

Synthesis and characterization of a medium-chain chlorinated paraffin (MCCP) mixture

Christoph Gallistl, Walter Vetter

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70593 Stuttgart, Germany

Introduction

Chlorinated paraffins (CPs) are a class of polyhalogenated compounds produced on a large scale since the early 1930s.¹ In different industries they are used as high pressure lubricants in metal working fluids and especially as plasticisers and flame retardants in polymers, paints and sealants.¹ CPs can be sub-divided into three classes depending on the length of their carbon backbones, i.e. short-chain CPs (SCCPs, C₁₀-C₁₃) which are strictly regulated by law, as well as medium-chain (MCCPs, C₁₄-C₁₇) and long-chain CPs (LCCPs, >C₁₇).^{2,3}

CPs were rated as the most complex class of polyhalogenated contaminants in the environment and their analysis is still challenging.⁴ The group of MCCPs alone may include >100.000 congeners due to different chlorination degree and position of the chlorine atoms.⁵ Due to the high complexity, technical CP congeners and their residues in environmental samples cannot be separated by gas chromatography and the resulting co-elutions of many CP congeners lead to signal overlapping, even when mass spectrometry is used as detection technique.⁶

The aim of this study was to synthesize less complex MCCP mixtures by means of the chlorination of one individual backbone (*n*-tetradecane) to different chlorine content. The synthesis mixtures were analyzed by different methods (NMR, EA-IRMS, GC/FID, GC/ECD, GC/ECNI-MS and GC/EI-MS).

Materials and methods

Chemicals and standards. Sodium sulfate (water free, p.a., ≥ 99%), *iso*-octane (for pesticide residue analysis) and sulfonyl chloride (≥ 97%) were from Sigma-Aldrich (Steinheim, Germany). *n*-Tetradecane was from Alfa Aesar (Karlsruhe, Germany), whereas *n*-hexane (HPLC grade, ≥ 95%), dichloromethane (p.a., 99.8%) and acetonitrile (HPLC grade, ≥ 99.9%) were from Th. Geyer (Renningen, Germany). Deuterated chloroform (CDCl₃, 99.8%) was from Deutero (Karlsruhe, Germany). The internal standard perdeuterated α -hexachlorocyclohexane (α -PDHCH) was synthesized in our lab.⁷

Synthesis of MCCPs. Photochlorination of *n*-tetradecane was executed based on the method of Tomy *et al.*⁸ *n*-Tetradecane (2 mL) was combined with 15 mL sulfonyl chloride and 10 mL dichloromethane in a 50 mL-quartz glass beaker. For irradiation, the beaker was stored at 20 °C in a water bath in front of a water cooled 150 W mercury medium pressure lamp (TQ 150, Heraeus Noblelight, Hanau, Germany) at a distance of 10 cm. To investigate the reaction process, 1 mL aliquots of the reaction solution were taken after 25 min, 70 min, 120 min, 250 min and 300 min of irradiation. The reaction was stopped by adding 10 mL of water and shaking in 30 mL glass flasks. The solutions were extracted twice with 5 mL of *n*-hexane in a separating funnel. The combined hexane phases were washed alternately with 15 mL of 10% sodium bicarbonate solution (w/w) and 15 mL of bidest. water until pH neutralization. The organic phase was concentrated to ~1 mL by rotary evaporation and solvent was removed by a gentle stream of nitrogen.

Gas chromatography coupled with electron-capture negative ion mass spectrometry (GC/ECNI-MS). Analyses were performed on a 7890/5975C system (Agilent, Waldbronn, Germany) equipped with a HP-5 MS column (30 m x 0.25 mm i.d., 0.25 μ m d_f, Agilent, Waldbronn, Germany). The carrier gas helium 5.0 (Westfalen, Münster, Germany) was used at a constant flow of 1.2 mL/min. Methane 5.5 (Air Liquid, Bopfingen, Germany) was used as the reagent gas at 1.6x10⁻⁴ Torr. Temperatures of ion source and transfer line were set at 150 °C and 300 °C, respectively. Splitless injections (1 μ L) were performed with a PTV injector (CIS-4, Gerstel, Mülheim, Germany) in pulsed pressure mode (25 psi, 1 min). The PTV temperature program was 80 °C (0.01 min), then at 700 °C/min to 300 °C (2 min) and finally cooled with 10 °C/min to 260 °C until the end of the run. The GC oven was programmed at 50 °C (1 min), then at 10 °C/min to 300 °C (14 min). Measurements were run in the full scan mode (*m/z* 50-800) or in SIM mode by recording the two most abundant isotope peaks of the [M-Cl]⁻ and [M-HCl]⁻ ions of Cl₄- to Cl₁₂-chlorinated tetradecanes.

