

Study of the use of Negative Chemical- and Electron Impact- Ionization modes for the analysis of Short- and Medium- Chlorinated Paraffins by High-Resolution Mass Spectrometry

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1 Introduction

Chlorinated paraffins, CPs, are also known as chlorinated alkanes. Depending on the different carbon-chain lengths CPs are generally classified into three groups: short-chain (SCCPs, C₁₀–C₁₃), medium-chain (MCCPs, C₁₄–C₁₇), and long-chain (LCCPs, C₁₈–C₃₀)¹. In 2017, SCCPs were classified as persistent organic pollutants (POPs) under the UN Stockholm Convention (UNEP, 2017)². Even so, they are produced in higher volumes than other POPs and this gives rise to their ubiquitous presence in the environment³.

The analysis of CPs has been a challenge in analytical chemistry for over three decades⁴. This arises from the presence and analytical interferences of other POPs^{5,6}, and from the high complexity of CPs⁷ that consist of complex mixtures of several thousands of individual congeners with varying numbers of chlorine atoms and different chain lengths⁴. According to the numerical estimation of Vetter et al.³, there are around $8.91 \cdot 10^7$ congeners of SCCPs and $2.28 \cdot 10^{10}$ of MCCPs.

High-resolution mass spectrometric techniques (HRMS) such as gas chromatography (GC)-Orbitrap-HRMS can resolve homologs yielding ions, can reduce background interference, and can improve detection accuracy. Also, these can study in-source fragmentation and thermal degradation for CPs with high detail. Negative chemical ionization (NCI) is the most applied ionization mode for CPs⁸. This “soft” ionization method enhances the sensitivity of the dominant fragment ion which is useful in the analysis of CP homologs with complex composition⁹. On the other hand, a “hard” ionization, electron impact (EI), yields high fragmentation and it is often difficult to find homolog-specific ions with sufficient intensity for use as quantification masses¹⁰. However, when it comes to the low chlorinated CPs (\leq Cl₅), they cannot be detected at high enough sensitivity by NCI, while EI can be used to detect and quantify them¹¹.

Matsukami et al.¹¹ could detect tetra- and penta-chlorinated homologs using GC-EI-orbitrap-HRMS, while they analyzed the higher chlorinated ones applying NCI. Harada et al.¹² also used both ionization modes for the analysis of SCCPs, and they were able to quantify congeners with 5 chlorine atoms in pooled 24 h food. McGrath, Poma, Matsukami, et al.¹³ analyzed CPs by EI too, and they found that the lowest chlorinated CPs showed the highest relative abundance in polyvinylchloride and rubber consumer products and toys.

This work aims to study both ionization modes for the analysis of CPs. In this way, the distribution of the different congeners in technical mixtures and their sensitivity were analyzed to achieve an accurate description of the CP mixtures that could be applied for further quantification methods of different indoor environmental matrices. Considering that indoor environments are a potentially important source of human exposure to pollutants, especially indoor dust, and the limited information available¹, a test on dust samples was also performed for both ionization modes.

2 Materials and Methods

2.1 Standards

For the study of the ionization mode influence on the congener distribution and building the calibration curve, three technical SCCP mixtures (C₁₀–₁₃) containing 51.5%, 55.5%, and 63% chlorine and three MCCP mixtures (C₁₄–₁₇) containing 42%, 52%, and 57% chlorine were used. ¹³C₁₀-labeled 1,5,5',6,6',10-hexachlorodecane (¹³C₁₀-hexachlorodecane) was used as an injection standard.

2.2 Sampling and sample pretreatment

Indoor dust samples were taken in Örebro in July 2019. The sampling was done using a vacuum cleaner coupled with a <0.7 μm pore size glass fiber filter. The dust was transferred to a previously sterilized amber vial and stored at -20 °C until analysis. 0.10 ± 0.03 g of dust were weighed and transferred to a 10 mL glass test-tube and spiked with 10 ng of carbon-13 labeled ¹³C₁₀-hexachlorodecane. 1.5 mL of dichloromethane (DCM): hexane (3:1, v/v) were added to the tubes and they were vortexed for 10 seconds prior to sonification for 15 minutes, while every 5 minutes the tubes were vortexed for 5 seconds. Then, the samples were centrifugated for 8 minutes at 3000 rpm and the supernatant was collected. This procedure was done three times and all of them were combined. The extracts were concentrated to 1 mL under N₂ stream. For the clean-up, columns consisting of 0.5 g of silica gel, 0.5 g of florisil, and 0.5 g of dried sodium sulfate were used. The analytes were eluted with 15 mL of DCM: hexane (1:1, v/v) and then concentrated to 0.5 mL under N₂ stream prior to analysis. The same procedure was also done using sodium sulfate instead of dust and used as a procedural blank.

