ulSynthesis and characterization of C_{10}-C_{17} chloroparaffins with single chain lengths and their use for quantification via GC/ECNI-MS-SIM

Sprengel J, Vetter W

Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, D-70593 Stuttgart, Germany, jannik.sprengel@uni-hohenheim.de

Introduction

Chlorinated paraffins (CPs) are highly complex mixtures of chlorinated *n*-alkanes with different chain lengths and degrees of chlorination. CP mixtures are nowadays classified regarding their chain length ranges into (i) short-chain chlorinated paraffins (SCCPs, C_{10}-C_{13}), (ii) medium-chain chlorinated paraffins (MCCPs, C_{14}-C_{17}) and long-chain chlorinated paraffins (LCCPs, $>C_{18}$).[1] CPs are high production volume chemicals (HPVCs) with an estimated annual production figure of currently 1.3 million metric tons[2] (~total production volume of polychlorinated biphenyls (PCBs) in 30 years[3]). While SCCPs have recently been regulated and characterized as persistent organic pollutants (POPs)[4–6], no regulations exist for MCCPs and LCCPs.

Although CPs have been analyzed in environmental samples for decades, no definitive quantification method has been established yet. CPs generally consist of many thousands compounds which cannot even be separated via tandem chromatography.[7] One of the most frequently used methods to quantitate CPs via low resolution mass spectrometry (LRMS) is gas chromatography (GC) with electron capture negative ionization mass spectrometry (ECNI-MS).[8] Measurements are performed in the selected ion monitoring (SIM) mode using the most prominent isotope peaks of the [M-Cl]⁻-fragment ion.[8] The total CP amount is determined by linear regression via the correlation between Cl content and response factor.[8] This method allows to quantify samples whose Cl content does not closely resemble a technical standard through linear regression.[8] However, problems may arise through overlapping of several *m/z* values of ions of different chain lengths and degrees of chlorination.[9] The goal of this study was to synthesize CP mixtures with individual chain lengths and checking them for potential mass overlaps with other chain lengths. This information was used to inspect the GC/ECNI-MS-SIM quantification procedure of Reth *et al*.[8] on possible improvements.

Materials and methods

Chemicals and standards. *n*-hexane (HPLC grade) was from Th. Geyer (Renningen, Germany). *n*-Decane (95%), *n*-dodecane (98%), *n*-hexadecane (*puriss, p.a.* standard for GC), 2,2,4-trimethylpentane and dichloromethane (both for pesticide residue analysis), and sodium chloride were from Fluka Analytics (Seelze, Germany). *n*-Undecane (98%), *n*-tridecane (98%), *n*-pentadecane (98%), *n*-heptadecane (98%) and sulfuryl chloride (97%) were from Sigma-Aldrich (Steinheim, Germany). *n*-Tetradecane (99%) was obtained from Alfa Aesar (Karlsruhe, Germany). Sodium hydrogen carbonate (*p.a.*, ≥99.5%) and sodium sulfate (≥99%) were from Carl Roth (Karlsruhe, Germany). Commercial analytical standard mixtures of SCCPs (51.5%, 55.5% and 63% Cl content) and MCCPs (42%, 52% and 57% Cl content), each at 100 ng/µL, were from Dr. Ehrenstorfer (Augsburg, Germany). The internal standard, perdeuterated α-hexachlorocyclohexane (α-PDHCH) was synthesized in our work group.[10]

Synthesis of CPs. The synthesis followed the procedure described by Tomy *et al*.[11] and Gallistl *et al*..[12] In short, ~0.7 g of the respective *n*-alkane, 10 mL dichloromethane and 15 mL freshly distilled sulfuryl chloride were irradiated for 180 min (for the C_{14}- to C_{16}-alkanes) and 220 min (for the C_{10}- to C_{13}- and C_{17}-alkanes). Crude

products were extracted and purified as described before[12], yielding ~1 g of colorless and viscous liquids. Purity was determined by GC/FID and GC/ECNI-MS, and Cl contents were determined by EA/IRMS.

GC/ECNI-MS. Quantitative measurements were performed on a 7890/5975C system (Agilent, Waldbronn, Germany) equipped with an HP-5ms UI column (30 m x 0.25 mm i.d., 0.25 µm d_f) (Agilent, Waldbronn, Germany). Helium 5.0 (Westfalen, Münster, Germany) was used as carrier gas at a constant flow of 1.2 mL/min. Methane 5.5 (Air Liquide, Bopfingen, Germany) was used as the reagent gas at 1.6×10^{-4} Torr. Temperatures of ion source and transfer line were set at 150 °C and 300 °C, respectively. The GC oven program was described elsewhere.[12] Measurements in full scan mode covered *m/z* 50 to 800 and in the SIM mode the two most abundant isotope peaks of the [M-Cl]⁻ ions of Cl_4- to Cl_{10}-congeners as reported by Reth *et al.*[9] were measured.

