On: 11 March 2012, At: 04:57
Publisher: Taylor & Francis
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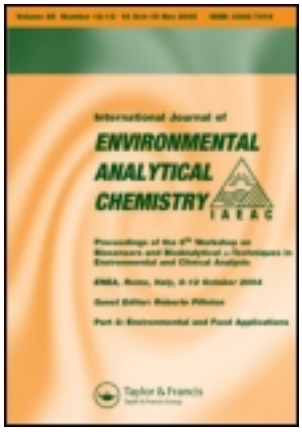

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

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Available online: 14 Mar 2011

To cite this article: Tatsiana P. Rusina, Peter Korytár & Jacob de Boer (2011): Comparison of quantification methods for the analysis of polychlorinated alkanes using electron capture negative ionisation mass spectrometry, International Journal of Environmental Analytical Chemistry, 91:4, 319-332

To link to this article: http://dx.doi.org/10.1080/03067311003602583

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 4, 10 April 2011, 319–332

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Comparison of quantification methods for the analysis of polychlorinated alkanes using electron capture negative ionisation mass spectrometry

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(*Received 3 June 2009; final version received 5 January 2010*)

Four quantification methods for short-chain chlorinated paraffins (SCCPs) or polychlorinated alkanes (PCAs) using gas chromatography electron capture negative ionisation low resolution mass spectrometry (GC-ECNI-LRMS) were investigated. The method based on visual comparison of congener group patterns of external standards used for quantification and fish samples was very sensitive for the choice of the quantification standard. Two other methods used the existing relation of the response factors with the chlorine content of SCCP mixtures for quantification. Results from the three methods above deviated from nominal values less than 20%. This was ~50% when individual PCA standards were applied for quantification of SCCPs. The deviation is probably caused by the fact that only C_{10} carbon chain length standards with 5–9 chlorine atoms could be used. However, quantification using individual PCA standards is a promising method provided more standards will become commercially available. The clear advantage is that the standards are defined, which makes quantification comparable between different laboratories. Application of all four quantification methods to the analysis of four different fish samples gave results that agreed with the median values within ±40%.

Keywords: polychlorinated alkanes; short-chain chlorinated paraffins; response factors; quantification

1. Introduction

Chlorinated paraffins (CPs) are complex technical mixtures of polychlorinated alkanes (PCAs) with carbon lengths of C_{10}–C_{30} and a chlorination degree of 30–70%. They have been produced since the 1930s [1]. They are used as extreme-pressure additives in industrial cutting fluids, plasticisers and flame retardants for polyvinyl chloride (PVC) and other plastics (polyester, polyolefins, polystyrene), rubbers (neoprene), and as additives in paints and sealants [1]. They are classified by carbon chain lengths as short-chain CPs (SCCPs, C_{10}–C_{13}), medium-chain CPs (MCCPs, C_{14}–C_{17}) and long-chain CPs (LCCPs, $>C_{17}$). CPs are ubiquitous, persistent and bioaccumulative environmental pollutants. Therefore, several countries have imposed regulations on SCCPs use and singled them out as priority pollutants (e.g. European Water Framework Directive [2]). The 25th Adaptation

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067311003602583
http://www.informaworld.com

to Technical Progress to the Dangerous Substances Directive (67/548/EEC) has formally classified SCCPs as Category 3 carcinogens (R40) and as Dangerous for the Environment (R50/53). They are also under consideration to be included in the Stockholm Convention on Persistent Organic Pollutants (POPs) [3]. Nevertheless, CP production is still substantial and ongoing, in particular in India, China, the US and EU [3]. The regulations and ongoing production create needs for monitoring of these compounds in environment. However, the analysis and quantification of CPs is extremely difficult due to the complex composition ($>10{,}000$ congeners) of CP products. Limited information about concentrations in the environment requires establishing routine analytical quality assured methods. CPs are mainly analysed by gas chromatography (GC) coupled to low- (LR) or high-resolution (HR) mass spectrometry (MS) with different ionisation methods: electron capture negative ionisation (ECNI) [4,5], electron ionisation (EI) [6], metastable atom bombardment (MAB) [7]. Occasionally, other methods like dechlorination of CPs followed by GC analysis with a flame ionisation detector (FID) [8] have been used or electron capture detection (ECD) [9]. CPs were also analysed by liquid chromatography (LC) using chloride-enhanced atmospheric pressure chemical ionisation [10].

