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Optimization and validation of an analytical method for the quantification of short- and medium-chained chlorinated paraffins in food by gas chromatography-mass spectrometry

Thomas J. McGrath^{a,*}, Giulia Poma^a, Jasper Bombeke^a, Franck Limonier^b, Els Van Hoeck^b, Laure Joly^b, Adrian Covaci^{a,*}

^aToxicological Centre, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium

^bSciensano, Chemical and Physical Health Risks Department, Rue Juliette Wytsman 14, B-1050 Ixelles, Belgium

*corresponding authors

Abstract

This work describes the optimization and validation of an analytical method for the quantification of short- and medium-chained chlorinated paraffins (SCCPs and MCCPs, respectively) in a range of food matrices using gas chromatography-electron capture negative ionization-mass spectrometry (GC-ECNI/MS). A dispersive solid phase extraction (dSPE) method was optimized for fish, meat, oil, milk and whole-grain cereal followed by clean-up with concentrated sulfuric acid and acid silica. Fractionation using silica cartridges efficiently removed a number of potentially interfering halogenated compound classes from sample extracts while retaining 96% of Σ SCCPs and 99% of Σ MCCPs. Limits of quantification (LOQs) estimated for food samples ranged from 0.7 to 6.0 ng/g wet weight (ww) for Σ SCCPs and 1.3 to 12 ng/g ww for Σ MCCPs. The applicability of the optimized protocol was assessed in each of the described food matrices via repeated analysis (n=3) of samples fortified with SCCP 55.5%CI and MCCP 57%CI technical mixtures at two concentration levels and spiked lard samples from a recent European Union Reference Laboratory (EURL) interlaboratory study on CPs in food. The EURL's accuracy criteria was met for both homologue groups in all food matrices with overall accuracy in the range of 76 to 130% for in-house spiked samples and 57 to 150% for the EURL lard analysis. Excellent precision was observed for most samples with relative standard deviation (RSD) between replicates (n=3) $\leq 12\%$ for Σ SCCPs and $\leq 17\%$ for Σ MCCPs in all food matrices analysed. The selection of the internal standard was a significant factor in the accuracy of the method and highlights the strong need for more appropriate isotopically labelled CP standards.

Keywords; chlorinated paraffins, chlorinated alkanes, gas chromatography, mass spectrometry, food.

1. Introduction

Chlorinated paraffins (CPs) are a complex mixture of thousands of individual compounds which have been used as plasticizers, flame retardants and additives to various lubricants, adhesives and metal working fluids (ECHA, 2009). Manufactured since the 1930s, CPs are high production volume chemicals with an estimated total production exceeding 1 million tons per year as of 2016 (Glüge *et al.*, 2016). CPs are typically classified by carbon chain length as short-chain (SCCPs; C₁₀-C₁₃), medium-chain (MCCPs; C₁₄-C₁₇) and long-chain (LCCPs; C_{>17}) and by degree of chlorination on a weight/weight basis (30-70% w/w). As semi-volatile compounds, CPs may be released to the environment via volatilisation or leaching from treated polymers during their manufacture, use, or after-life (ECHA, 2009) and have been observed to accumulate in soils, sediments, water, and wildlife (van Mourik *et al.*, 2016; Glüge *et al.*, 2018). CPs have also been detected in human serum (Li *et al.*, 2017; van Mourik *et al.*, 2020) and milk samples (Xia *et al.*, 2017). SCCPs were classified as H351 potential carcinogens under EU Regulation No. 1272/2008 (EU, 2008b) and have exhibited neurotoxic and endocrine disrupting properties (Zhang *et al.*, 2016; Gong *et al.*, 2018; Yang *et al.*, 2019). According to these environmental and health hazards, SCCPs were registered as Stockholm Convention Persistent Organic Pollutants (POPs) in 2017 (UNEP, 2017). SCCPs have also been restricted to 0.15% w/w in articles on the European market (EU, 2015) and included in the EU Water Framework Directive (EU, 2008a), although no regulations exist in relation to CPs in food or feed, internationally, to the authors' knowledge. Such legislative instruments are thus driving apparent shifts to increasing application of other CP formulations, namely MCCPs and LCCPs (Glüge *et al.*, 2018).

While data on human exposure to CPs generally remain scarce, a number of studies have suggested that ingestion of contaminated food could be a major intake pathway (Li *et al.*, 2020). Fridén (2010) indicated that dietary intake may account for as much as 85% of non-occupational exposure to SCCPs in Sweden, while assessments in Canada (CEPA, 2008) and China (Gao *et al.*, 2018) have each drawn similar conclusions. Both plant and animal food products may become contaminated with CPs via bioaccumulation in the environment (Li *et al.*, 2019; Dong *et al.*, 2020; Guan *et al.*, 2020), though research has also evidenced transfer from food processing equipment (Yuan *et al.*, 2017) and food packaging (Wang *et al.*, 2019). Studies from Asia and Europe have determined SCCPs and MCCPs in a variety of foodstuffs, including fish, meat, oil, milk and plant-based foods (NFA, 2017; Huang *et al.*, 2018; Krätschmer *et al.*, 2019; Sprengel *et al.*, 2019; Dong *et al.*, 2020; Li *et al.*, 2020). Despite these findings, further broad-ranging dietary studies are still required to fully evaluate the contamination status of CPs in food.

Among the barriers prohibiting our understanding of the true health risks posed by CPs is the lack of reliable analytical methods. Commercial CP formulations consist of very complex mixtures of thousands of isomers with different carbon chain lengths and chlorination degrees, as well as branched alkanes and other hydrocarbon impurities (van Mourik *et al.*, 2015). Chromatographic separation of individual congeners has proven to be a major challenge, while interferences from other halogenated contaminants, like polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), can also impede reliable quantification. Furthermore, a lack of appropriate commercially available chemical standards and reference materials has further hindered CP quantitation (Schinkel *et al.*, 2018; van Mourik *et al.*, 2018).