GC/FID. All samples were measured on an HP 5890 series II GC equipped with an HP 7673 A autosampler (Agilent, Waldbronn, Germany). The conditions were described elsewhere.[13]

Elemental analysis coupled to isotope-ratio mass spectrometry (EA/IRMS). Cl contents of CPs were determined via elemental analysis coupled to isotope-ratio mass spectrometry EA/IRMS as reported elsewhere.[12]

Quantification of the synthesized CPs. CP solutions of each single chain length were prepared at both ~100 ng/µL and ~50 ng/µL in *i*-octane. Furthermore, 1+1 (v:v) mixtures of each combination of two chain lengths and three mixtures each of SCCPs and MCCPs (∑CP ~100 ng/µL, **Tab. 1**) were prepared and analyzed. For each chain length, the two most abundant isotope peaks of the [M-Cl]⁻ homolog clusters with 4-10 Cl atoms were measured. In addition, Cl_{11} congeners were also measured for C_{17}-CPs (**Tab. 2**). Samples were quantified using the method by Reth *et al.*[8] (method 1). In short, the amount of CPs is calculated via linear regression between the Cl content and a response factor determined in technical standards of a given concentration. A modification of this method was introduced by using exponential instead of linear regression to calculate the response factor (new

Tab. 1: chain length distribution of three SCCP- and three MCCP-mixes (∑CP ~ 100 ng/µL)

	SCCP mixes				MCCP mixes		
	mix 1	mix 2	mix 3		mix 1	mix 2	mix 3
C_{10}	7%	5%	24%	C_{14}	66%	66%	23%
C_{11}	32%	41%	23%	C_{15}	24%	30%	30%
C_{12}	30%	28%	23%	C_{16}	7%	4%	30%
C_{13}	31%	25%	31%	C_{17}	4%	2%	24%

method 2, **Tab. 2**). A third quantification method took advantage of the average amount of Cl atoms per molecule. Here, calibration curves were determined separately for each single chain length. For that, approximate concentrations of single chain length CPs in the technical standards were calculated (new method 3, **Tab. 2**).

Results and discussion

Characterization of the synthesized CPs. The Cl contents of the eight synthesized single chain length CPs (C_{10}-C_{17}) as determined via EA/IRMS ranged from 51.1% (C_{15}-CPs) to 61.3% (C_{17}-CPs) (**Tab. 2**). This corresponded with 6-7 Cl atoms per carbon chain for C_{10}- to C_{16}-CPs. Only C_{17}-CPs showed a higher average Cl content of ~10 Cl atoms. No impurities or by-products were detected by GC/FID and GC/ECNI-MS.

Quantification of single chain length CP-solutions. GC/ECNI-MS quantification of single chain length CPs (~100 ng/µL) with linear regression according to Reth *et al.*[8] deviated between -73% and +107% from the target value (**Tab. 3**). Four chain lengths with an average of ~6 Cl atoms per carbon chain (C_{10}-, C_{11}-, C_{14}- and C_{15}-CPs) were underestimated (<50% of the target concentration), whereas the higher chlorinated chain lengths (~60% or more, C_{12}- C_{13}- and C_{17}-CPs) were generally overestimated. This was likely due to the fact that the GC/ECNI-MS response of homolog groups strongly depends on the degree of chlorination.[8] Generally, results obtained with exponential regression (new method 2) were closer to the true value for C_{10}-, C_{11}-, C_{14} and C_{15}-CPs while

method 3 additionally was more accurate for C_{13}-CPs (**Tab. 3**). Although single chain length concentrations in the standards could only be estimated, quantifying each chain length separately (new method 3) showed the smallest quantification errors for four out of seven chain lengths.

Tab. 2: Cl contents and homolog patterns of the synthesised CPs and formulas used for new methods 2 and 3.