Single-column GC fails by far to separate all individual congeners and its isomers. The chromatograms have a characteristically unresolved and broad profile, which clearly indicates the presence of a very large number of co-eluting and partly overlapping peaks. In GC–ECD, the only way of reporting results is as a total [(short + medium + long)-chain] CP concentration and, frequently, many interfering halogenated compounds also present in the samples are included in that total sum. If MS detection in the electron ionisation (EI) mode is used, again it is only possible to report 'total CPs', with the additional drawback of lower sensitivity compared to ECD. This is because EI leads to a strong fragmentation of the CP compounds, with mass spectra giving no compound-specific information [11]. The use of EI-MS/MS detection increases the selectivity and avoids matrix interferences, which allows detection at a lower level [6]. Nevertheless, a congener and homologue-specific analysis is also not possible with this method. Recently comprehensive two-dimensional GC coupled with ECNI time-of-flight mass spectrometry (GC × GC-ECNI-TOF-MS) has been used and showed an improved separation of CPs [12]. However, more accurate quantification of CPs, as a complex mixture, remains unsolved. GC-ECNI-LRMS is one the most frequently used techniques for the determination of CPs, because it provides sufficient sensitivity and enables distinguishing congener groups by carbon chain length and chlorination degree ($C_{10}H_{17}Cl_5$, $C_{10}H_{16}Cl_6$, etc.). However, the main drawback of the technique is the dependence of the response factor under ECNI conditions on the number of chlorine atoms in the molecule. Furthermore, congener groups with less than five chlorine atoms are discriminated by the technique [13]. For qualitative and quantitative analysis by ECNI characteristic ions $[M–Cl]^-$, $[M–HCl]^-$ or $[M+Cl]^-$ can be monitored. However, the results are usually too high due to mass overlap among the CP congeners because of application of LRMS [14]. In addition, interferences from, e.g. toxaphene and chlordane-related compounds, which have molecular masses similar to short- and medium-chain CPs and are difficult to separate during clean up, further contribute to this bias. Today, such problems can be avoided by application of GC-ECNI-HRMS (resolution, ca. 12 000) [15] but the cost level of this technique is too high for routine analysis. In ECNI-MS the response is very much dependent on the chlorination degree and therefore, the most critical point in the analysis is the proper selection of an external CP standard for quantification, since composition and in particular chlorine content of the

CPs in the samples and in the external standards can be very different. In the last decade, various quantification methods have been reported for GC-ECNI-MS [16–18].

In this work, four quantification methods for SCCPs were compared including two new approaches. CP mixtures, individual PCA standards and several fish samples were measured by GC-ECNI-LRMS and results were discussed.

2. Experimental

2.1 *Materials*

Residue analysis grade solvents were purchased from Promochem (Wesel, Germany). SCCP standard mixtures with 51.5%, 55.5% and 63% chlorine (100 ng/μl solutions in cyclohexane), were obtained from Dr. Ehrenstorfer, Augsburg, Germany and a SCCP mixture 60% chlorine from Sigma-Aldrich, Zwijndrecht, The Netherlands. Additionally, a SCCP solution with 61.5% chlorine was prepared by mixing the 60 and 63% SCCP standard mixtures (1 : 1, v/v). SCCP standard mixtures of different carbon chain lengths and chlorination degrees with concentrations of 10 ng/μl in cyclohexane (C10 44.82%, 50.18%, 55.00%, 60.09%, 65.02%; C11 45.50%, 50.21%, 55.20%, 60.53%; C12 45.32%, 50.18%, 55%, 65.08%, 69.98%; C13 44.9%, 50.23%, 55.03%, 59.98%, 65.18%) were purchased from Dr. Ehrenstorfer, Augsburg, Germany. Individual PCA standards as listed in Table 1 were obtained from Chiron AS, Norway and from Dr. Ehrenstorfer, Augsburg, Germany. $^{13}C_6$-*gamma*-HCH and CB112 (2,3,3′,5,6-pentachlorobiphenyl)

Table 1. Relative response factors (RRF) of individual PCA congeners.