Gas chromatography with electron-capture detection (GC/ECD) and with flame ionization detector (GC/FID). Analysis were performed both on a 5890 series II system (Hewlett Packard, Waldbronn, Germany), equipped with a DB-5 column (30 m x 0.32 mm i.d. x 0.25 μ m d_f). The operation parameters are reported elsewhere.⁹

Response factors of chlorinated *n*-tetradecanes in different detectors. The synthesized product mixtures were diluted in *iso*-octane to ~500 ng/ μ L for GC/FID and GC/EI-MS measurements and to ~50 ng/ μ L for GC/ECD and GC/ECNI-MS measurements, respectively. α -PDHCH was added as internal standard and response factors of the different detectors were calculated from the internal standard corrected peak areas of the MCCP hump peak to the corresponding mass concentrations of the synthesis aliquots. The responses were normalized to the highest response (= 100%) of each detector type.

Elemental analysis coupled to isotope-ratio mass spectrometry (EA-IRMS). The chlorine content of the synthesis mixtures were determined by EA-IRMS using a Euro EA elemental analyzer (Eurovector, Redavalle, Italy) coupled to a Finnigan DELTAplus XP system (Thermo Fisher Scientific, Bremen, Germany). The oxidation and reduction tube temperatures were set at 1000 and 650 °C, respectively. A molecular sieve was used as column and the oven temperature was set at 90 °C with a purge flow of 80 mL/min.⁹ Aliquots (~ 2 mg dissolved in 20 μ L *n*-hexane) were filled in 2.0 x 5.0 mm tin capsules (99.9% Sn) for liquid samples (IVA Analysentechnik, Meerbusch, Germany) and the solvent was vaporized over night at room temperature before the capsules were sealed.¹⁰

^1H and ^{13}C nuclear magnetic resonance spectroscopy ($^1\text{H}/^{13}\text{C}$ -NMR). ^1H -NMR and ^{13}C -NMR spectra were recorded with a 300 MHz INOVA system (Varian, Palo Alto, California, USA). For ^1H -NMR and for ^{13}C -NMR about 20 mg and 150 mg synthesized chlorinated *n*-tetradecane mixtures were dissolved in 300 μL CDCl_3 . Chemical shifts were referenced to the residual signal of CHCl_3 (^1H : 7.26 ppm / ^{13}C : 77.2 ppm).

Results and discussion

Photochlorination of *n*-tetradecane

Our goal was to synthesize MCCP mixtures with same chain length but varying chlorination degree. This was achieved by photochlorination of *n*-tetradecane with sulfonyl chloride. Five aliquots were taken during the synthesis at different time points. The first sample, taken after 25 min, showed a chlorination degree of 44.3% according to elemental analysis (EA). Subsequent aliquots taken after 70 min, 120 min, 250 min and 300 min reaction time had chlorine contents of 51.4%, 55.4%, 55.4%, 58.7% and 59.5%, respectively. GC/ECNI-MS chromatograms of the five aliquots showed no isolated peaks but unresolved “hump” peaks (**Fig. 1a**) although the mixtures were less complex due to the same carbon backbone in comparison to technical MCCP mixtures. Moreover, the elution range was shifted to higher retention time with increasing Cl content.