A range of analytical methods have been described to date, utilizing either gas or liquid chromatography (GC and LC, respectively) coupled with a variety of mass spectrometric (MS) detectors. Good sensitivity has been achieved using electron impact (EI) ionization on GC tandem MS instruments, although extensive fragmentation and low abundance of larger ion fragments means that CP homologue groups are

indistinguishable (Zencak & Oehme, 2006). Two dimensional GC has been shown to provide a greater degree of chromatographic separation at the expense of complex instrumentation and data processing requirements (Xia *et al.*, 2016). More recently, LC has been coupled with quadrupole time-of-flight (QTOF)-MS detection for analysis of SCCPs, MCCPs and LCCPs simultaneously (Bogdal *et al.*, 2015; Li *et al.*, 2017). The superior selectivity offered by high resolution (HR) MS, such as QTOF or Orbitrap MS allows for congener specific analysis with significantly reduced interference between homologue groups and other compounds (Bogdal *et al.*, 2015; Xia *et al.*, 2016; Krätschmer *et al.*, 2018; Mézière *et al.*, 2020). However, despite the promising advances of HRMS technologies, such instruments are not yet widely found in routine analytical laboratories due to prohibitive monetary expenses and the need for highly trained expert operators.

To date, the most common approach for SCCP and MCCP analysis has been the use of GC coupled to single quadrupole, low resolution (LR) MS operated in electron capture negative ionization (ECNI) mode (van Mourik *et al.*, 2018). This method is highly sensitive and provides information on congener group patterns, but also requires multiple injections and extensive sample clean-up to minimize interferences between CP homologues and from other organohalogen contaminants (Yuan *et al.*, 2019). Despite the drawbacks in selectivity, the popularity of this technique also partly derives from the simplicity of single quadrupole GC-MS operation and the widespread adoption of these instruments. Given the commonplace use of GC-ECNI/MS for CP quantification, it is important that comprehensive validation of this instrumental setup is undertaken to evaluate analytical performance. To the authors knowledge, this is the first study to perform an extensive validation for the quantification of SCCPs and MCCPs in food using GC-ECNI/MS.

The objective of this study was to develop a sensitive and reliable procedure for the analysis of SCCPs and MCCPs in a wide variety of food matrices based on dispersive solid phase extraction (dSPE), acid clean-up, and GC-ECNI/MS quantification. The key parameters affecting accuracy and precision of SCCP and MCCP quantification via GC-ECNI/MS and chlorine-degree calibration were thoroughly explored. Finally, the optimized protocol underwent comprehensive validation via repeated analysis of a range of fortified food matrices to assess overall analytical performance.

2. Materials and Methods

2.1. Standards and reagents

Technical mixtures of SCCPs with chlorination degrees of 51.5, 55.5 and 63% and MCCPs with chlorination of 42, 52 and 57% were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). An isotopically labelled CP standard of 1,5,5,6,6,10-hexachlorodecane (¹³C-HCD) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA), while individual standards of ε-hexachlorocyclohexane (ε-HCH) and 6-methoxy-2,3,3',4,4',5'-hexabromodiphenyl ether (6-MeO-BDE-157) were obtained from Dr. Ehrenstorfer and AccuStandard (New Haven, USA), respectively. Dichloromethane (DCM) was purchased from Sigma Aldrich (Darmstadt, Germany), *n*-hexane and acetone from Acros Organics (Geel, Belgium) and 2,2,4-trimethylpentane (*iso*-octane) from VWR (Leuven, Belgium). Concentrated sulfuric acid (H₂SO₄, 98%) was obtained from Sigma Aldrich and silica gel (SiO₂, 70-230 mesh) was from VWR. 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.

2.2. Sample extraction and clean-up

In this study, five food matrices were selected for optimization and validation experiments to represent a wide variety of food matrices. Individual samples of fish (smoked salmon), meat (pork sausage), oil (extra-virgin olive oil), milk (whole-fat dehydrated cow's milk) and cereal (whole-wheat breakfast cereal) were purchased from supermarkets in Antwerp, Belgium. The fish and meat samples were lyophilized prior to homogenization using a mortar and pestle, while the dried milk and cereal samples containing negligible amounts of water, were homogenized without a freeze-drying step. The lipid and water content of each sample was determined gravimetrically according to the method previously described by Xu *et al.* (2015).

Extraction and clean-up steps are presented schematically and pictorially in Supplementary Information (SI), Figure S1 and Figure S2, respectively. Five or 10 g of each dried sample (Table 1) were weighed into 50 mL glass centrifuge tubes and spiked with 5 ng of ^{13}C -HCD internal standard (IS). A volume of 20 mL of *n*-hexane:DCM (3:1 v/v) was added to samples, which were then vortexed for 1 min, sonicated for 10 min and centrifuged for 3 min at 2000 rpm. The supernatant was transferred to new vials and the extraction repeated with 20 mL fresh solvent mixture. Extracts were treated by addition of 3 mL of concentrated sulfuric acid, vortexed for 15 s, centrifuged for 3 min at 2000 rpm and the supernatant transferred to new vials. This step was repeated with a further 3 mL of sulfuric acid, again transferring to new vials. Finally, 6 g of acid silica (44% w/w) was added to each extract vial, vortexed and centrifuged as with previous steps and the resulting supernatant transferred to new vials. Purified extracts were evaporated to incipient dryness and reconstituted in 0.5 mL of *n*-hexane. Fractionation was performed using 3 mL Agilent Bond Elut 500 mg silica cartridges which were first conditioned with 6 mL of DCM, air-dried via positive pressure and then washed with a further 6 mL of *n*-hexane. Sample extracts were loaded onto cartridges with two rinses of 0.5 mL *n*-hexane and then eluted in two fractions. Fraction 1 was eluted with 6 mL *n*-hexane and contained interfering organohalogenes to be discarded. A second fraction was eluted using 12 mL of DCM containing all targeted SCCPs and MCCPs for analysis. Fraction 2 was evaporated to dryness under a gentle nitrogen stream and reconstituted in 100 μL of *iso*-octane containing ϵ -HCH and 6-MeO-BDE-157 recovery standards (RS) at 50 ng/mL each.