	C_{10}-CP	C_{11}-CP	C_{12}-CP	C_{13}-CP	C_{14}-CP	C_{15}-CP	C_{16}-CP	C_{17}-CP
Cl-content (Cl%)	60.5	58.3	59.9	59.6	53.2	51.1	53.0	61.3
Cl atoms per chain	5.9	6.0	6.9	7.4	6.2	6.1	7.0	10.3

Homolog pattern (stacked bar chart, legend: 12 Cl, 11 Cl, 10 Cl, 9 Cl, 8 Cl, 7 Cl, 6 Cl, 5 Cl, 4 Cl):

Cl group	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}
top	5%	6%	6%	1%		3%	2%	2%
	21%	22%	22%	26%	14%	13%	11%	13%
				8%	36%	7%	28%	32%
	48%	47%	37%	33%		25%	30%	29%
					39%	36%		
	24%	22%	30%	26%			24%	5%
					11%	16%		16%
bottom	2%	1%	5%	5%			5%	2%

exponential function for method 2	total response factor $= a * e^{[Cl\%]*b}$
calculation of single chain length concentration in technical standards	$c(C_x\text{-CP}) = c(\textstyle\sum CP) * \dfrac{\text{total } C_x\text{-CP peak area}}{\text{total } \sum CP \text{ peak area}}$; (x = 10-17)

Combination of two or more chain lengths. Typically, results for single chain lengths were only slightly influenced by the presence of other chain lengths (e.g. the quantity obtained for C_{11}-CPs in presence of other chain lengths varied less than 8% from the pure C_{11}-CP solution). Only selected mixtures with C_{13}-, C_{15}- and C_{16}-CPs resulted in variations of up to >20% relative to the pure solutions. Hence it was unexpected that self-prepared SCCP mixes (combination of C_{10}- to C_{13}-CP standards) and MCCP-mixes (combination of C_{14}- to C_{17}-standards) with the same chain lengths present as in the analytical standards deviated less from the expected amount of CPs than the single chain standards of the same concentration (**Tab. 3**). Technical standards, quantified as if they were samples, showed the same (lower) quantification deviations as self-prepared mixtures. It thus appeared that the presence of additional chain lengths improved the overall result most likely via overlapping effects of different chain lengths.

Tab. 3: Deviations of calculated CP-concentrations from target value (~100 ng/µL) by quantification methods 1-3

	pure CP solutions							SCCP mixes				MCCP mixes			
	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	mix 1	mix 2	mix 3	Std 55%	mix 1	mix 2	mix 3	Std 52%
method 1	-73%	-59%	3%	107%	-56%	-51%	14%	-20%	-23%	-23%	-10%	-52%	-50%	-43%	-48%
method 2	-63%	-38%	56%	152%	-35%	-13%	106%	8%	4%	10%	17%	-30%	-24%	-34%	-17%
method 3	50%	12%	49%	48%	12%	-34%	-65%	4%	3%	5%	20%	-13%	-8%	-44%	-9%

Examination of overlapping effects. For mixtures of more than one chain length, numerous overlapping effects are inevitable when using LRMS. Interferences between homologs that are five carbon atoms and two Cl atoms or two carbon atoms and one Cl atom apart were already observed and examined before.[9] Analysis of the single chain length CP standard solutions by GC/ECNI-SIM ions specific for the other chain lengths allowed to discover several additional mimicking effects of CPs. Those were distributed into three groups in the dependence of the grade of deviation in the ratio of quantifier and qualifier ion from the theoretical value (IR_{dev}): (i) IR_{dev} <20%, (ii) IR_{dev} 20-50% and (iii) IR_{dev} >50% (or forming only the quantifier or qualifier ion) for the measured chain length. The individual chain lengths had an impact on at least 10 homolog groups of other chain lengths, and the maximum of 26 interferences was observed for C_{17}-CPs. As expected, all single chain length CP standards gave response for CPs with three carbon atoms more and/or less with mimicking effects of group (ii) and (iii). MCCP chain lengths also showed eleven overlaps which affected only the quantifier ions (but not the qualifier ions) of other MCCP chain lengths which falsified their IR. This problem was much more pronounced as for SCCPs which only showed two such overlaps. Effects on only the qualifier ions was almost exclusively observed between SCCP and MCCP species. The only exception within MCCPs was noted for C_{15}- and C_{16}-CPs which mimicked the presence of Cl_8 congeners of MCCPs with one additional carbon atom, i.e. $C_{16}Cl_8$- and $C_{17}Cl_8$-CPs.

Although the peak areas caused by these effects were relatively small, they may disrupt exact quantification of minor chain lengths in CP mixtures as well as qualitative characterization via homolog patterns when using GC/ECNI-MS with low resolution. These effects were likely the main reason for the lower deviations in the SCCP and MCCP mixtures compared to the individual chain lengths. At the moment it is difficult to estimate if this will change when different compositions of CPs are found in environmental samples. GC/ECNI-HRMS might be helpful to investigate these issues more in detail.[14]

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