2.3 Instrumental setups and data acquisition

A TRACE 1310 GC system coupled to a Q-Exactive mass spectrometer fitted with a chemical ionization (CI) source was used with a 30 m × 0.25 mm, 0.25 μm DB-5MS column. The same oven temperature program was used for EI and NCI methods: 100 °C for 5 minutes and then increased to 300 °C with a slope of 20 °C/min and hold for 10 minutes. For the EI method the MS transfer line and the ion source temperature were both set at 300 °C and for NCI at 280 °C and 180 °C, respectively. Methane was used as CI gas with a 1.2 mL/min flow.

For both methods, the data was acquired in a full scan mode (m/z 100–700) with a resolving power of 60 000. Based on previous studies, for the EI method, the signals of the three most abundant isotopologues of the $[M - Cl - 2HCl]^+$ were monitored for each homolog¹¹. For the NCI method, $[M - Cl]^-$ ions were monitored for the congeners that contained seven and more chlorines, and for those that contain six or less $[M - HCl]^-$ ¹⁴. The targeted homologs were C_nCl_m with $n=10-17$ and $m=3-n$. Signals were extracted at ±5 ppm, using TraceFinder 4.1 EFS software.

2.4 Quantification method

The quantification method used in the present work was based on the one proposed by Reth et al.¹⁵ that relates the total response factor, RF, to the chlorine content. A six-point calibration curve was built for each SCCPs and MCCPs mixtures with different chlorine contents at 5 ppm in n-hexane. Three points of each calibration curve were the diluted technical mixtures and the other three were 1:1 mixes of two of them, obtaining then the chlorine content in between.

3 Results

A different congener distribution was obtained for the same standard mixtures depending on the used ionization mode. When analyzing SCCPs in EI mode, the most abundant congeners were those containing four or five chlorines. Using this ionization mode, the congeners that contain between three and nine chlorines were detected. However, the sensitivity for the congeners with nine Cl was too low and they were only seen for SCCPs of 12 and 13 carbons. As can be seen in Figure 1, there was a clear tendency in the chlorine distribution depending on the carbon chain length. When the carbon chain increased, the abundance of the higher chlorinated SCCPs also increased. This trend was clearly shown by the congeners containing 3 chlorines, whose relative abundance decreased when the number of carbons increased, and the congener with 8 chlorines which became more abundant when the carbon chain became longer. On the other hand, when NCI was used, the most abundant congeners contained seven or eight chlorines. Using this ionization mode, the detected number of chlorines ranged from five to ten chlorines, but the sensitivity for the congeners with five Cl was mostly too low. Using NCI, the same trend was seen, since the abundance of the seven chlorine congeners decreased while the nine Cl congeners increased with the increment of the carbon chain length. The congener distribution of MCCPs showed the same trend that was seen for SCCPs. Using EI lower chlorination degrees had higher relative abundance than applying NCI mode, while the more chlorinated congeners showed higher abundance with NCI. For EI, the most abundant MCCP congeners contained six or seven chlorines, while when NCI was used the most abundant were those with eight or nine chlorines.

