# DETECTION AND QUANTIFICATION OF CHLORINATED PARAFFINS IN FOOD SAMPLES USING GC-ORBITRAP MASS SPECTROMETRY

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**Introduction**

Chlorinated paraffins (CPs) are complex mixtures of various constitutional and optical isomers as well as a wide range of carbon chain lengths and degrees of chlorination. CPs can be classified either by grade of chlorination or by carbon chain length, resulting in three categories, i.e. short chain CPs (SCCPs, $C_{10}$-$C_{13}$), medium chain CPs (MCCPs, $C_{14}$-$C_{17}$) and long chain CPs (LCCPs, $C_{18}$-$C_{30}$) [1]. Although there are reports of other organohalogen compounds being naturally produced in the (mostly marine) environment, there is currently no indication of natural sources of CPs [2]. Even though the production of SCCPs has been phased out in Europe, the U.S. and Canada, a simultaneous increase of MCCP production resulted in a total CP production estimated at more than 1.1 million tonnes/year for 2012, newer data is currently unavailable [3]. Because of their persistence and believed harmful effects on exposed humans and the environment, SCCPs have been listed as candidates of the Stockholm Convention POPs list and are expected to be added to the list in mid-2017. To enable enforcement and monitoring of restrictions for only one type of CPs, a robust and highly selective analysis is necessary. The quantification of CPs poses analytical challenges due to the high complexity of the mixtures, the overlap of short and medium chained CPs which cannot be resolved purely chromatographically (Figure 1) as well as possible mass interferences with other halogenated pollutants.

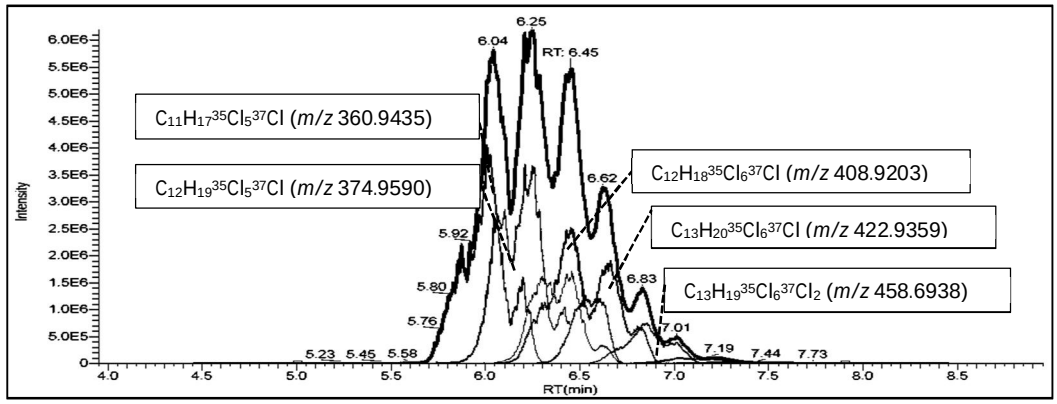


**Figure 1.** GC/NCI-MS chromatogram (Thermo Scientific Q Exactive GC at a resolution of 60,000 measured as FWHM at *m/z* 200) of a SCCP technical mixture (55.5% Cl) on a 15 m x 0.25 mm i.d. capillary column. Inserted are examples of extracted ion chromatograms for the most abundant isotopes of various congeners ($C_{11}$-$C_{13}$ with various chlorination degrees) which result in the total ion chromatogram (TIC) shown in bold trace.

To this day there is no consensus on an analytical procedure for SCCPs and MCCPs in food samples, resulting in many different methods with barely comparable results. The most commonly used method, GC/ECNI-MS, shows interferences with other halogenated compounds and other CPs, making analysis time consuming and complicated [4]. Other methods like GC/EI-MS, GC/FID or GC/ECD are unable to determine congener patterns while offering a way to screen for CPs in general [5]. Less used or newer approaches like direct injection APCI-qTOF-MS are able to determine congener group patterns within a single run [6]. High resolution mass spectrometry is particularly valuable for in-depth studies of congener patterns and fulfilling regulatory demands in connection to the impending world-wide ban of SCCPs. In this study, the performance of a novel bench top, high resolution accurate mass Orbitrap-based GC/MS was tested for the analysis of both SCCP and MCCP in standards and salmon samples. Using full-scan acquisition and negative chemical ionisation (NCI), focus was put on assessing linear dynamic range, selectivity and sensitivity.