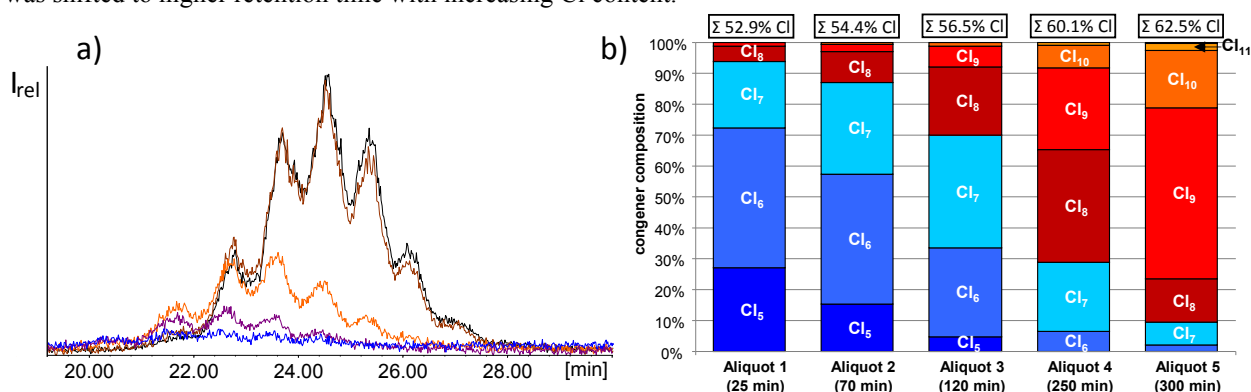


Fig. 1: (a) Overlay of GC/ECNI-MS full scan chromatograms of the synthesis products obtained after 25 min (aliquot 1, blue), 70 min (aliquot 2, violet), 120 min (aliquot 3, orange), 250 min (aliquot 4, brown) and 300 min (aliquot 5, black) reaction time and (b) the corresponding polychlorotetradecane isomer composition (in %) as well as the resulting calculated chlorination degree of the mixture ($\Sigma\%Cl$).

Congener composition of the polychlorotetradecane synthesis mixtures

The congener composition of the polychlorotetradecane mixtures was estimated by integrating the hump peaks of the most abundant isotope peaks of $[\text{M}-\text{Cl}]^+$ of tetra- to dodecachlorinated isomers in the GC/ECNI-MS chromatograms. The resulting peak areas were divided by the number of chlorine atoms to take into account the chlorine depending response in GC/ECNI-MS measurements. This procedure was also suggested by Tomy *et al.* for determination of the congener composition of C_{10} - C_{13} SCCPs.¹¹ For example, the area of the $[\text{M}-\text{Cl}]^+$ ion of $\text{C}_{14}\text{H}_{24}\text{Cl}_6$ (m/z 369) was divided through a factor of five. The percentage of the congeners was calculated by the ratios of this corrected peak areas to the sum of all corrected peak areas. The average chlorination degree calculated out from the congener composition agreed well with the degree of chlorination determined by elemental analysis (**Fig. 1b**). Accordingly, division of the peak areas by the number of chlorine atoms resulted in an acceptable approximation. Synthesis aliquots 1 and 2 were dominated by Cl_6 -tetradecane congeners, whereas synthesis aliquot 3 was dominated by Cl_7 -, aliquot 4 by Cl_8 - and aliquot 5 by Cl_9 -tetradecanes. With increasing reaction time the amount of Cl_5 - and Cl_6 -tetradecanes was continuously decreasing in favor of Cl_9 - to Cl_{11} -isomers.

Comparison of normalized GC/FID, GC/ECD, GC/EI-MS and GC/ECNI-MS response factors

Quantification of CPs is usually performed by GC/ECD, GC/EI-MS or GC/ECNI-MS.¹² Average response factors of the synthesized polychlorotetradecane mixtures in these GC detectors were determined after normalized to the highest response of each detector type (see Materials and methods). Furthermore, GC/FID responses were corrected regarding the average carbon content of the mixtures in order to eliminate carbon content depending effects.¹³ GC/ECD and GC/ECNI responses showed a nearly exponential increase depending on the average