No.	Individual congener		Batch	Producer	Isomers present	RRF[a]
1	1,1,1,3,8,9	$C_9H_{14}Cl_6$	3925	Chiron	isomer1	0.022
2	1,1,1,3,9,10	$C_{10}H_{16}Cl_6$	3744	Chiron	isomer mix	0.012
3	1,1,1,3,10,11	$C_{11}H_{18}Cl_6$	3745	Chiron	isomer mix	0.012
4	1,1,1,3,11,12	$C_{12}H_{20}Cl_6$	3746	Chiron	isomer mix	0.008
5	1,1,1,3,12,13	$C_{13}H_{22}Cl_6$	3747	Chiron	isomer mix	0.009
6	1,1,1,3,6,8,8,8	$C_8H_{10}Cl_8$	3748	Chiron	isomer 1	0.002
					isomer 2	0.002
7	1,1,1,3,8,10,10,10	$C_{10}H_{14}Cl_8$	3857	Chiron	isomer 1	0.024
8	1,1,1,3,9,11,11,11	$C_{11}H_{16}Cl_8$	3749	Chiron	isomer 1	0.012
9	1,1,1,3,10,12,12,12	$C_{12}H_{18}Cl_8$	3761	Chiron	isomer 1	0.008
10	1,1,1,3,11,13,13,13	$C_{13}H_{20}Cl_8$	3762	Chiron	isomer 1	0.008
11	1,1,1,3,12,14,14,14	$C_{14}H_{22}Cl_8$	3763	Chiron	isomer 1	0.015
12	1,2,5,6,9	$C_{10}H_{17}Cl_5$	CP-3	Dr. Ehrenstorfer	diastereoisomer 1	0.009
					diastereoisomer 2	0.010
13	1,2,4,5,9,10	$C_{10}H_{16}Cl_6$	CP-6	Dr. Ehrenstorfer	isomer 1	0.15
14	1,2,5,6,9,10	$C_{10}H_{16}Cl_6$	CP-4	Dr. Ehrenstorfer	isomer 1	0.14
15	1,2,5,6,9,10	$C_{10}H_{16}Cl_6$	CP-5	Dr. Ehrenstorfer	mixture of 2 diastereoisomers	0.063
16	1,2,4,5,6,9,10	$C_{10}H_{15}Cl_7$	CP-7	Dr. Ehrenstorfer	diastereoisomer 1	0.30
					diastereoisomer 2	0.40
					diastereoisomer 3	0.27
17	2,3,4,5,6,7,8,9	$C_{10}H_{14}Cl_8$	CP-9	Dr. Ehrenstorfer	isomer 1	1.32
18	1,2,3,4,5,6,7,8,9	$C_{10}H_{13}Cl_9$	CP-10	Dr. Ehrenstorfer	isomer 1	2.01

[a]RF relative to added $^{13}C_6$-*gamma*-HCH syringe standard.

(Dr. Ehrenstorfer, Augsburg, Germany) served as syringe standards. $^{13}C_{10}$-*trans*-chlordane (CIL Cambridge Isotope Laboratories, Apeldoorn, The Netherlands) was used as an internal standard. Florisil® PR (60–100 mesh) was obtained from Fluka (Zwijndrecht, The Netherlands), anhydrous sodium sulphate and silica gel 60 (70–230 mesh) extra pure from Merck, Amsterdam, The Netherlands.