**Materials and methods** *Standards and samples*. Two standard solutions resembling technical mixtures of SCCP (100 mg/L in cyclohexane, $C_{10}$-$C_{13}$, 55.5% Cl) and MCCP (100 mg/L in cyclohexane, $C_{14}$-$C_{17}$, 42% Cl) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). For calibration, solutions of SCCP and MCCP with the concentration 0.1, 0.5, 1, 5, 10, 15 ng/µL and the addition of 0.1 ng/µL 1,5,5,6,6,10-$^{13}C_{10}$-hexachlorodecane and 0.05 ng/µL ε-HCH were prepared in cyclohexane. Standard solutions of native mono-*ortho* PCBs and di-*ortho* PCBs (Cambridge Isotope Laboratories, Tewksbury, MA, USA) were diluted with cyclohexane to 0.015-1.5 ng/µL for individual congeners. Salmon (*Salmo salar*) samples (n = 6) of various origins were acquired from supermarkets and vendors in Baden-Wuerttemberg (Southern Germany) as part of the official food control and homogenized according to the demands of appendix I of Regulation (EC) 396/2005 for salmon. Lipids were cold extracted with *n*-hexane/dichloromethane (1:1, v/v). Further sample clean-up was based on the procedure described by Reth *et al.* elsewhere [7].

*Instrumental analysis*. Most experiments were performed with a Thermo Scientific Q Exactive GC Orbitrap mass spectrometer coupled with a Thermo Scientific TRACE 1310 GC. Sample introduction was performed with a Thermo Scientific TriPlus RSH autosampler. Compound separation was achieved on a Thermo Scientific TraceGOLD TG-5SilMS 15 m x 0.25 mm I.D. x 0.25 µm film capillary column. The mass spectrometer was tuned and calibrated using FC43 to achieve mass accuracy of < 0.5 ppm. The oven temperature program started at 60 °C (holding time 2 min). Then the temperature was raised at 50 °C/min to 300 °C and held for 5 min. Helium was used as a carrier gas at a flow rate of 1.4 mL/min. The split-/splitless injector was used in splitless surge mode with a pressure of 9 psi for 1 min for the injection of 1.3µL. The system was operated in negative ionization mode (NCI) using methane gas as reagent gas. Data was acquired in full scan mode with 60,000 mass resolution (full width at half maxima (FWHM), measured at *m/z* 200). Data processing was done using the Thermo Scientific TraceFinder software.

**Results and discussion**

Measurements of SCCP and MCCP standards at different concentrations for the assessment of linearity and dynamic range resulted in an excellent linearity with $R^2 > 0.99$ (Figure 2). CP congeners with chain lengths between $C_{10}$-$C_{17}$ and chlorinated with five to eleven chlorine atoms were detected using the theoretical masses of the most and second-most abundant isotope peaks of $[M\text{-}Cl]^-$ fragment ions (Figure 1). It should be noted that lower chlorinated CPs resulted in a higher abundance of $[M\text{-}HCl]^-$ ions, which were then screened instead of the

[M-Cl]$^-$ species. Depending on chain length and chlorination degree, congeners were detected in standards assessed at concentrations as low as 0.1 ng/µL.

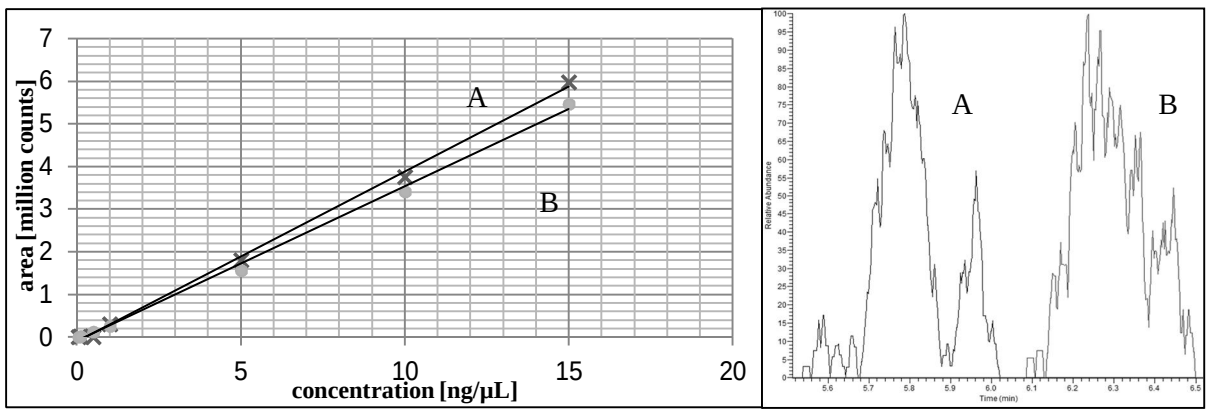


**Figure 2**. Calibration line of CP standards (0.1 – 15 ng/µL) (left panel) and GC/HR-NCI-MS ion chromatograms (right panel, 0.1 ng/µL level, R = 60,000) of the most abundant isotope peaks of the [M-Cl]$^-$ fragment ion of $C_{10}H_{15}Cl_7$ (A) and $C_{14}H_{24}Cl_6$ (B) extracted from the full scan run.

