

A new quantification procedure for the analysis of chlorinated paraffins using electron capture negative ionisation mass spectrometry

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Introduction

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated *n*-alkanes containing thousands of different isomers, enantiomers and diastereomers. CPs are subdivided according to their carbon chain length into short chain chlorinated paraffins (SCCPs, C₁₀-C₁₃), medium chain CPs (MCCPs, C₁₄-C₁₇) and long chain CPs (LCCPs, C_{>17}). The degree of chlorination can vary between 30 and 70% depending on the field of application¹.

Currently, worldwide only a few laboratories analyse CPs². Normally, high resolution gas-chromatography (HRGC) combined with high- or low-resolution mass spectrometry (MS) in the electron capture negative ionisation (ECNI) mode is employed. Quantification is usually performed with technical CP mixtures, since other reference standards are not available. Congeners with 5-9 chlorine atoms are determined in environmental samples¹.

ECNI-MS as a suitable technique for the analysis of polychlorinated compounds is well documented³. However, there are some problems in the quantification of CPs, since the response factors of the congeners in these complex mixtures vary considerably and are unknown. The most common quantification procedure, which tries to overcome these problems, has been extensively described by Tomy et al⁴. However, this approach is still hampered by the strong dependence of the results on the degree of chlorination of the applied standard. Therefore, this work describes an alternative quantification procedure, which compensates for the influence of different response factors and makes results independent from the chlorine content of the reference standard.

Methods and Materials

Chemicals and standards. Three technical SCCPs (C_{10-13} , 51%, 55.5% and 63% chlorine, 100 ng/ μ l, solutions in cyclohexane) and two technical MCCPs (C_{14-17} , 52% and 56% chlorine, 100 ng/ μ l, solutions in cyclohexane) were obtained from Ehrenstorfer (Augsburg, Germany). Additionally, a 1+1 solution of the SCCP mixtures with 51% and 55.5% (53% Cl) and a 1+1 solution of the SCCP mixtures with 55.5% and 63% were prepared (59% Cl). A pure SCCP mixture, Hordalub 80 (56% Cl), from Hoechst (Germany) was diluted to 100 ng/ μ l in cyclohexane. The SCCP mixtures Cereclor 60 L (59% Cl) and Cereclor 70 L (69% Cl) were produced by Imperial Chemical Industries (ICL, England) and diluted to 107 ng/ μ l in cyclohexane. A 1+1 mixture of them (65% Cl) was used as linearity control. $^{13}C_{10}$ -*trans*-chlordane (100 ng/ μ l, solution in nonane, purity 99%) was purchased from Cambridge Isotope Laboratories (Andover, USA) and employed as internal standard (ISTD).

Reference solutions for quantification contained 1500 ng of CPs and 10 ng of $^{13}C_{10}$ -*trans*-chlordane in 150 μ l of cyclohexane. Purity and pretreatment of chemicals and solvents for the clean-up of the fish sample (Cod liver, *Gadus morhua*, Baltic Sea) as well as the applied procedure are described in detail elsewhere^{5,6}.

Instrumentation. Chromatographic separations were carried out on an HP 5890II (Hewlett Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 μ m thick film of DB5-MS (5% phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). Sample volumes of 1.5 μ l were injected in the splitless mode (2 min) at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at a column inlet pressure of 68.9 kPa (10 psi). The temperature programme was as follows: 100 °C, isothermal for 2 min, then 10 °C/min to 280 °C and isothermal for 8 min.

An HP 5989B (Hewlett Packard, Palo Alto, USA) mass spectrometer was employed in the ECNI mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 120 Pa (0.9 Torr). The electron energy was 100 eV. The ion source temperature was set to 200 °C, the quadrupole temperature to 100 °C and the transfer line temperature to 280 °C. EI-MS/MS analysis was performed on a CP-3800 gas chromatograph coupled to a 1200 triple quadrupole MS (Varian, Walnut Creek, USA). More details about the EI-MS/MS analysis are given elsewhere⁷.