2.2 *Fish samples*

Hake (*Merluccius merluccius*) was caught south off the coast of Ireland in 2004, cod (*Gadus morhua*) was caught in the southern North Sea in 2007 and farmed salmon (*Salmo salar*) was obtained from Scotland in 2007. Hake and cod were dissected and the livers were isolated. Twenty-five livers of male and female cods of about the same weight and age of 1–2 years were homogenised and stored in glass jars at −20°C until the analysis. One hake liver and a salmon tissue were used for analysis. Internal reference material (IRM) was prepared from a batch of homogenised fillets taken from 30–50 cm long eels (Anguilla anguilla). The eels were caught in the Meuse river close to Eijsden in The Netherlands in 1999.

2.3 *Extraction and clean up*

An extraction and clean up method according to Reth *et al.* [19] was used. About 5–8 g fish liver or tissue, corresponding to 1.5–4 g of fat, was homogenised with sodium sulphate. Sub-samples of cod liver were spiked with the SCCP 63% standard mixture. An amount of 10 ng (absolute) of $^{13}C_{10}$-*trans*-chlordane was added as internal standard for a check of the fractionation step as slight changes in humidity can cause shifting of the elution windows on the Florisil® column. $^{13}C_{10}$-*trans*-chlordane elutes just before the SCCPs, so a low recovery of $^{13}C_{10}$-*trans*-chlordane indicates a possible loss of the SCCPs during fractionation. $^{13}C_{10}$-*trans*-chlordane was not used for quantification. The sample was extracted with 500 ml of *n*-hexane/dichloromethane (DCM) (1 : 1, v/v) in a Soxhlet apparatus for 24 h. The extract was concentrated on a Kuderna-Danish apparatus to a volume of approximately 2 ml. Then lipids were removed on a 40 g silica gel column impregnated with 44% concentrated sulphuric acid by elution with 130 ml of *n*-hexane/DCM (1 : 1, v/v). The lipid free sample was concentrated on a Kuderna-Danish apparatus to a volume of approximately 2 ml. Subsequently, the sample was fractionated on an 18 g Florisil® column (deactivated with 1.5% (w/w) of water) with 90 ml of *n*-hexane (fraction 1 containing the PCBs, toxaphenes, heptachlor, *alpha*-chlordene, *gamma*-chlordene, *cis*-chlordane and *trans*-nonachlor) and 120 ml *n*-hexane : DCM (3 : 1, v/v) resulting in fraction 2 containing the CPs, $^{13}C_{10}$-*trans*-chlordane, *oxy*-chlordane, *trans*-chlordane, a residue of *cis*-chlordane and the HCH isomers. The CP fraction was concentrated on a Kuderna-Danish apparatus after addition of 20 ng (absolute) of PCB 112 as a syringe standard to correct for concentration and injection variability. Finally the extract was concentrated down to 200 µl with a gentle flow of nitrogen.

2.4 *Instrumentation*

All the measurements were performed on an Agilent 6890/5973 GC-ECNI-LRMS (Agilent, USA) equipped with a capillary column (DB-5, crosslinked 5%-phenyl-95%-methylpolysiloxane, 15 m, 0.25 mm i.d., 0.25 µm film) and a split/splitless injector using

pulsed splitless mode (splitless time 1.5 min). The carrier gas was He at a constant flow rate of 0.9 ml min^{-1} and an initial column pressure of 10 kPa. A volume of 2 µl was injected at a temperature of 275°C. The temperature program was as follows: 90°C, hold for 2 min, 10°C/min to 280°C and hold for 20 min. The transfer line temperature was set at 280°C. The MS was operated in the ECNI mode using methane with 10% ammonia (99.99% purity) as a reagent gas (1.75 ml min^{-1}). The chemical ionisation (CI) gas pressure in the ion source was $2.4*10^{-7}$ bar ($1.8*10^{-4}$ Torr). Tuning of the MS was done at m/z 185, 351, 449 using perflouro-5,8-dimethyl-3,6,9-trioxidodecane (PFDTD). The ion source temperature was 150°C and the quadrupole temperature 100°C. The electron energy was 70 eV and the emission electron current 50 µA. CP congeners with 10–13 carbon atoms and 5–10 chlorine atoms were analysed. For each group of CP congeners the signal of the two most abundant isotopes of the $[M–Cl]^-$ ion clusters [14] were recorded in the selected ion monitoring (SIM) mode with dwell times of 40 ms. Integration was done using MS-Chemstation software.