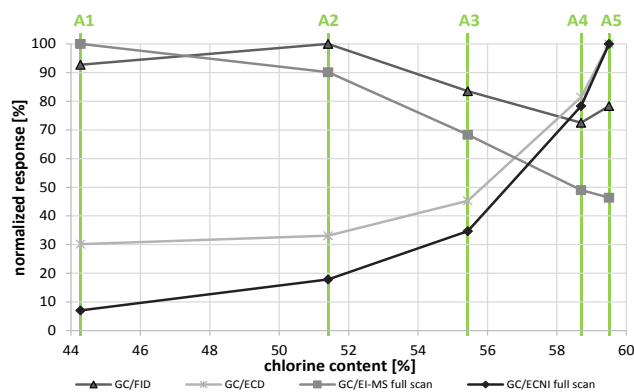


Fig. 2: Normalized GC/FID (carbon content corrected), GC/ECD, GC/EI-MS and GC/ECNI-MS responses of five synthesized polychlorinated *n*-tetradecanes (aliquots 1-5, abbreviated by A1 to A5), depending on their average chlorine content.

chlorine content of the mixtures (Fig. 2). In contrast, GC/EI-MS and GC/FID responses decreased although the GC/FID responses were expected to be independent of the chlorine content after the correction for the carbon content. This effect was most likely due to the high complexity of the mixtures with several thousand co-eluting congeners. The average response factors of MCCP mixtures are heavily influenced by the average chlorine content of the CP mixture and small deviations between the degree of chlorination of a standard mixture and residues in environmental samples can falsify the quantification result.

Measurements of mass-to-charge ratios

The isotope clusters of polychlorotetradecanes in GC/ECNI-MS full scan mode were characterized by overlaps of $[M-Cl]^+$ and $[M-HCl]^+$ fragment ions of isomers (data not shown). In the GC/ECNI-MS SIM mode, the two most abundant isotope peaks of the $[M-Cl]^+$ fragment ions were selected for quantification (Tab. 1). The measured abundance ratio of the isotope peaks of congeners with more than seven chlorine atoms agreed well with the theoretical isotope ratio (Tab. 1).

Tab. 1: Theoretical and measured ratios of two most abundant signals of the respective $[M-Cl]^+$ isotope clusters of polychlorinated *n*-tetradecanes in the GC/ECNI-MS-SIM mode as well as the corresponding percentage amount of the congeners in synthesis aliquot 1 and 5.

molecular formula	most abundant isotope <i>m/z</i>	second abundant isotope <i>m/z</i>	theoretical isotope ratio	Aliquot 1		Aliquot 5	
				congener composition [%]	measured isotope ratio	congener composition [%]	measured isotope ratio
C ₁₄ H ₂₅ Cl ₅	335	333	100 : 78	27.2	100 : 89	n.d.	n.d.
C ₁₄ H ₂₄ Cl ₆	369	371	100 : 64	45.2	100 : 73	1.90	100 : 43
C ₁₄ H ₂₃ Cl ₇	403	405	100 : 80	21.5	100 : 103	7.40	100 : 70
C ₁₄ H ₂₂ Cl ₈	437	439	100 : 96	5.00	100 : 101	14.1	100 : 89
C ₁₄ H ₂₁ Cl ₉	473	471	100 : 89	1.10	100 : 88	55.3	100 : 91
C ₁₄ H ₂₀ Cl ₁₀	507	505	100 : 78	n.d.	n.d.	18.6	100 : 78
C ₁₄ H ₁₉ Cl ₁₁	541	543	100 : 86	n.d.	n.d.	2.50	100 : 85
C ₁₄ H ₁₈ Cl ₁₂	575	577	100 : 96	n.d.	n.d.	0.20	100 : 95

n.d.: not detectable

In contrast, lower chlorinated congeners in synthesis aliquot 1 showed higher ratios than the theoretical isotope (Tab. 1, highlighted in red when ratios were $\geq 5\%$ than theoretical ratios) ratios which was most likely due to the overlapping of $[M-Cl]^+$ with $[M-HCl]^+$ fragment ions. However, in the presence of a high amount of high-chlorinated congeners such as in aliquot 5, the measured isotope ratios of the hexa- to octachlorinated tetradecanes were lower than the theoretical values (Tab. 1, highlighted in blue when ratios were $\leq 5\%$ than theoretical ratios). This could be the result of the mass overlap of $[M-Cl]^+$ with $[M-Cl-HCl]^+$ fragment ions of isomers with one chlorine more (e.g. $[M-Cl]^+$ of hexachlorinated with $[M-Cl-HCl]^+$ of heptachlorinated *n*-tetradecanes).