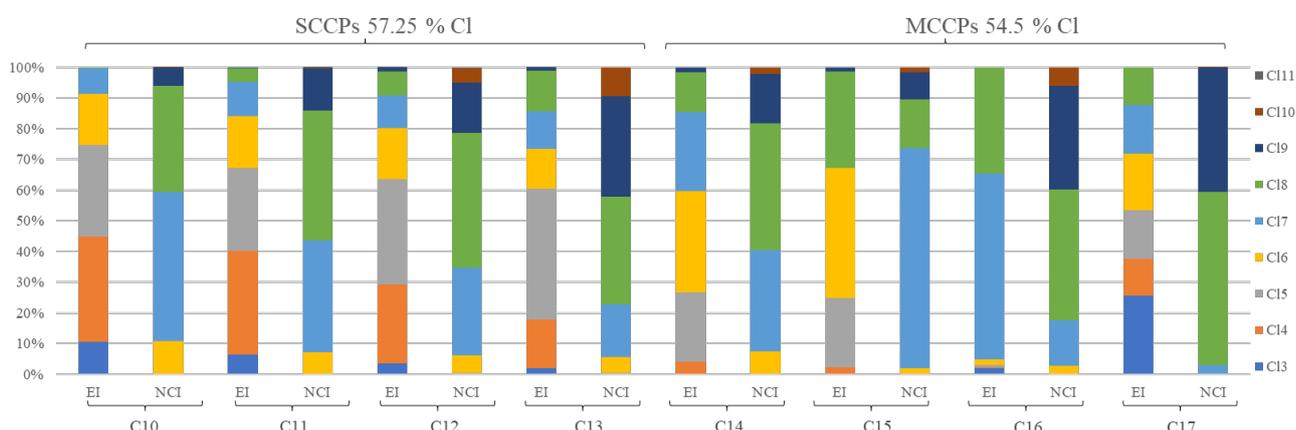


Figure 1: Relative abundance of each congener for a standard mixture of SCCPs with 57.25% of chlorine and MCCPs with 54.5% of chlorine when EI and NCI ionization modes are used

As described above, EI showed better sensitivity for the determination of tri-, tetra-, and penta-chlorinated homologs, while NCI was more suitable for those with a higher number of chlorines. Still, some congeners could be analyzed by both ionization methods. However, the sensitivity for those congeners was not the same using both ionization modes. Congeners with six and more chlorine atoms, using EI showed noisy peak shape and lower values of peak area and signal-to-noise ratio compared to the peaks of the same congeners when they were obtained by NCI. On the other hand, homologs with five or fewer chlorines showed better peaks when they were ionized by EI compared to NCI. Figure 2 shows examples of chromatographic peaks when both ionization modes were used for $C_{11}H_{17}Cl_7$ from a SCCPs standard mixture with 53.5 % of chlorine content and $C_{14}H_{25}Cl_5$ from a MCCP standard mixture with 49.5

% of chlorine. As it can be seen, for $C_{11}H_{17}Cl_7$ the signal-to-noise ratio and the peak area were ten times higher than when NCI was used, while for $C_{14}H_{25}Cl_5$ using EI the peak area was 100 times bigger than by using NCI.

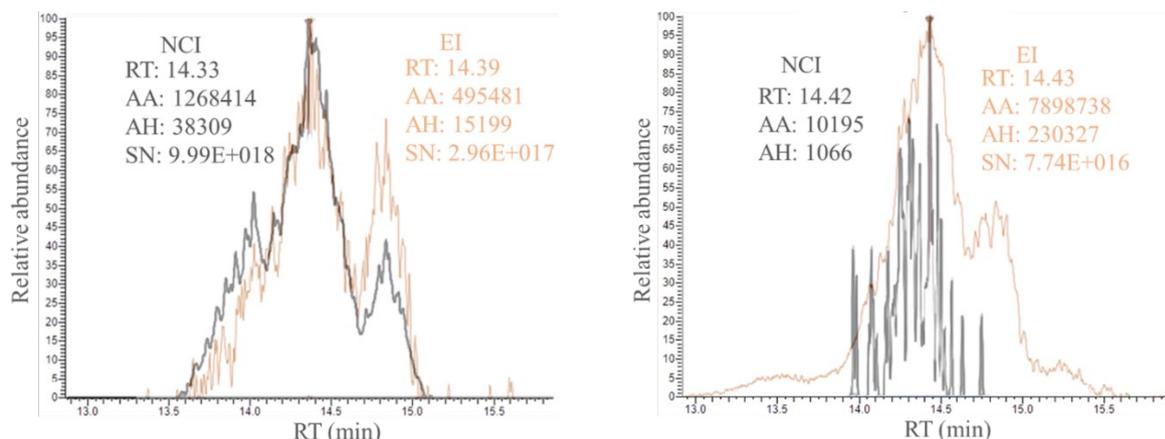


Figure 2: Chromatographic peak of $C_{11}H_{17}Cl_7$ from a SCCPs standard mixture with 53.5 % of chlorine content (left) and $C_{14}H_{25}Cl_5$ from a MCCPs standard mixture with 49.5 % of chlorine (right) when EI and NCI methods are used. Abbreviations: retention time (RT), peak area (AA), peak height (AH), and signal to noise (SN) values.