2.3. Instrumental analysis and quantification

All analyses were performed using an Agilent 7000D series gas chromatograph-mass spectrometer equipped with a DB5-MS capillary column (15 m, 0.25 mm internal diameter, 0.1 μm film thickness). The instrument was operated in electron capture negative ionization (ECNI) mode using methane as reagent gas. Injections of 2 μL were carried out in pulsed splitless mode with a hold of 0.04 min at 92°C before ramping at a rate of 700°C/min to 300°C. The oven temperature program entailed a hold of 1.25 min at 90°C followed by a ramp of 25°C/min to 180°C and then 10°C/min to 325°C, with a final hold of 6.5 min for a final run time of 25.85 min. The flow rate of the helium carrier gas was 1 mL/min for 17.85 min and then increased to 2.5 mL/min. The temperature of the transfer line was 300°C, the quadrupoles set to 150°C and the source temperature 150°C. Analysis was conducted in single ion monitoring (SIM) mode using the most abundant isotope of $[\text{M}-\text{Cl}]^-$ and $[\text{M}-\text{HCl}]^-$ ions for quantification and a second isotope for qualitative purposes (Table S1). CP congener groups with carbon-chains ranging from C_{10} to C_{17} and chlorine numbers ranging from Cl_5 to Cl_{10} were monitored to total 24 SCCPs and 24 MCCPs, herein referred to as CP $\text{C}_n\text{-Cl}_m$ for n carbon atoms and m chlorine atoms (e.g. CP10-7 for $\text{C}_{10}\text{H}_{15}\text{Cl}_7$). To maximize sensitivity, CP homologues were

grouped into four separate injections as per methods previously described (Zeng *et al.*, 2011) with acquisition conducted in 6 or 7 time segments (Figure S3, Figure S4). These groupings imply that CPs with five more carbons and two less chlorines share the same nominal mass, thus creating pairs of SCCP and MCCPs which can be analysed using the same m/z integers. Congener groups were identified by careful comparison of retention time, peak shape and quantifier/qualifier ion ratios between sample chromatograms and those of analytical standards. Congener groups were quantified when signal to noise ratio of the quantitative ion exceeded 10. Quantification was conducted according to the chlorine-content calibration procedure described by Reth *et al.* (2005) with minor modifications as detailed in Section S1 of the SI. The finalized quantification procedure used the area of the 6-MeO-BDE-157 recovery standard to calculate relative areas for targeted CP congener groups in equations 1-5, Section S1.

2.4. Assessment of method performance

Performance of the fully optimized method was assessed by repeated ($n=3$) extraction and analysis of each food matrix fortified with CPs at two concentrations. Each sample was spiked with a combination of SCCP 55.5%CI and MCCP 57%CI technical mixtures at a mass rate of 300 ng each for low spikes and 1000 ng each for high spikes (Table 2). Food samples were also analysed in triplicate without CP spikes and the mean Σ SCCP and Σ MCCP concentrations in non-spiked samples were subtracted from individual measurements in spiked samples. Fortified lard samples from the recent European Reference Laboratory (EURL) interlaboratory study (ILS) on CPs in food (Krätschmer & Schächtele, 2019) were also analysed in triplicate as part of this method validation. A comprehensive description of the EURL lard sample preparation is reported by Krätschmer and Schächtele (2019) and Σ SCCP and Σ MCCP concentrations in the fortified lard are provided in Table 3. The EURL samples were obtained after the completion of the study and the results presented herein do not represent participation in the ILS. Procedural blanks ($n=10$) were extracted on ten separate days and spiked procedural blanks ($n=4$) were prepared using the same CP spike as food samples at the low spike dose. Accuracy of the method was expressed as i) %accuracy (measured concentration divided by spiked concentration multiplied by 100) and also ii) z-scores computed according to the EURL's General Protocol for EU Proficiency Tests on Pesticide Residues in Food and Feed (EURL, 2018) (described in Section S2, SI). Accuracy criteria described in the EURL document stipulate that results of $|z| \leq 2.0$ are acceptable, $2.0 < |z| < 3.0$ questionable, and $|z| \geq 3.0$ unacceptable. Relative standard deviation (RSD) of replicate extractions ($n=3$) was used as a measure of precision. Relative standard error (RSE) and %error were used to assess the working range according to methods described by the United States EPA Method 8000D (USEPA, 2018) (detailed in Section S2, SI).