¹H- and ¹³C-NMR measurements of synthesis aliquots 1-5

¹H-NMR spectra of the synthesis mixtures showed poorly resolved multiplets with chemical shifts ranging from 0.8 to 5.2 ppm (Fig. 3a). Signals at 0.8 to 1.15 ppm originated from terminal methyl groups whereas non-chlorinated methylene groups $[-CH_2-]$ gave rise to broad multiplet peaks at 1.15 to 3.2 ppm. Monochloromethylene groups $[-CHCl-]$, chloromethyl groups $[-CH_2Cl]$ and dichloromethylene groups $[-CCl_2-]$ were found at lower field (3.4 to 5.2 ppm).

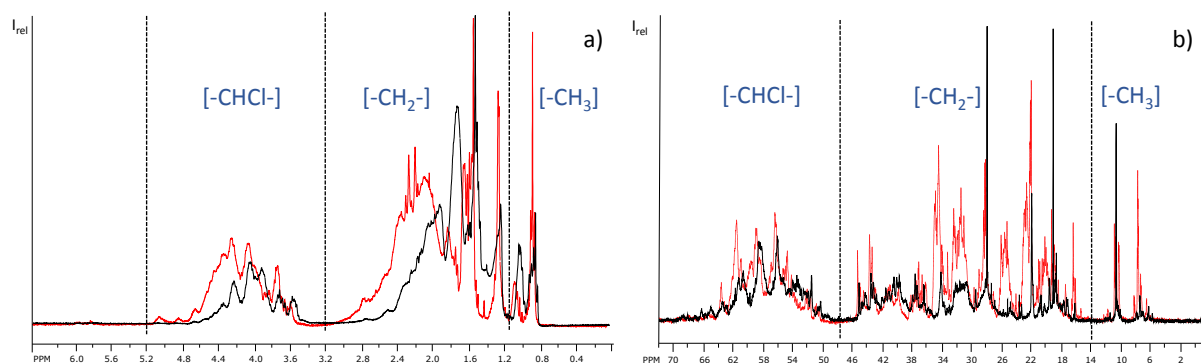


Fig. 3. Overlay of (a) ^1H NMR and (b) ^{13}C NMR spectra of synthesis aliquot 2 (70 min reaction time, black) and synthesis aliquot 4 (250 min reaction time, red). Unresolved signal groups were found in the range of methyl groups [-CH₃], methylene groups [-CH₂-] and monochloromethylene groups [-CHCl-].

The chemical shift ranges in the aliquots were in accordance with those of technical MCCP mixtures¹⁴ and polychlorohexadecane mixtures of varying chlorination degree.¹⁵ Likewise, ^{13}C -NMR spectra were characterized by poorly resolved multiplets of (various) methyl (5-12 ppm), methylene (15-45 ppm) and monochloromethylene groups (50-68 ppm) (**Fig. 3b**).

Integration of the specific methylene and monochloromethylene peak groups of polychlorinated *n*-tetradecanes allowed us to estimate the average chlorination content of the mixtures. Based on the general molecular formula for polychlorinated *n*-tetradecane congeners (i.e. C₁₄H_{30-y}Cl_y), the average number of chlorine substituents (y) in the synthesis aliquots was calculated from the percentage area ratios (X) of the corresponding methyl-, methylene- and monochloromethylene-signals by:

$$y = 30 - (14 \cdot (X[-\text{CHCl-}] + 2 \cdot X[-\text{CH}_2\text{-}] + 3 \cdot X[-\text{CH}_3]))$$

Accordingly, the average chlorine content (Cl%) was determined by using the molecular weight of carbon (M_C), hydrogen (M_H) and chlorine (M_{Cl}) by:

$$\text{Cl}\% = \frac{y \cdot M_{\text{Cl}}}{14 \cdot M_{\text{C}} + (30-y) \cdot M_{\text{C}} + y \cdot M_{\text{Cl}}} \cdot 100\%$$

