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**OPTIONS FOR ANALYSING CHLORINATED PARAFFINS IN ENVIRONMENTAL MATRICES**

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## Introduction

Chlorinated paraffins (CPs), complex industrial mixtures of >1000 polychlorinated n-alkanes, are extensively used for numerous applications (e.g. metal drilling, flame retardants) and produced in high amounts. As a result, they are present in almost every environmental compartment, including remote areas[1]. However, international classification and regulations are still curbed by insufficient information on their levels and fate, and toxicity potential, while robust analytical tools to assess this are missing. Limited data shows that of all CP groups, short-chained CPs ($C_{10-13}$; SCCPs) have the highest toxicity, bioaccumulation and long range transport potential and are therefore under particular scrutiny. Data for medium-chained CPs ($C_{14-17}$; MCCPs) is inconclusive and information on long-chained CPs ($C_{>18}$; LCCPs) is lacking[2].

This is mainly due to the difficulties that arise with CP analysis[3]. While only semi-quantitative analysis is possible with the most commonly applied technique (GC-qMS)[4], the number of studies reporting new and improved methods has rapidly increased since 2010. For example, Bogdal et al.[5] developed a novel and promising use of high resolution time of flight MS ($CH_2Cl_2$-APCI-QTOF-MS) and Xia et al.[6] screened SCCPs with a two dimensional GC combined with a micro electron capture detector (GCxGC-μECD) with a thermal modulator. Furthermore, a method that distinguishes between SCCPs and MCCPs on GC-ECNI-qMS has been developed[7]. Therefore, our aim was to compare the suitability of current and new instrumental techniques to determine CPs in environmental matrices. First, we identified key challenges in CP analysis on the most commonly applied technique (GC-qMS). Then, an instrumental technique was developed for GCxGC-μECD with a flow modulator and, next to the $CH_2Cl_2$-APCI-QTOF-MS, it was tested on how they cope with the identified key challenges. This was done by analysing CP mixtures, other polychlorinated compounds and environmental samples.

## Materials & Methods

Eight commercial SCCP, LCCP and MCCP mixtures (100 ng/ μL) with different chlorine contents were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Collection, extraction and clean-up procedures of samples (sole, cockle shells and suspended solid matter) are described elsewhere[8].

Instrumental techniques

An Agilent 6890N gas chromatograph (GC) coupled to a quadrupole mass spectrometry (GC-qMS) system in electron capture negative ionisation (ECNI) mode with a DB5 (15m x 0.25mm i.d. x 0.1μm) GC column, and methane as reagent gas was used. The MS was run in selected ion monitoring mode (SIM) mode and the CP congener groups were quantified by the [M-Cl] fragments.

Then, the Agilent 7890A GCxGC with differential flow technology as modulator coupled to a micro electron capture detector (μECD) with a scan speed of 100 Hz was used. One μL of the sample was introduced using a splitless injection. The GC temperature programme started at 100 ºC held for 2 min, then increased by 10 ºC/min to 200 ºC and 1.5 ºC/min to 200 ºC, and held for 8 min. A DB-5 (15 m x 0.25 mm i.d. x 0.25 μm) was used as primary column, and a ZB-50 (5 m x 0.25 mm i.d.x 0.25 μm) was used as secondary column. The modulation period was 4 seconds, and helium served as carrier gas using a flow rate of 0.4 mL/min (first dimension and 35 mL/min second dimension).

Lastly, a quadrupole time-of-flight high resolution mass spectrometer (qTOF-HRMS) (Triple TOF 5600 AB/Sciex, Concord, Ontario, Canada) running in negative atmospheric pressure chemical ionization (APCI) mode was also used. The injection was performed with an Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan) and acetonitrile as eluent with an isocratic flow of 250 μL/min. Dichloromethane ($CH_2Cl_2$) with a flow rate of 40 μL/min was mixed with the eluent just before the

injection entered the ion source, to increase the sensitivity of the CP detection in negative APCI mode. Adding $CH_2Cl_2$ leads to an excess of $Cl^-$ ions in the ion source, enhancing the formation of $[M+Cl]^-$.