3. Results and Discussion

3.1. Sample extraction and clean-up

In this study, a dispersive solid phase extraction (dSPE) method was developed using common laboratory equipment to avoid the need for complex apparatuses, such as Soxhlet or Accelerated Solvent Extraction systems. A multistep clean-up using sequential washes with concentrated sulfuric acid and mixing with acid silica was optimized to efficiently remove matrix components such as lipids and pigments. Combinations of 1:1, 3:1 and 9:1 (v/v) *n*-hexane:DCM were tested to determine the optimal solvent ratio for achieving comprehensive extraction of all targeted SCCP and MCCP congener groups without excess co-

208 extraction of lipids. These solvents have often been used for CP extraction (Yuan *et al.*, 2019) and are also
209 compatible with acid treatments. Recoveries of SCCPs and MCCPs spiked into fish tissue were similar for
210 the 1:1 and 3:1 solvent mixtures but markedly lower using the 9:1 combination. Visual inspection of
211 concentrated extracts revealed the 3:1 *n*-hexane:DCM mixture to result in cleaner extracts and this
212 combination was selected for all subsequent experiments. A total extraction volume of ~40 mL was used to
213 ensure that the proportion of solvent lost during transfers of supernatant during clean-up steps was
214 minimized and to reduce the formation of emulsions between the sulphuric acid and organic layers. The use
215 of two sequential additions of 3 mL sulfuric acid was found to result in superior purification of extracts than a
216 single 6 mL treatment as less of the acid-lipid fraction adhered to the walls of the extraction vial. It was also
217 observed that the use of glass vials instead of polypropylene conical centrifuge tubes for extraction and
218 clean-up steps greatly reduced background noise in final chromatograms (Figure S5).

219 Due to the broad GC retention times of SCCP and MCCP congener groups and low specificity of single
220 quadrupole ECNI/LRMS measurement, steps to remove potentially interfering contaminants such as PCBs
221 and OCPs were required during sample clean-up. Whereas most studies have utilized multi-layer column
222 chromatography to separate compound classes (van Mourik *et al.*, 2015; Yuan *et al.*, 2019), this work used
223 solid-phase extraction (SPE) as a means of refinement. Methods described by Roosens *et al.* (2010) for the
224 fractionation of hexabromocyclododecane (HBCD) and polybrominated diphenyl ethers (PBDEs) using 500
225 mg silica cartridges were found to separate targeted CPs from a number of organohalogen pollutant classes
226 including PCBs, PBDEs, and OCPs. Initial trials showed losses of up to 20% of individual SCCP congener
227 groups with the lightest compounds most affected, while all MCCP groups were recovered at rates greater
228 than 95%. Altering elution volumes was not able to improve recovery of SCCPs, and tentative additions of
229 cyclohexane or pentane (up to 100%) in fraction 1 also failed to generate more favourable results.
230 Eventually, the use of an initial DCM conditioning step and ensuring that all traces of DCM were removed
231 from the cartridge and extract prior to loading provided Σ SCCP and Σ MCCP fractionation recoveries of 96
232 and 99%, respectively. Application of the optimized fractionation procedure in spiking experiments
233 demonstrated successful removal of PCBs, PBDEs, Dechlorane Plus and some OCPs including chlordanes,
234 hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene
235 (DDE) from the CP analysis fraction. Compounds which partitioned with CPs included α -, β -, γ -, ϵ -HCHs,
236 MeO-PBDEs and some novel brominated flame retardants (NBFRs), including 2-ethylhexyl-2,3,4,5-
237 tetrabromobenzoate (EH-TBB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and bis(2-ethylhexyl)
238 tetrabromophthalate (BEH-TEBP). The fractionation procedure also provided secondary benefits by retaining
239 remaining traces of lipids, acids and silica particles from the preceding processes.

240

241 3.2. Instrumental acquisition

242 The instrumental acquisition parameters used in this study were modified from previously described
243 methods (Tomy *et al.*, 1997; Reth & Oehme, 2004; Zeng *et al.*, 2011). Both 15 and 30 m DB5-MS analytical
244 columns were tested in this study. Chromatographic separation of co-eluting SCCPs and MCCPs was not
245 found to be achievable with the longer GC column or with longer analytical run times using slower oven ramp
246 programs. As such, the shorter column was employed with faster ramps to gain sensitivity by increasing
247 signal to noise in CP humps. This also increased the throughput of the samples, which is an important factor
248 given that each extract must be injected four times. While all targeted CPs eluted within 17.85 min under the

current program, GC runs required an extra 8 min in order to fully elute high-boiling matrix components which had otherwise been observed to interfere with subsequent injections.

Formation of $[M-Cl]^-$ and $[M-HCl]^-$ ions was evaluated at MS source temperatures of 140, 170, 200, 230, and 250°C for the SCCP and MCCP target groups. Abundances of both ions decreased substantially at temperatures of 200°C and above, while $[M-Cl]^-$ formation was generally favoured at 170°C and $[M-HCl]^-$ at 140°C. These findings mirror those previously described on GC-ECNI instruments (Tomy *et al.*, 1997; Yuan *et al.*, 2016). A source temperature of 150°C was then selected to achieve an overall optimal response across each of the targeted CPs and monitoring of $[M-Cl]^-$ ions was adopted for all congener groups except for SCCPs with 5 and 6 chlorines, which use $[M-HCl]^-$ (Table S1). A high degree of interference was observed between chain-length groups due to overlapping isotope clusters. This was true for combinations of SCCPs and MCCPs, but also for interferences *within* single technical mixture standards. For all congener groups, the most abundant isotopes were used for quantification. The second most abundant isotope was used for qualitative purposes for most congener groups, while in some cases the third most abundant isotope was selected due to lower levels of apparent interference with other homologues (example in Figure S6). A comparison between SIM measurements using MS1 and MS2 of the triple quadrupole instrument revealed MS2 acquisition to be notably more sensitive. As such, MS1 was configured to transmit all ions and mass filtering was conducted using MS2.