3. Quantification

3.1 *Quantification using matching of patterns and correction factors (Method 1)*

Tomy *et al.* [15] quantified SCCP mixtures by GC-ECNI-HRMS assuming that the response of a congener group would be proportional to the number of chlorine atoms as well as to its molar concentration. The method was based on the generation of congener group patterns per carbon chain length of the samples and of the available SCCP mixtures that served as external standards. The signal of the most abundant $[M–Cl]^-$ ion of each congener group was integrated and related to the natural isotopic fraction and to the number of chlorines in a congener group. That gave the relative abundance of a congener group. The congener patterns of the sample and the standards were compared. The standard that resembled the sample the most was used for quantification. The most prominent congener group of the sample and that of the suitable external standard was used for quantification. The average molecular mass and the relative abundance of the congener group were used in calculations as correction factors for the differences between the patterns. Tomy *et al.* [15] emphasised that the external standard should resemble the sample as much as possible to obtain reliable results.

In this work several SCCP mixtures (SCCP 51.5%, 55.5%, 60% and 63% Cl content) and the fish samples were measured by GC-ECNI-LRMS and quantified according to this procedure.

3.2 *Quantification using relative response factor (Method 2)*

This method was based on the determination of the relation between the relative total response factor (RTRF) of a CP mixture and the chlorine content [18]. The peak areas of the most abundant $[M–Cl]^-$ ion of each congener group was used to calculate the relative response factors, which were combined to obtain the RTRF as described by Reth *et al.* [18]. The chlorine content of the CP mixture was calculated using peak areas of congener groups and molar masses of chlorines present [18]. The calibration line of RTRF as a function of the chlorine content was composed. For the quantification of the sample first the chlorine content of the sample has to be calculated using the aforementioned approach. Knowing the chlorine content, the RTRF of the sample was

obtained using the calibration line. Once, the RTRF is determined, the CP concentration in the sample can be calculated. The advantage of the method is that it is independent from the chlorine content of the CP standard used and requires only the establishment of a relation between the RTRF and the chlorine content of the CP standards prior to the analysis.

3.3 *Combined quantification method of Coelhan and Reth (Method 3)*

Coelhan *et al.* [17] suggested to quantify SCCPs according to carbon chain lengths (i.e. $C_{10}, C_{11}, C_{12}, C_{13}$) applying several SCCP standard mixtures with different chlorine contents and carbon chain lengths. The choice of the external standard for quantification was based on the visual comparison of the patterns of the standards and the samples. The mixture with a composition best corresponding to the sample was used for quantification. Coelhan *et al.* [17] showed that application of the SCCP standard mixtures with several chlorine contents and homolog group distributions that differ from those in the sample leads to quantification errors of 90 to 1,100%. The complication of the Coelhan method is that it is difficult to select a standard with a SCCP composition that exactly corresponds to that of the sample.

In this work a combination of the Coelhan and Reth quantification methods was tested. A SCCP mixture was quantified according to carbon chain length as proposed by Coelhan *et al.* [17]. The Reth *et al.* [18] approach was used to compensate for the differences between the chlorine content of the SCCP standard mixtures and environmental samples. To enable that correction, the RTRF of CP mixture of each carbon chain length and chlorine content was calculated by:

$$\text{RTRF (CP mix of carbon chain length } x) = \sum_i \frac{\text{A}_{i(CONGENER\_GROUP)}/nCl}{\text{A}_{i(IS)}} \times \frac{N_{IS}}{N_{CP}} \quad (1)$$