Results and Discussion

Selection of standards. The most critical point in the analysis of CPs by ECNI-MS is the proper selection of a CP reference standard, since composition and in particular chlorine content of the CPs in environmental samples are unknown prior to analysis. Tomy et al.⁸ and Coelhan et al.⁹ already reported that deviations of 100% and more are possible, if the chlorine content of the standard mixture and of the sample do not fit. As can be seen from Table 1, quantification of standards against those of different chlorine content produced easily deviations of 59-846%.

Table 1: Obtained CP amounts and relative deviations when quantifying different SCCP standards (51%, 55% and 63% Cl) against each other.

Sample	SCCP standard		
	51% Cl	55% Cl	63% Cl
51% Cl	1500 ng	616 ng (59 %)	159 ng (89%)
55% Cl	3655 ng (144%)	1500 ng	386 ng (74%)
63% Cl	14185 ng (846%)	5822 ng (288%)	1500 ng

The quantification against a standard with the same chlorine content had a precision of <5%. In contrast, the use of a mixture with 70% chlorine content for a standard with 51% caused an error of 1113%. Table 1 also shows, that the quantified amount was too low, if the employed standard had a higher chlorine content and vice versa.

Calculation of response factors for CP mixtures. For the quantification of CPs in environmental samples the relative total CP area (see Formula 1) is needed to determine the total response factor of the standard CP mixture (2):

$$\text{Rel. total CP area} = \sum_i \frac{\text{area}_i(\text{analyte})}{\text{area}_i(\text{ISTD})} \quad (1)$$

“i” stands for one CP congener group. The amount of internal standard in the sample and in the standard solutions is equal and therefore not relevant for a relative calculation.

$$\text{Total response factor (CP mixture)} = \frac{\text{rel. total CP area (Std.)}}{\text{amount CPs (Std.)}} \quad (2)$$

For ECNI conditions the total CP area is mainly dependent on the chlorine content of the applied CP standard and much less from the carbon chain length. The chlorine content of a CP mixture can be calculated as shown in equation (3).

$$\text{Chlorine content (CP mixt.)} = \sum_i \frac{\text{rel. area (congener group } i) \cdot \text{chlorine content (congener group } i)}{\text{rel. total CP area}} \quad (3)$$

The relative total CP area increases proportionally to the chlorine content of the standard. Seven SCCP mixtures with different chlorine content (51-70%) and different composition were analysed to investigate the correlation between the total response factor of a CP mixture (see Formula 2) and the chlorine content of a CP mixture.

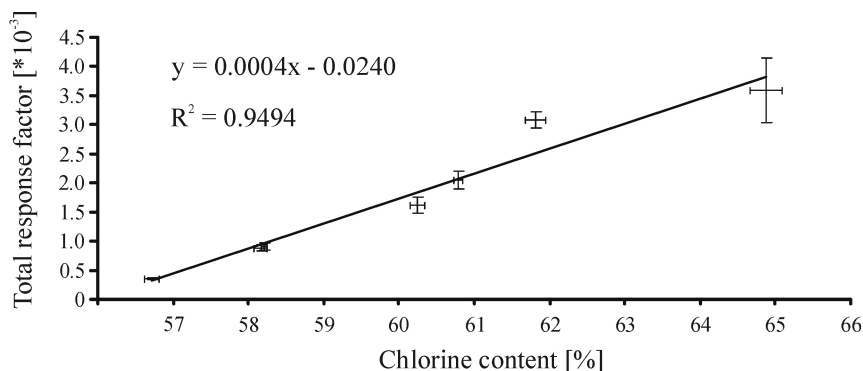


Figure 1: Dependence of the total response factor on the degree of chlorination for seven different SCCP mixtures (51-70% chlorine, average of five measurements).

As can be seen from Figure 1, a linear correlation exists between the chlorine content of the CP mixture and the total response factor. Analyses were repeated

five times (on five different days). Slopes and axis intercepts deviated not more than 13%, and R^2 was always >0.9 .