3.3. Working range and limits of quantification

The working range of the analytical method was assessed by preparing analytical standards at seven concentrations (0.3, 0.6, 1, 3, 6, 10 and 20 µg/mL) and five degrees of chlorination for each of the Σ SCCP and Σ MCCP groups in *iso*-octane (totalling 35 calibration points each, Figure 1). Technical standards were mixed in 1:1 ratio to achieve midway Cl% points between those of the supplied SCCPs (51.5, 55.5 and 63%) and MCCPs (42, 52 and 57%) as described in Section S1 of the SI. The calibration points fit exponential regression curves with minimum R^2 of 0.971, which were then used to quantify each of the points. Although linear regression is often used for chlorine-content calibration (Reth *et al.*, 2005; van Mourik *et al.*, 2018), such calibration was deemed unsuitable due to extreme inaccuracy observed in the lowest Cl% standards. Indeed, exponential regression has been applied successfully in a number of studies (Sprengel & Vetter, 2019; van Mourik *et al.*, 2019; Yuan *et al.*, 2019). The %error was calculated for each point and RSE determined for the overall performance of the calibration. Σ SCCPs measurements for all 35 points met the accuracy criteria of $\leq 30\%$ error recommended by the EPA's Method 8000, Determinative Chromatographic Separations guide (USEPA, 2018). For MCCPs, 91% of calibration points fell within the $\leq 30\%$ error limitations, although all measurements were $\leq 35\%$ error except for one outlier. RSE values calculated for calibration across the full concentration and Cl% ranges were 13 and 20% for Σ SCCPs and Σ MCCPs, respectively. These RSE values fall well within the EPA's prescribed limit for 'poor performing compounds' ($\leq 30\%$), while Σ SCCPs also met the criteria for 'good performing compounds' ($\leq 20\%$). These results ensure that there is a strong relationship between instrument response, concentration and chlorine content to indicate that the calibration procedure can account for the variety of Cl% degrees observed in real samples. van Mourik *et al.* (2019) reported an RSE of 23% for GC-ECNI/MS measurements of Σ SCCPs over the same Cl% span and a concentration range of 1-50 µg/mL. The same study reported a similar RSE for GCxGC- μ ECD determined in the same manner and an RSE of 12% for APCI-QTOF-MS in the range of 0.1 to 20 µg/mL. To the authors' knowledge, RSEs for MCCPs have not been reported in the literature.

Procedural blanks were extracted and analysed on 10 separate days in order to assess background contamination within the method. Despite rigorous solvent cleaning of laboratory equipment and overnight baking of glassware (400°C), trace levels of some SCCP congener groups were identified in a small number of blanks (C10, CI-7-8 and C11, CI-7-8) and MCCPs were detected more uniformly (C14, CI-6-8 and C15, CI-6-8). To ensure that background contamination did not contribute significantly to measurements of CPs in real samples, the instrumental LOQs for Σ SCCPs and Σ MCCPs were defined as the lowest calibration concentration to exceed the mean levels in blanks by a 99% confidence interval. Instrumental LOQs calculated in this way were 0.3 and 0.6 µg/mL for Σ SCCPs and Σ MCCPs, respectively. These values were used to derive matrix specific LOQs on wet weight and lipid weight bases by integrating total extracted dry mass of samples with respective water and lipid contents. Wet weight LOQs ranged from 0.7 to 6 ng/g ww for Σ SCCPs and 1.3 to 12 ng/g ww for Σ MCCPs, while lipid weight LOQs ranged from 6 to 320 ng/g lw and 12 to 630 ng/g lw for Σ SCCPs and Σ MCCPs, respectively (Table 1). LOQs of the current method were broadly similar to those described for fish and oil samples using GC-ECNI/MS (Sprengel *et al.*, 2019; van Mourik *et al.*, 2019), while Huang *et al.* (2018) reported LOQs approximately one order of magnitude lower for meat using GCxGC-ECNI/TOFMS. Although superior instrumental sensitivity has been demonstrated by high resolution methods, LOQs for SCCPs and MCCPs in fish analysed by atmospheric pressure chemical ionization (APCI)/QTOF-MS were similar to the present study (Yuan *et al.*, 2018) and approximately one order lower by GC-ECNI/Orbitrap-MS methods (Krätschmer *et al.*, 2019). The matrix specific LOQs estimated in this study were below the vast majority of values reported in recent analysis of fish and oil from Europe (Krätschmer *et al.*, 2019; Sprengel *et al.*, 2019) to indicate that the method is sufficiently sensitive for quantification of SCCPs and MCCPs in real samples.

3.4. Internal standards

In the present study, internal standard selection was limited by the partitioning behaviour of different compounds during the fractionation and clean-up. Many of the compounds often used as internal standards for CP quantification (in carbon-labelled form) were effectively removed from sample extracts, including HCB, PCBs, chlordanes and Dechloranes (van Mourik *et al.*, 2015). Of the compounds which did partition with SCCPs and MCCPs, EH-TBB, BEH-TEBP and BTBPE were also disqualified due to low stability in acid used during the clean-up, while HCHs and MeO-BDEs appeared to have favourable characteristics as potential internal standards. Furthermore, HCHs eluted from the GC prior to all CP congener groups so as not to interfere during analysis and MeO-PBDEs were well separated from CPs mass spectrally (Figure S3 and Figure S4). Of the many possible HCH and MeO-PBDE isomers, ϵ -HCH and 6-MeO-BDE-157 were selected for further investigation as they are not detected in the environment, and thus unlikely to be present in food samples prior to spiking. Preliminary testing of these compounds in spike and recovery experiments showed their use in quantification to greatly overestimate CP concentrations, by as much as 760 and 410% for ϵ -HCH and 6-MeO-BDE-157, respectively. This may suggest that these compounds are recovered from extraction and clean-up processes to a lesser extent than the measured CP congener groups and, thus, over-correct for losses in the quantification procedure. The comparatively lower vapour pressure of ϵ -HCH may have contributed to extra losses due to evaporation steps, while the greater polarity of 6-MeO-BDE-157 than SCCPs and MCCPs may have affected its recovery. As such, ϵ -HCH and 6-MeO-BDE-157 were each utilized in the analytical method as recovery standards, added to sample extracts post-extraction. Standards

added at this stage of analysis can be used in quantification to correct for instrumental and matrix effects, while not accounting for losses during extraction and clean-up.