$A_{i(CONGENER\ GROUP)}$ and $A_{i(IS)}$ are the areas of congener group i in the CP mixture of carbon chain length x and the internal standard, respectively. N_{IS}, N_{CP} are the amounts of the internal standard and CP mixture, respectively. The area of each congener group divided by the number of chlorine atoms in congener group ($n\ Cl$) is the quantification correction. The chlorine content of the CP mixture was calculated as:

$$\text{Cl content (CP mix of carbon chain length } x) = \sum_i \frac{Rel\_\text{A}_{i(CONGENER\_GROUP)} \times Cl_{i(CONGENER\_GROUP)}}{\text{Rel\_}Total\_CP\_A} \quad (2)$$

in which $Rel\_A_{i(CONGENER\ GROUP)}$ is the area of the congener group i in CP mixture of carbon chain length x divided by the area of the internal standard. The chlorine fraction Cl of congener group i was calculated by using the molar masses of chlorines present. $Rel\_Total\_CP\_A$ is the relative total CP area: the sum of the relative areas of all congener groups of carbon chain length x.

$$Rel\_Total\_CP\_A = \sum_i \frac{A_{i(CONGENER\_GROUP)}}{A_{i(IS)}} \quad (3)$$

Thus, for each carbon chain length CP mixture ($C_{10}, C_{11}, C_{12}, C_{13}$) the relation between RTRF and the chlorine content was established. For the quantification of samples the

chlorine content of CPs of each carbon chain length has to be calculated using Equation (2). Knowing the chlorine content of the sample the RTRF of the sample for each carbon chain length can be obtained from the relation mentioned above. Subsequently, the determined RTRFs are used to calculate the amount of CPs of each carbon chain length using the formula:

$$N_{\mathrm{CP}}(\text{sample carbon chain length } x) = \frac{Rel\_Total\_CP\_A}{\mathrm{RTRF}(sample)} N_{IS} \quad (4)$$

Applying this method, CP mixtures can be characterised by the carbon chain length.

3.4 *Quantification based on the individual PCA congeners (Method 4)*

An interesting issue for the quantification of SCCPs is the exploration of response factors (RF) of individual PCA congeners with different carbon chain lengths and chlorine contents, including their isomers. If different congeners with the same number of chlorine atoms would have identical RFs (a difference of 20–30% is acceptable), this would allow using one congener as an external standard for the quantification of congener groups with the same chlorine content. Beaume *et al.* [20] studied RFs of only C_{10} individual PCA standards with 5–9 chlorine atoms and used them for quantification of C_{10}-chloroalkane residues in fish. In this work individual PCA standards of different carbon chain lengths with 5–9 chlorines in molecule and different chlorine patterns as listed in Table 1 were used. The advantage of the method would be that the standards are defined. Nevertheless, the availability of individual PCA standards is limited. Synthesis of some PCA standards was discussed by Coelhan [21]. Another limitation is possible presence of several isomers in individual PCA standards.

4. Results and discussion

4.1 *Quantification by Method 1*

For assessment of quantification errors that may arise from application of SCCP mixtures with the same or different chlorine content than that in the sample four available SCCP mixtures were successively used as an external standard to quantify the other three as samples. In the first instance, the SCCP mixture with 51.5% Cl was chosen as a standard for the quantification of the 55.5%, 60%, and 63% Cl mixtures. Then, the SCCP mixture with 55.5% Cl was used as the standard, and so on. As congener group patterns differed in selected SCCP standards the calculation procedure did not always follow the principle of their resemblance as required by Tomy *et al.* [15]. Repeatability of the estimated amounts in SCCP mixtures was $\leq$10% for three replicate injections. SCCP mixtures with almost similar chlorine contents, for instance 51.5 and 55.5% Cl, 60 and 63% Cl, respectively, had deviations of $<$50% from the nominal value (Figure 1). If the SCCP standard mixtures with higher chlorine contents (63% or 60% Cl) were used for the quantification of the lower chlorinated ones (51.5%, 55.5% Cl), the deviations from the nominal value were $<$100%. Application of lower chlorinated standards (51.5%, 55.5% Cl) for quantification of the higher ones resulted in underestimated nominal values and deviations of $>$300%. Figure 1 shows that the outcome of the Tomy quantification method is extremely dependent on selection of the external standard.