**Results and discussion**

Key challenges with GC-qMS
Two key challenges arise with GC-ECNI-qMS, the response and the separation. Compared to other polychlorinated compounds, the response of SCCPs is low (Fig. 1a), that of MCCPs even lower. LCCPs as well as lower chlorinated congeners (CPs with $<Cl_5$) are usually not even detected. Chromatographic separation between CPs and of CPs from other compounds is not achieved by one dimensional GC and CPs appear as big lump in the chromatogram. Separation and quantification of congener groups by mass spectrometry is difficult too, as high resolving power is needed (10 000) to separate within CP congener groups and CPs from other polychlorinated compounds (Table 1), which is not the case with qMS (resolving power 1000), creating mass overlap and potential errors in quantification. Extensive identification and quantitation procedures have been developed to minimize these interferences[4,7], however require multiple injections, are tedious and time consuming (analysis time 120 min). Furthermore, suitable individual congener standards are currently not available yet and therefore only CP mixtures can be used for quantification

GCxGC-μECD & $CH_2Cl_2$-APCI-QTOF-MS
Both techniques show great potential of improving CP analysis significantly in terms of detection and separation. Although the response was low, LCCPs were detected by the GCxGC-μECD. CPs were separated to some degree with SCCPs, MCCPs and LCCPs separated from each other as well as from other polychlorinated compounds (Fig. 1b). Also lower chlorinated congers can be detected.
With the QTOF, LCCPs were also detected and particularly the response of MCCPs was improved (Figure 2), suggesting this technique is highly suitable for MCCP analysis. MCCP congener groups with 4 chlorine atoms were found in biota samples as well as MCCP mixtures, indicating that lower chlorinated CPs can be detected with this technique. As QTOF has a resolving power of 40 000, CP congener groups can be separated by mass spectrometry. Apart from the nominal masses of two congener groups, no interfering response of other polychlorinated compounds was found when using nominal masses of the CP congener groups (Fig. 2). Furthermore, the QTOF technique has a rapid analysis time (2 min).

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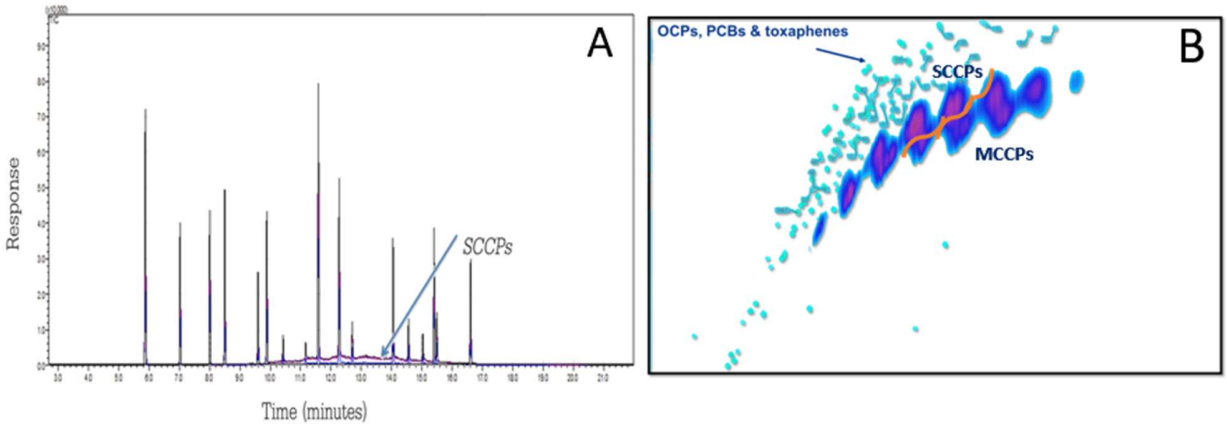