At present, only two isotopically labelled CPs are commercially available from chemical standard manufacturers. While labelled analogues are usually the 'gold standard' for internal standard quantification, the available ^{13}C -hexachlorodecane (^{13}C -HCD) and ^{13}C -octachlorododecane (^{13}C -OCDD) each have substantial m/z overlaps with native CPs which are indistinguishable by ECNI-LRMS (Figure S7). ^{13}C -HCD was purchased for this study on the basis that the isotope cluster of its $[\text{M}-\text{Cl}]^-$ ion had fewer m/z interferences with native SCCPs and MCCPs than did the alternative mass-labelled standard and has been used more widely for GC-ECNI/MS analysis (Krätschmer & Schächtele, 2019).

3.5. Accuracy and precision

Mean %accuracy in triplicate analyses of spiked food matrices calculated using the ^{13}C -HCD internal standard ranged from 133 to 149% for ΣSCCPs and 122 to 187% for ΣMCCPs . According to the EURL z-score accuracy limits, all ΣSCCP measurements were acceptable, while only 20% of ΣMCCP results met these criteria, with 50% in the questionable range and 30% considered unacceptable (Figure 2). Results calculated using ^{13}C -HCD were uniformly overestimated for both ΣSCCPs and ΣMCCPs , though this effect was more apparent for the MCCPs. Although internal standard recovery was generally very good (80 to 113%), this might suggest that ^{13}C -HCD was recovered from the extraction process at lower rates than the congener groups which constitute ΣSCCP and ΣMCCP values overall. As ^{13}C -HCD is, in fact, an isomer of one of the lightest CP congener groups measured in this study, CP10-6, losses during evaporation and fractionation were possibly greater than those of other homologues. It is also likely that interferences between short- and medium-chain homologue groups contributed to overestimation of results due to ion clusters overlapping with selected quantification m/z values.

Given the poor performance of ΣMCCP accuracy using the ^{13}C -HCD internal standard, an alternative quantification procedure was sought by substituting the areas of ^{13}C -HCD with those of the recovery standards in *relative total area* calculations (equations 1-5, Section S1). As a further alternative, *relative total area* was computed by using the area of ^{13}C -HCD divided by the area of a recovery standard to test the effect of correcting against the response of both standards simultaneously.

The use of the ϵ -HCH recovery standard for quantification resulted in slightly better %accuracy values for ΣSCCPs , ranging 107 to 133%, although none of the ΣMCCP results met the EURL acceptability criteria, with %accuracy ranging 168 to 229%. Measurements calculated using the 6-MeO-BDE-157 recovery standard, however, showed a marked improvement in accuracy for both homologue groups with ΣSCCP %accuracy ranging from 76 to 97% and ΣMCCPs ranging from 87 to 130%. All results computed using 6-MeO-BDE-157 fell within the EURLs accuracy acceptability criteria for both ΣSCCPs and ΣMCCPs (Figure 2). The variation between results calculated using the ϵ -HCH and 6-MeO-BDE-157 recovery standards indicated that matrix enhancement effects may also be contributing to overestimation of results. Tests involving post-extraction spiking of fish extracts with targeted CPs and each of the internal and recovery standards confirmed this to be the case and demonstrated that enhancement effects for 6-MeO-BDE-157 were similar to that of native CPs (Figure S8). Correction against both the ^{13}C -HCD internal standard and either of the recovery standards resulted in a greater proportion of acceptable z-scores than using ^{13}C -HCD alone, though the broader spread of results was likely caused by the extra uncertainty introduced by a second correcting factor.

374 Due to the superior accuracy of measurements quantified by 6-MeO-BDE-157 recovery standard
375 correction, this procedure was adopted in the final protocol. Final %accuracy, z-scores and RSD from
376 triplicate spiked food analyses quantified by 6-MeO-BDE-157 are presented in Table 2. With this final
377 protocol, \sum SCCP RSD values were $\leq 5\%$ for each of the five food matrices, at both spiking levels, while
378 \sum MCCP RSD was $\leq 8\%$ for all high spikes and $\leq 17\%$ for all low spikes.

379

380 *3.6. Application to EURL interlaboratory study samples*

381 Lard samples (denoted A to E) from a recent EURL interlaboratory study on CPs in food were analysed
382 in triplicate using the final optimized method as a further assessment of accuracy and precision. Samples B
383 to E were spiked with SCCPs and MCCPs of varying C-chain length to approximate patterns observed in
384 real salmonid fish, egg and meat samples. Samples D and E were additionally fortified with PCBs and
385 PBDEs to reflect observed occurrences of these compounds in real food samples. Sample A received no
386 external CP spike. Full details on the preparation of lard samples is available in Krätschmer and Schächtele
387 (2019). Results of the EURL sample analysis are presented in Table 3. Mean %accuracy of \sum SCCP
388 measurements in samples B to E ranged from 57 to 82%, while \sum MCCP results ranged from 119 to 150%
389 and all mean z-scores met the EURLs accuracy acceptability criteria. The RSD between triplicate analyses
390 was $\leq 15\%$ for both \sum SCCP and \sum MCCPs for each of the B to E samples to indicate good precision (Table
391 3). CPs detected in sample A were below LOQ for both \sum SCCPs (<6.0 ng/g lw) and \sum MCCPs (<12 ng/g lw).
392 Successful analysis of these EURL samples provides further validation of the optimized method by
393 demonstrating acceptable measurements of \sum SCCPs and \sum MCCPs in samples displaying complex
394 congener group mixtures and organohalogen interferences as could be expected in real samples.