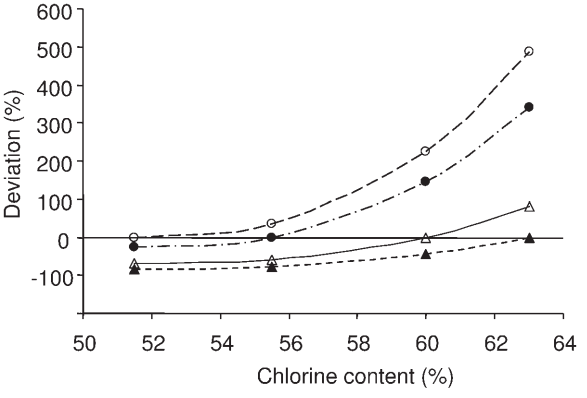

Figure 1. Plot of deviation of SCCP concentration from nominal value calculated by method 1 versus chlorine content. SCCP 51.5% Cl served as external standard (open circles); SCCP 55.5% Cl served as external standard (filled circles); SCCP 60.0% Cl served as external standard (open triangles); SCCP 63.0% Cl served as external standard (filled triangles).

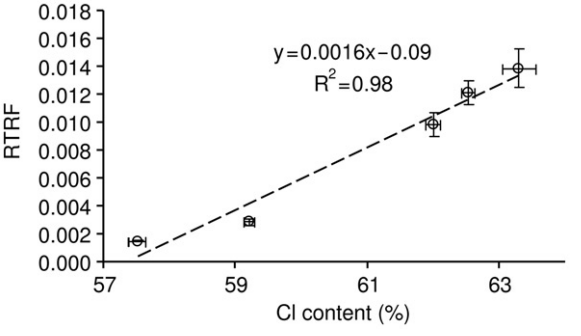

Figure 2. Dependence of the RTRF on the chlorine content for five SCCP mixtures (51.5 (57.5)%, 55.5 (59.2)%, 60.0 (62.0)%, 61.5 (62.5)% and 63.0 (63.3)%) using the Reth *et al*. [18] method. The calculated chlorine content of SCCP standard mixture is given in brackets.

4.2 *Quantification by Method 2*

Standard solutions of SCCP mixtures with chlorination degrees of 51.5, 55.5, 60, 61.5 and 63% Cl were analysed to establish a relation between the RTRF and the calculated chlorine content. For estimation of the intermediate precision of the RTRF and the calculated chlorine content each SCCP mixture represented by two concentration levels in the range of 5–20 ng μl^{-1} was analysed four times. The relative standard deviation of the RTRF was $\leq$15%, and the variability of calculated chlorination degrees was less than 0.5%. A good linear relation ($R^2 = 0.98$) was found and used for quantification (Figure 2). Two separately prepared SCCP mixtures with 60% Cl and 61.5% Cl were used to check a calibration line. The deviation of SCCP quantity in those mixtures from nominal values was 15%. The calculated chlorine content of lower chlorinated SCCP mixtures is systematically higher than that declared by the producers (Figure 2). This happens