This allows to determine the total response factor of a CP mixture as follows:

$$\text{Total response factor (CP mixture)} = \text{slope} \cdot \text{chlorine content} - \text{axis intercept} \quad (4)$$

Once the total response factor of the CPs in a sample is determined in this way, the total CP amount in the sample can be calculated as follows:

$$\text{CP amount (sample)} = \frac{\text{total area (sample)}}{\text{total response factor (Std.)}} \quad (5)$$

Compared to the usually applied CP quantification, this CP quantification procedure is independent from the chlorine content of the CP standard (see Table 2).

Table 2: CP quantification of different SCCP mixtures based on chlorine content corrected total response factors. Standard deviations for five parallels on five different days are also given.

CP mixture [% chlorine content]	Spiked amount [ng]	Found amount [ng]	Rel. standard deviation [%]
Standards used for establishment of linear function			
51	1500	1438	4
55*	1500	1417	6
55*	1500	1401	7
63	1500	1853	24
59	1500	1487	1
60	1605	1412	12
70	1605	1522	5
Control samples			
53	1500	1243	17
65	1070	836	22
Hordalub 80 (56% Cl)	1500	1741	16

* The CP mixture with chlorine content of 55% was analysed twice.

For CP mixtures, which were parts of the linearity, the average deviation was less than 9%. Control samples of other technical mixtures with 53, 65 and 56% showed deviations between 16 and 22.

Application to biota. As an example, the quantification of a cod liver from the Baltic Sea is discussed applying both the conventional and the modified quantification procedure mentioned above. Screening by EI-MS/MS showed a total CP concentration of 94 ng/g wet weight (ww) for the sum of SCCPs and MCCPs. In a second step the chlorine content of the liver sample was determined by ECNI-LRMS as well as of two SCCP standards (55.5% and 63% Cl content specified by the manufacturer) and one MCCP standard (52%) as control (see Table 3).

Table 3: Chlorine contents [%] determined by the modified quantification procedure and ECNI-LRMS for the SCCP standards (55% and 63%), the liver sample and the MCCP standard (52%).

	SCCP standard mixture (55.5%)	SCCP standard mixture (63%)	Sampl e	MCCP standard mixture (52%)
Calculated Cl content [%]				
for SCCPs	60.3	64.0	61.9	
for MCCPs			56.5	56.3

The calculated chlorine content of the MCCPs present in the sample was similar to the chlorine content of the MCCP standard. However, the calculated chlorine content of the SCCPs in the sample was between the two SCCP standards (55.5% and 63%). Table 4 shows the obtained differences between the proposed calculation and those based on two commercial reference standards with a deviating degree of chlorination.

Table 4: SCCP and MCCP concentrations in a cod liver obtained with the proposed method and based on two commercial standards of different degree of chlorination.

	Quantification		via total response factor
	according to Tomy et al. ⁴		
	SCCP standard (55.5%)	SCCP standard (63%)	
SCCP concentration [ng/g ww]	151	48	73 ^a
	MCCP standard (52%)		
MCCP concentration [ng/g ww]	68		72 ^b
SCCP+MCCP concentration [ng/g ww]	219	116	145

^a Linearity determined with three SCCP mixtures (51%, 55.5% and 63% Cl, $R^2 = 0.999$)

^b Linearity estimated with two MCCP mixtures (52% and 56% Cl)

For MCCPs both methods gave similar results, since the chlorine content of the CPs in the sample and in the standard was coincidentally similar. However, the SCCP concentration obtained with the conventional quantification method was highly dependent on the chlorine content of the applied standard as described before, whereas the result for the chlorine content corrected calculation was in between. EI-MS/MS screening is not dependent on the degree of chlorination⁷ and indicated also that ECNI-MS gave an erroneously too high result, when a standard of too low degree of chlorination was employed.

Conclusions. Prior to the extensive analysis of all CP congener groups in an environmental sample, the composition and the chlorine content of the CPs are unknown. Moreover, only a limited number of CP standard mixtures with different composition and different degree of chlorination are available. Since the total response factors of these mixtures vary considerably with the degree of

chlorination, it is important to use a standard mixture with the same chlorine content as that of the sample to avoid deviations of 100% or more. The here presented quantification procedure considers the linear dependence of the response factors on the chlorine content and makes the quantification independent from the chlorine content of the used standard mixture.

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