395

396 **4. Conclusions**

397 This work describes the optimization and validation of a GC-ECNI/MS analytical method for the
398 quantification of SCCPs and MCCPs in a range of food matrices. While the limitations of low-resolution mass
399 spectrometry for the analysis of chlorinated paraffins are well documented, GC-ECNI/MS analysis is likely to
400 remain as a common approach for collecting much needed data on SCCPs and MCCPs in environmental
401 matrices, including food. Through an exploration of the factors effecting CP measurement, this research
402 demonstrates that internal standard selection is a key consideration which can substantially impact method
403 accuracy. At present, just two isotopically labelled CP standards are commercially available, while very few
404 non-CP compounds are appropriate for use as internal standards due to complex clean-up requirements and
405 instrumental interferences. The findings of this work suggest that application of a number of labelled CP
406 congeners representing different C-chain lengths and CI-numbers as internal standards may enhance the
407 overall accuracy of \sum SCCP and \sum MCCP quantification by GC-ECNI/MS.

408

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589 **7. Tables**

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Table 1. Sample information and limits of quantification (LOQs) for individual food matrices.

Matrix	Water content (%)	Lipid content (%)	Extraction mass (g dw)	LOQ ng/g ww		LOQ ng/g lw	
				ΣSCCP	ΣMCCP	ΣSCCP	ΣMCCP
Fish	61	8.5	5	2.3	4.7	28	55
Meat	60	17	5	2.4	4.8	14	28
Oil	0.0	100	5	6.0	12	6.0	12
Milk	89 ^a	3.8	5	0.7	1.3	17	35
Cereal	5.0	0.9	10	2.9	5.7	320	630

Fish = smoked salmon, meat = pork sausage, oil = extra-virgin olive oil, milk = full-fat cow's milk and cereal = whole-wheat breakfast cereal. dw = dry weight, ww = wet weight and lw = lipid weight. Water and lipid content refer to wet weight calculations. ^aOriginal water content of full-fat milk estimated from Poma *et al.* (2018)

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Table 2. Mean accuracy (%), relative standard deviation (RSD %) and associated mean z-scores in spiked food matrices using the final optimized method.

Matrix	Spike Level	Spike mass (ng)*	Spike conc (ng/g ww)*	ΣSCCP		ΣMCCP	
				Mean ± RSD	z-score	Mean ± RSD	z-score
Fish	Low	300	23	88 ± 4	-0.49	87 ± 12	-0.51
	High	1000	78	97 ± 5	-0.13	130 ± 5	1.20
Meat	Low	300	24	87 ± 3	-0.50	126 ± 17	1.06
	High	1000	80	85 ± 2	-0.61	111 ± 3	0.44
Oil	Low	300	60	76 ± 2	-0.95	99 ± 5	-0.04
	High	1000	200	91 ± 2	-0.37	126 ± 5	1.04
Milk	Low	300	6.6	95 ± 4	-0.19	112 ± 5	0.50
	High	1000	22	93 ± 4	-0.29	116 ± 5	0.64
Cereal	Low	300	29	78 ± 2	-0.87	92 ± 5	-0.30
	High	1000	95	78 ± 5	-0.87	102 ± 8	0.07
Blank Spike	Low	300	-	101 ± 5	0.04	141 ± 6	1.65

All results calculated using 6-MeO-BDE-157 recovery standard. Extraction and analysis at each level and in each matrix performed in triplicate (n=3). Spike concentration is estimated using spike mass of CPs (ng), extracted mass of sample (g dry weight) and water content of sample (%). ww = wet weight. Z-scores are mean values from triplicate analyses calculated according to methods described by EURL, 2018. *Spike mass and spike concentrations of each of ΣSCCP 55.5%CI and ΣMCCP 57%CI.

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Table 3. Mean accuracy (%), relative standard deviation (RSD %) and associated mean z-scores from analysis of EURL interlaboratory study lard samples using the final optimized method.

Sample	Σ SCCPs			Σ MCCPs		
	Spike conc (ng/g lw)	Mean \pm RSD	z-score	Spike conc (ng/g lw)	Mean \pm RSD	z-score
Lard-B	69	57 \pm 12	-1.74	56	119 \pm 14	0.77
Lard-C	67	79 \pm 11	-0.82	203	150 \pm 10	1.99
Lard-D	31	82 \pm 9	-0.72	94	144 \pm 6	1.74
Lard-E	149	62 \pm 3	-1.53	121	126 \pm 15	1.02

All results calculated using 6-MeO-BDE-157 recovery standard. Extraction and analysis performed in triplicate (n=3). Lipid content of lard samples is > 99%. lw = lipid weight.

