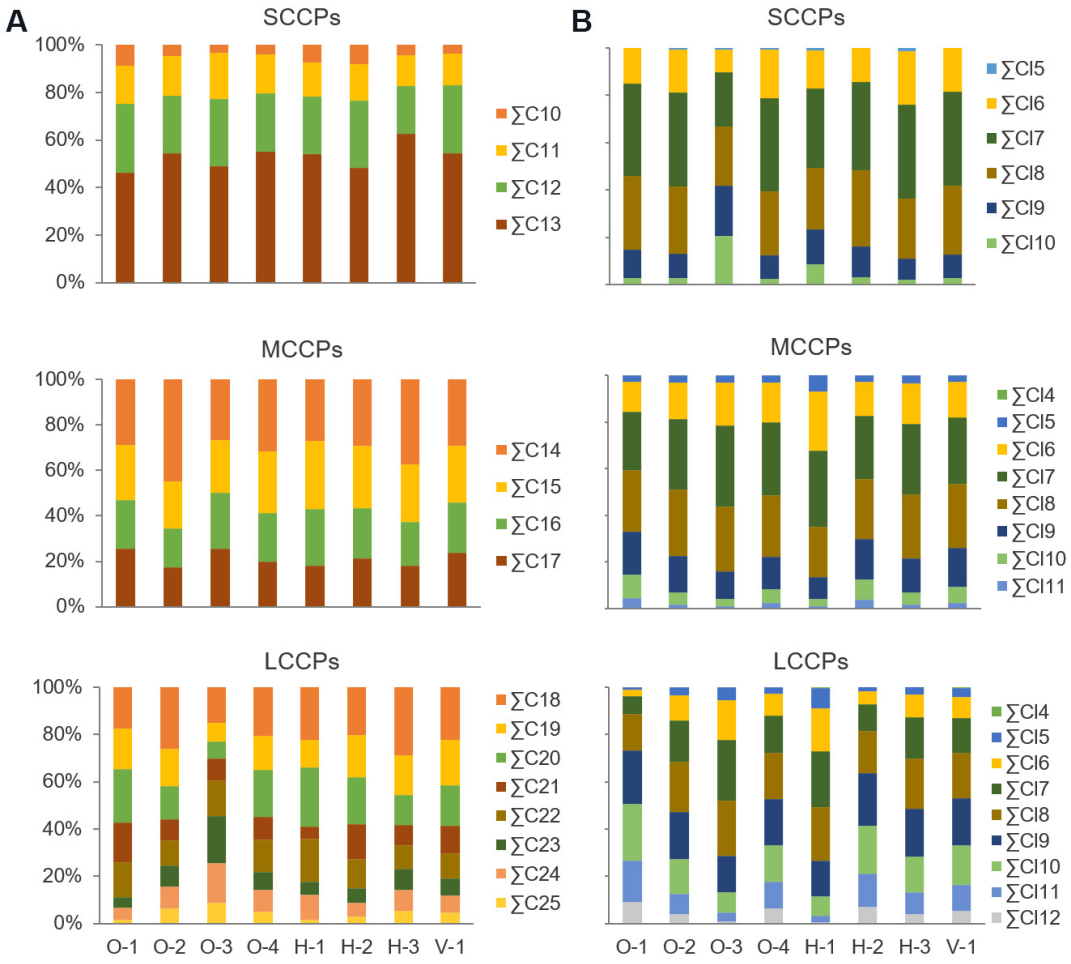

Figure 3. Proportional distribution of carbon-chain groups (A) and chlorine number (B) in office (O), home (H), and vehicle (V) dust from Melbourne, Australia, by relative response of individual CP congener groups.

Human exposure assessment

The concentrations of CPs measured in dust samples in the present study were used to calculate estimates of CP intake via indoor dust for adults and toddlers in Melbourne, Australia [Table 2]. Given the small number of samples, intake estimates were only computed at the median concentration level and provide a preliminary indication of exposure via dust which excludes contributions from time spent in vehicles. ∑CP(C_{10-20}) intake by adults according to mean and high dust ingestion scenarios was estimated to be 40 and 100 ng/kg bw/day, respectively, while toddlers were expected to experience higher exposure rates of 410 and 1700 ng/kg bw/day for the mean and high dust ingestion scenarios, respectively. Rates of exposure to ∑SCCPs and ∑LCCPs were similar for both age groups for both scenarios, while intakes of ∑MCCPs were estimated to be approximately 5 times greater for adults and 5.5-6 times higher for toddlers. The daily ∑CP intake estimates in this study were similar to those previously reported for Australian adults and toddlers, but higher than the rates estimated for Swedish populations[6,43]. Estimated rates of CP exposure from dust have been around 1-2 orders of magnitude greater in China, with much higher intake of SCCPs in particular[4,44]. With respect to dietary intake of SCCPs and MCCPs estimated by recent studies from Germany[11] and South Korea[45], it appears that CP ingestion via dust may represent a similar contribution to food intake for overall SCCP and MCCP exposure.

Table 2. Estimates of CP intake (ng/kg bw/day) via indoor dust ingestion for adults and toddlers

Age group	Dust ingestion scenario	∑SCCPs	∑MCCPs	∑LCCPs (C_{18-20})	∑CPs (C_{10-20})
Adult	Mean	6.1	30	6.1	40
	High	15	76	15	100
Toddler	Mean	63	350	56	410
	High	250	1400	220	1700

All estimates incorporate median CP concentrations derived by pattern deconvolution quantification for office and home samples and exclude exposure contribution from time spent in vehicles. Exposure estimates for adults (> 18 years old) and toddlers (6-24 months old) were calculated using body masses of 70 and 12 kg, respectively. Mean and high dust ingestion scenarios are calculated using intakes of 20 and 50 mg, respectively, for adults, and 50 and 200 mg, respectively, for toddlers. CPs: Chlorinated paraffins; LCCPs: long chain CPs; MCCPs: medium chain CPs; SCCPs: short chain CPs.