The chlorine content (Cl%) of the synthesized polychlorinated *n*-tetradecanes determined by ^1H - and ^{13}C -NMR agreed well with the results obtained by elemental analysis (EA) (**Tab. 2**). Due to the higher sensitivity of ^1H -NMR (~35fold lower detection limit) this technique appears more suitable for determination of the chlorine content. Compared to that, GC/ECNI-MS-SIM determinations by means of the [M-Cl]⁻ fragment ions deviated more from NMR and EA measurements (**Tab. 2**).

Tab. 2. Average chlorination degree by weight determined by ^1H -NMR, ^{13}C -NMR, elemental analysis (EA) and GC/ECNI-MS-SIM measurements of two synthesis mixtures after 70 min (aliquot 2) and 250 min (aliquot 4) reaction time.

Aliquot	reaction time [min]	^1H (^{13}C) signal area ratio [%]			chlorination degree [%] measured by		
		[-CHCl-]	[-CH ₂ -]	[-CH ₃]	^1H (^{13}C)-NMR	EA	GC/ECNI-MS
A2	70	30.7 (30.4)	65.3 (66.9)	4.0 (2.7)	51.4 (52.0)	51.4	54.4
A4	250	43.4 (44.3)	53.7 (53.4)	2.9 (2.4)	58.8 (59.4)	58.7	62.5

The synthesized polychlorotetradecanes have been characterized with regard to their chlorination degree and congener composition, therefore they can be used to test and compare established CP quantification techniques.

References

- (1) J. de Boer; P. Garrigues; J.-D. Gu; K. C. Jones; T. Knepper; A. Newton; D. L. Sparks. *The Handbook of Environmental Chemistry - Volume 10*; Springer: Berlin, **2010**.
- (2) Z. Zencak; M. Oehme. *TrAC - Trends in Analytical Chemistry* **2006**, 25 (4), 310–317.
- (3) Directive 2002/45/EC of the European Parliament and of the Council of 25 June 2002 amending for the twentieth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (short-chain chlorinated paraffins) **2002**.
- (4) M. L. Feo; E. Eljarrat; D. Barceló. *TrAC - Trends in Analytical Chemistry* **2009**, 28 (6), 778–791.
- (5) S. Shojania. *Chemosphere* **1999**, 38 (9), 2125–2141.
- (6) E. Eljarrat; D. Barceló. *TrAC - Trends in Analytical Chemistry* **2006**, 25 (4), 421–434.
- (7) W. Vetter; B. Luckas. *Journal of High Resolution Chromatography* **1995**, 18 (10), 643–646.
- (8) G. T. Tomy; B. Billeck; G. A. Stern. *Chemosphere* **2000**, 40 (6), 679–683.
- (9) W. Vetter; J. Kirres; P. Bendig. *Journal of Chromatography A* **2012**, 1263, 151–157.
- (10) S. Kaffarnik; M. Schröder; K. Lehnert; T. Baars; W. Vetter. *European Food Research and Technology* **2014**, 238 (5), 819–827.
- (11) G. T. Tomy; G. A. Stern; D. C. G. Muir; A. T. Fisk; C. D. Cymbalisty; J. B. Westmore. *Analytical Chemistry* **1997**, 69 (14), 2762–2771.
- (12) S. Bayen; J. P. Obbard; G. O. Thomas. *Environment international* **2006**, 32 (7), 915–929.
- (13) W. Zoller; W. Schafer; T. Class; K. Ballschmiter. *Fresenius Zeitschrift für Analytische Chemie* **1985**, 321 (3), 247–251.
- (14) H. Panzel; K. Ballschmiter. *Fresenius' Zeitschrift für Analytische Chemie* **1974**, 271 (3), 182–191.

(15) M. N. Gusev; Ya G. Urman; O. A. Mochalova; L. A. Kocharyan; I. Ya Slonim. *Bulletin of the Academy of Sciences of the USSR Division of Chemical Science* **1969**, 17 (7), 1456–1459.