Table 1 shows the equation and linearity of the curves obtained for SCCPs and MCCPs when both ionization modes were used. The calibration curves for EI showed a negative slope, while the ones obtained by NCI were positive. This means that the total RF increased with the chlorine content when NCI was used and decreased for EI. This further relies on the calculation of the real samples RF, because the same chlorine content mixtures led to different total RF values depending on which calibration curve was used. Regarding the linearity, calibration curves obtained by both ionization methods had a coefficient of determination above 0.90, while SCCPs showed better linearity than MCCPs in both modes.

Table 1: Equation and determination coefficient for the six-point calibration curve built based on the SCCPs and MCCPs technical mixtures, and average values and standard deviation of the concentration in ng/g and chlorine content in percentage of SCCPs and MCCPs in dust samples by using EI and NCI ionization modes

Analyzed	Ionization mode	Calibration curves		Dust samples	
		Equation	R ²	Chlorine content	Concentration
SCCPs	EI	RF = -15.28 · (Cl %) + 1005.13	0.94	52.3 ± 0.0	77.8 ± 2.9
	NCI	RF = 26.43 · (Cl %) - 1342.60	0.97	61.5 ± 0.3	40.4 ± 1.3
MCCPs	EI	RF = -1.62 · (Cl %) + 120.92	0.90	46.0 ± 0.7	121.6 ± 6.8
	NCI	RF = 7.90 · (Cl %) - 328.41	0.92	56.9 ± 0.6	178.1 ± 41.3

A test was carried out by analyzing two replicates of the same dust sample using both ionization modes. The equations of the calibration curves collected in Table 1 were further used for their quantification. Table 1 shows the mean value, after blank correction, and its uncertainty based on the standard deviation of both replicates. SCCPs were quantified in higher concentrations when EI was used compared to NCI mode. However, the measured values for MCCPs were higher when NCI mode was applied. Regarding the measured Cl % content, calculated by the equation reported by Reth et al.¹⁵, for both, SCCPs and MCCPs, the measured values were higher with NCI.

4 Discussion

Matching the results obtained by Matsukami et al.¹¹ and Harada et al.¹², both ionization modes (EI and NCI) have been demonstrated to be suitable for the analysis of SCCPs and MCCPs. However, the congener pattern obtained by each method does not match the other. This agrees with the results obtained by McGrath, Poma, Matsukami, et al.¹³, which showed varying congener group distribution patterns using ECNI and ESI. Matsukami et al.¹¹ used EI mode for the analysis of tetra- and penta- chlorinated CPs while NCI for those with higher chlorine content, the congener distribution shown in this work concurs with it. This is the reason why when EI was applied, the calibration curves had negative slopes because when the Cl % is increased the low chlorinated congeners are less abundant.

Testing real dust samples, when EI ionization mode was used, lower Cl content was measured compared to NCI. This agrees with previous studies and with the performed analysis of the standards. Moreover, SCCPs were measured in higher concentration by applying EI mode, and MCCPs showed higher values with NCI, which concurs with the same trend that has been exposed since MCCPs have a higher chlorinated degree than SCCPs.

5 Conclusions

The ionization mode's influence on the CPs mixtures' analysis was investigated. The measured chlorination degree, congener distribution, and estimation of the total RF value were not the same if EI or NCI were used, both for the standard solutions and dust samples. EI mode showed better sensitivity for the congeners with five or fewer chlorines and NCI for those with six and more.

Since the main aim of this project is to analyze samples from indoor environments, further studies are needed to achieve an optimal combination of both ionization methods based on their complementarity. According to the obtained results, EI should be applied for the CPs with $\leq Cl_5$, and NCI for those with $\geq Cl_6$.

6 References

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