*Figure 1: A) Total ion chromatogram of polychlorinated compounds (organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and SCCPs 63% Cl content by weight) and B) GCxGC-ECD contour plot of MCCPs 57% Cl (below orange line) and SCCPs 63% Cl (above orange line), as well as polychlorinated compounds (OCPs, PCBs and toxaphenes), indicated by blue dots.*

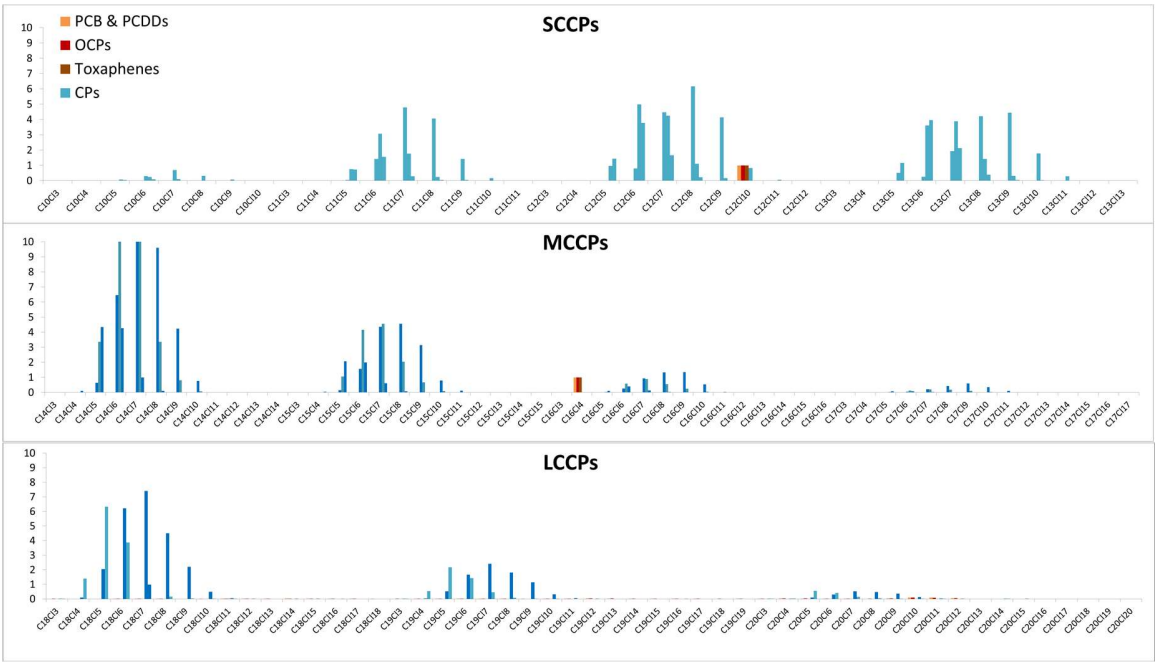


*Figure 2: Measured response of CP mixtures and other polychlorinated compounds (e.g. PCBs, OCPs PCDDs and toxaphene) on nominal masses from CP congener groups measured by $CH_2Cl_2$-APCI-QTOF-M.*

**Table 1: Separation of CP congener groups by mass spectrometry***

| | *Nominal mass* | *Unresolved ion range by a resolving power of 1000* | *Unresolved ion range by a resolving power of 10 000* |
|---|---|---|---|
| **SCCP** $C_{10}H_{12}Cl_{10}$ | 450.8077 | 450.3569 - 451.2585 | 450.7626 - 450.8528 |
| **MCCP** $C_{15}H_{24}Cl_8$ | 450.9668 | 450.5158 - 451.4178 | 450.9217 - 451.0119 |

**Calculated by the formula:* $unresolved\ ion\ range = nominal\ mass + \frac{nominal\ mass}{resolving\ power}, nominal\ mass - \frac{nominal\ mass}{resolving\ power}$