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677 **8. Figures**

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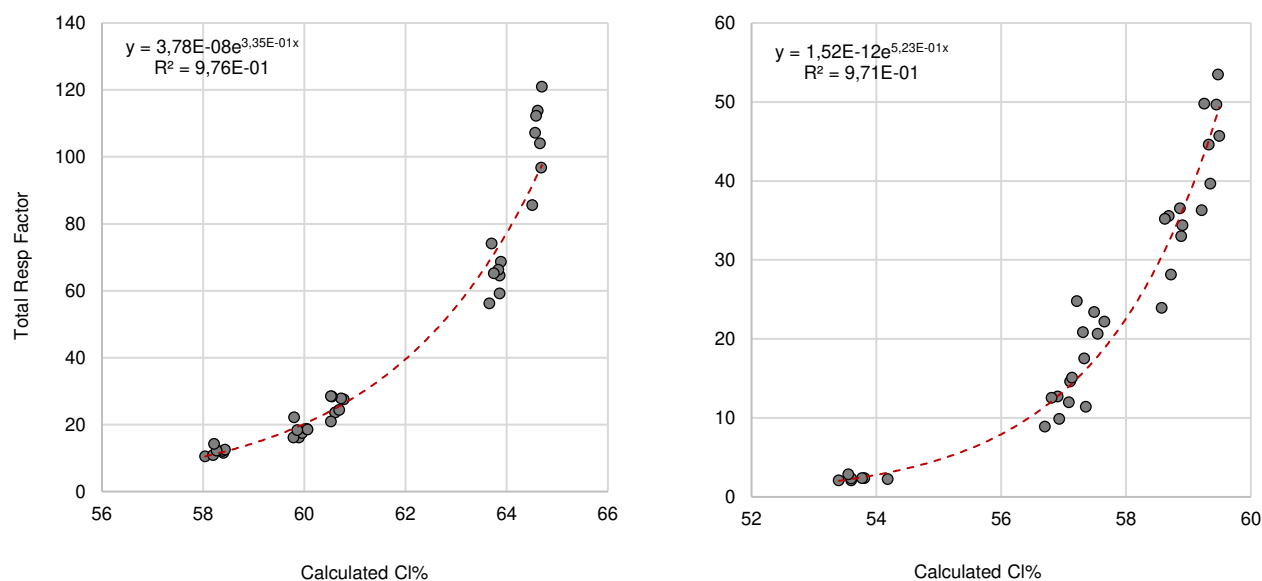


Figure 1. Calibration curves for ΣSCCPs (left) and ΣMCCPs (right) at seven concentrations ranging from 0.3 to 20 µg/mL.

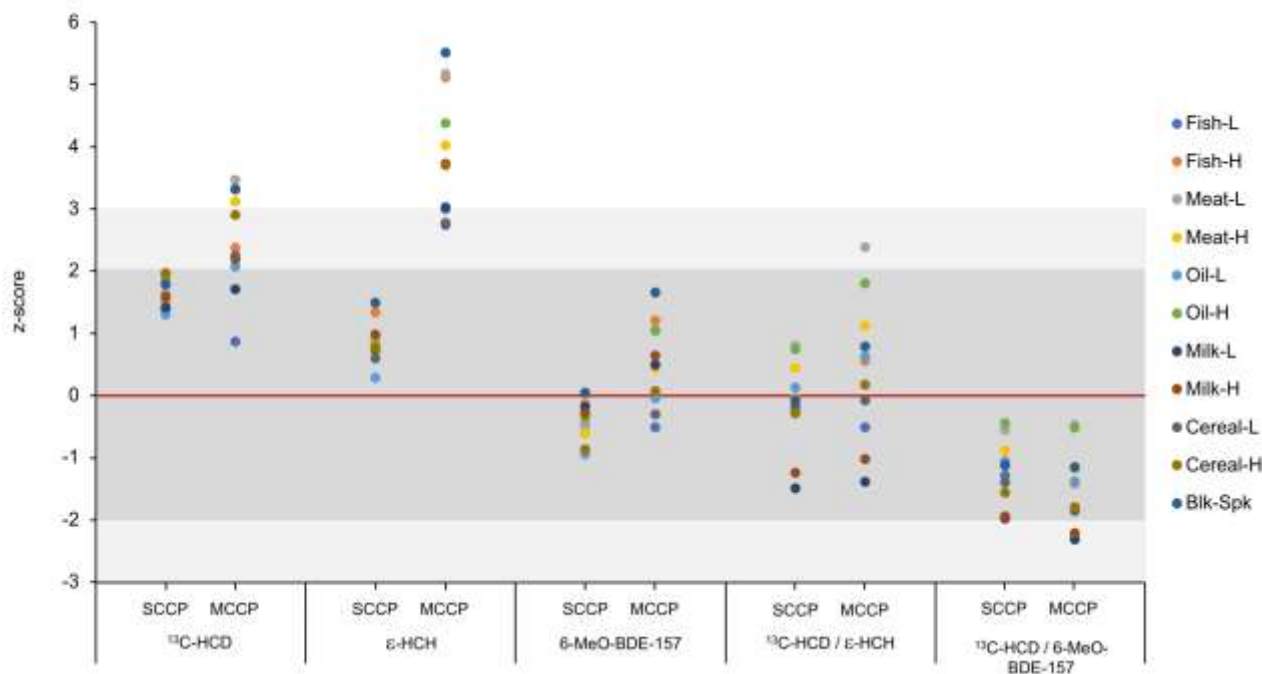


Figure 2. Mean z-scores from triplicate analysis of spiked food matrices using different internal and recovery standards for quantification of ΣSCCPs and ΣMCCPs. L= low spike, H= high spike; matrix specific spike concentrations are detailed in Table 2. Results of $|z| \leq 2.0$ are acceptable, $2.0 < |z| < 3.0$ questionable, and $|z| \geq 3.0$ unacceptable according to EURL (2018).

9. Supplementary Information

Section S1. Quantification by chlorine-content calibration.

Section S2. Assessment of method performance.

Table S1. Mass spectral acquisition parameters for SCCPs and MCCPs.

Figure S1. Diagram of extraction, clean-up and fractionation protocol.

Figure S2. Images of extraction and clean-up process.

Figure S3. Gas chromatograph retention regions of SCCPs and MCCPs.

Figure S4. Total ion chromatograms of a mixed standard analysed using the four separate acquisition methods.

Figure S5. Comparison of total ion chromatograms from analysis of procedural blanks extracted using glass vials vs using polypropylene tubes for each of the extraction and clean-up steps.

Figure S6. Example of isotopic interference between SCCP congener groups.

Figure S7. Theoretical mass spectra of $[M-Cl]^-$ ions for commercially available isotopically labelled CP analytical standards and potentially interfering native CP congener groups.

Figure S8. Matrix enhancement of SCCPs, MCCPs, internal standard and recovery standards in a fish tissue extract.