Spiking experiments with high levels of native mono- and di-*ortho*-PCBs and mixtures of MCCP and SCCP standards (see Materials and methods) showed no significant effect on peak profiles of extracted congeners (Figure 3). Such excellent selectivity is crucial for accurate quantification of individual CP homologue groups in real samples where different groups of CPs as well as other contaminants (such as PCBs) are likely present. Therefore, the high resolution of GC-Orbitrap-MS allows for the quantification of both SCCPs and MCCPs even in the presence of high amounts of PCBs in samples without congeners being overestimated due to mass overlaps.

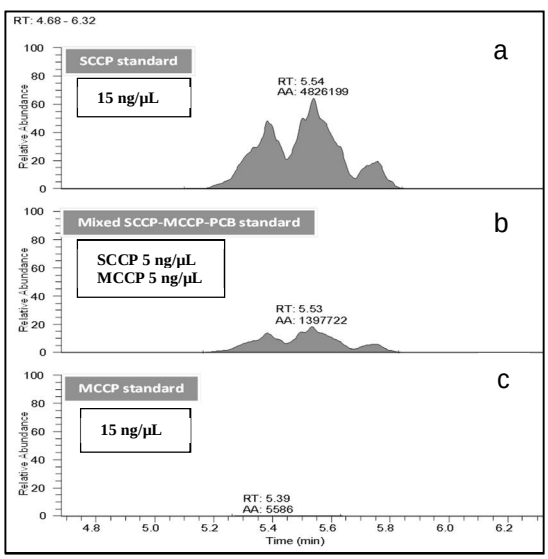


**Figure 3**. GC/NCI-HRMS ion chromatograms extracted from the full scan with (a) the most abundant [M-Cl]$^-$ ion of $C_{10}H_{16}Cl_6$ (*m/z* 312.96651) of a SCCP standard, (b) a 1:1:1 (v/v) mixture of SCCP, MCCP and mono-/di-*ortho*-PCBs and (c) of a MCCP standard. The extracted mass shows no changes in the peak profile between SCCP standard and mixture and no interferences from either MCCPs or PCBs. This particular congener was not detected in the MCCP standard.

Noteworthy, salmon (*Salmo salar*) samples from aquaculture in Norway (#1-4) showed similar CP homologue patterns which differed both from the CP pattern of a farmed salmon sample from Scotland (#5) and a wild salmon sample (#6) from Denmark/FAO 67. For instance, the Scottish salmon sample was characterized by high amounts of SCCPs of all chain lengths along with a four-fold higher relative abundance of hexachloropentadecane isomers compared to the other samples (Figure 4). These first results indicated that an assignment of areas of origin to salmon samples may be possible using the retrieved CP homologue patterns.

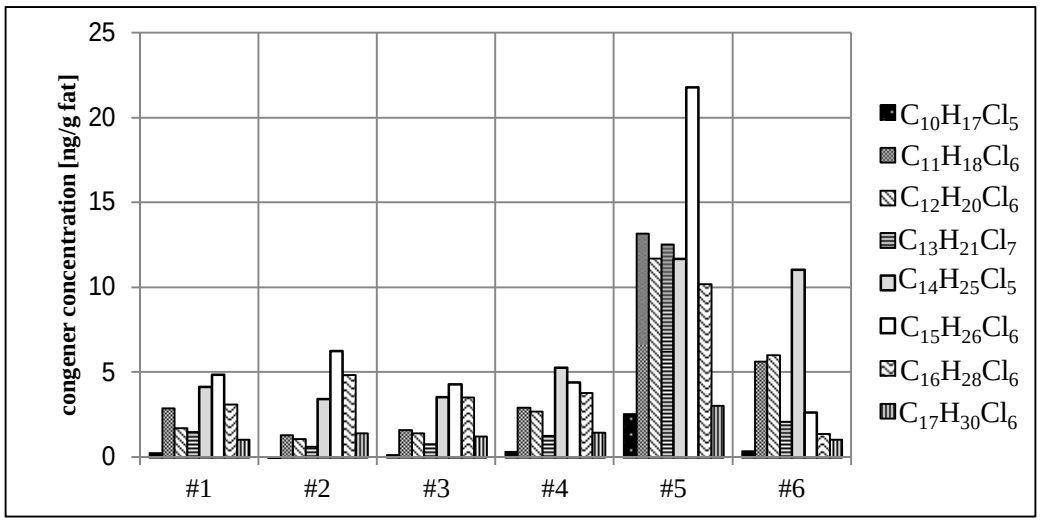


**Figure 4**. Concentrations of selected SCCP und MCCP homologues (represented by their most abundant isotope peaks of the dominant congener) in salmon samples.

These results demonstrate that GC in combination with high resolution, accurate mass Orbitrap-MS enables insights into the pattern and content of CPs without having to fear mass interferences from both other CP homologues and PCBs, indicating the same for other halogenated compounds. Preliminary results suggest that determination of both CPs and PCBs in the same sample is possible in one run, presenting a potential for shorter sample preparation and quicker analyses of prevalent chlorinated POPs in food.

**References**

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