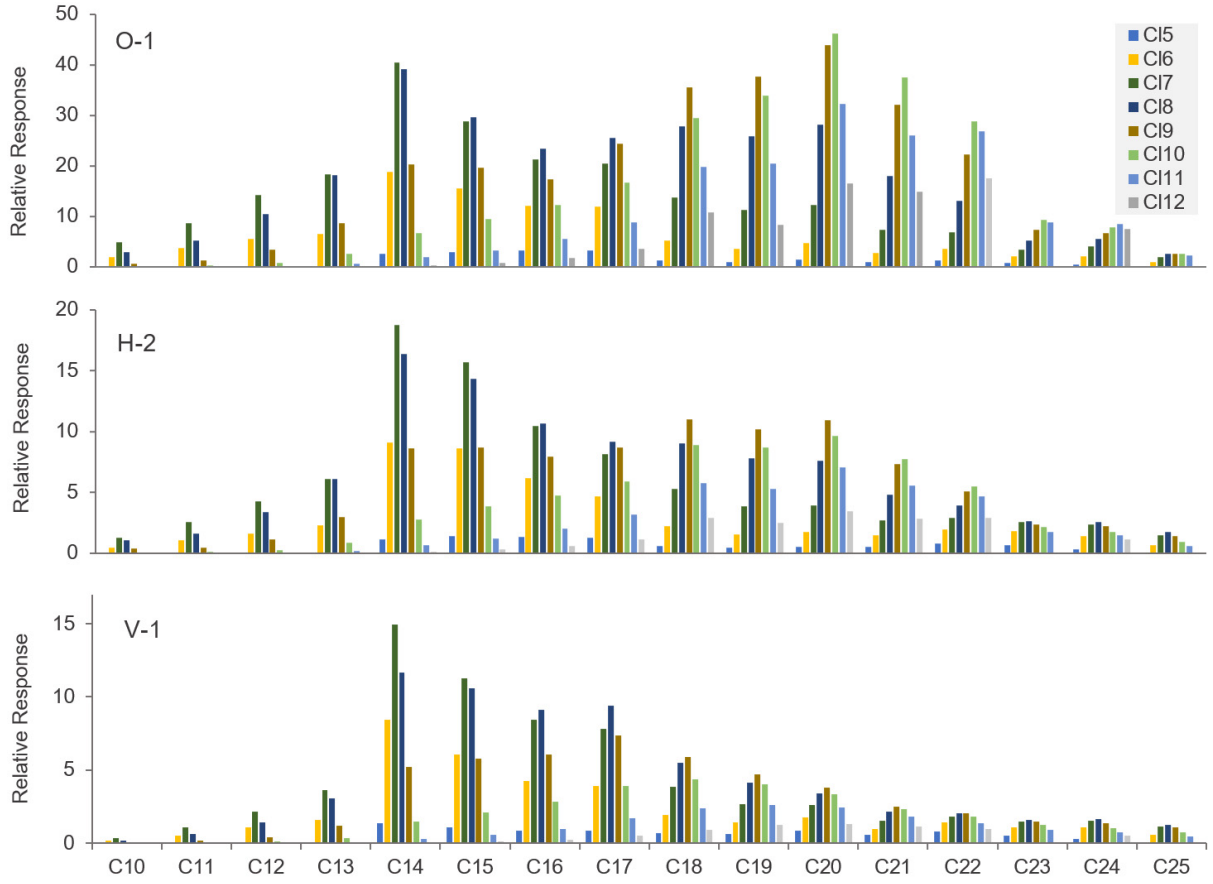

Figure 4. Relative responses of individual CP congener groups in selected office (O), home (H), and vehicle (V) indoor dust samples from Melbourne, Australia.

CONCLUSIONS

This study provides a comprehensive method validation for the extraction and analysis of SCCPs, MCCPs, and LCCPs in indoor dust by LC-QTOF-HRMS. A direct comparison of two common quantification strategies for CPs was performed by applying each of the protocols to the same set of LC-QTOF acquisition data so that the relative strengths of pattern deconvolution and chlorine-content calibration could be evaluated. This assessment showed that results generated by pattern deconvolution quantification met the EURL's accuracy criteria in all applied tests and exceeded the performance of the chlorine-content procedure in terms of both trueness and precision measures. Application of the analytical method to real

indoor dust samples demonstrated the sensitive and simultaneous measurement of SCCPs, MCCPs, and LCCPs with low levels of deviation between results obtained by the two quantification methods. This first report of CP concentrations in indoor dust from Melbourne, Australia, found MCCPs to be dominant, while ubiquitous detection of LCCP congener groups extending to carbon-chain lengths outside the range of all available commercial standards exposed the strong need for appropriate analytical standards. A preliminary human exposure assessment indicated that CP intake via ingestion of indoor dust in Melbourne might represent a significant exposure pathway for both adults and toddlers.

DECLARATIONS

Authors' contributions

Conceptualization, methodology, formal analysis, investigation, writing - original draft preparation, review and editing, visualization, funding acquisition: McGrath TJ

Conceptualization, methodology, resources, writing - review and editing, supervision, project administration, funding acquisition: Covaci A

Conceptualization, writing - original draft, review and editing, visualization, funding acquisition: Poma G

Availability of data and materials

Additional data for this study are presented in the supplementary information.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for Publication

Not applicable.

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