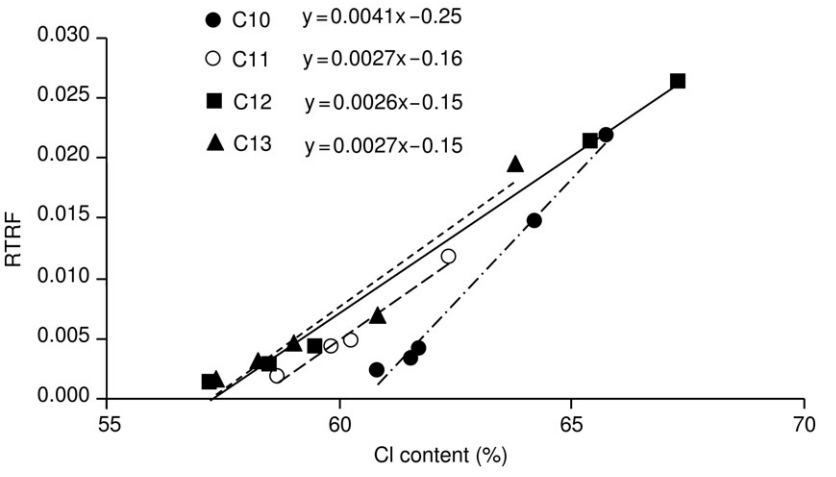

Figure 3. Correlation between the RTRFs and the chlorine contents of SCCPs of different carbon chain lengths (C_{10} 44.82 (61.55)%, 50.18 (60.85)%, 55.00 (61.75)%, 60.09 (64.22)%, 65.02 (65.75)%; C_{11} 45.50 (58.69)%, 50.21 (59.86)%, 55.20 (60.28)%, 60.53 (62.38)%; C_{12} 45.32 (57.21)%, 50.18 (58.50)%, 55 (59.50)%, 65.08 (65.39)%, 69.98 (67.31)%; C_{13} 44.9 (57.34)%, 50.23 (58.24)%, 55.03 (59.01)%, 59.98 (60.83)%, 65.18% (63.80)). The calculated chlorine content of SCCP standard mixture is given in brackets.

because congeners with three and four chlorine atoms are not detected by ECNI. Therefore, the relative abundances of higher chlorinated congeners are overestimated and contribute more in calculation of chlorine content [18]. This method of quantification does not depend on visual comparison of congener group patterns and accounts for the chlorine content of the measured sample in relation with the RTRF.

4.3 *Quantification by Method 3*

SCCP mixtures of C_{10}, C_{11}, C_{12} and C_{13} carbon chain lengths with different chlorination degrees were analysed and processed according to Method 3, as described in Section 3.3 above. For each individual carbon chain length the RTRFs were related to the calculated chlorine contents resulting in four relations (Figure 3). Repeatability of the triplicate measurements was $< 10\%$. Additionally, C_{11} and C_{13} SCCP mixtures were measured on different days showing an intermediate precision of RTRFs $< 15\%$. Good relations between RTRF and calculated chlorine content were obtained ($R^2 > 0.94$) showing, except for C_{10} SCCPs, similar slopes (0.0027) and intercepts (−0.15) (Figure 3). The reason for the steeper slope for C_{10} could be higher concentrations of tri and tetra chlorinated SCCPs in this mixture, which are not detected by ECNI. Therefore, the relative abundance of higher chlorinated C_{10} SCCPs is overestimated resulting in a much higher calculated chlorine content of 61.55% compared to the declared 44.82%. In the C_{11}–C_{13} SCCP mixtures the presence of low chlorinated congeners is apparently lower. Indeed, as was shown by Korytar [22] in the C_{10}–C_{13} CP mixtures of 55% Cl the congeners with 3–5 chlorine atoms were more present in the C_{10} CP mixture. Each relation was checked by the C_{10} 55.0% Cl, C_{11} 60.53% Cl, C_{12} 69.98% Cl and C_{13} 59.98% Cl separately prepared standard solutions. The deviation from the nominal values were 5–15%.

The method allows reporting concentrations of SCCPs by carbon chain length while the quantification error is reduced to the level comparable to Method 2. Application of this method does not require matching of patterns.

4.4 *Response factors of individual PCA congeners (Method 4)*

The relative response factors (RRF) of a series of available individual congeners were studied and the results are presented in Table 1. The RRFs of congeners with monochlorine substituted carbon atoms increase with the number of chlorine atoms in the molecule. For instance, the RRF for pentachlorodecane (1,2,5,6,9-$C_{10}H_{17}Cl_5$) is 0.01 against 2.01 for nonachlorodecane (1,2,3,4,5,6,7,8,9-$C_{10}H_{13}Cl_9$). This observation is not unexpected. However, addition of a second and third chlorine atom at terminal carbon atom does not increase the RRF from hexa to octa chlorine congeners ($RRF = 0.01 - 0.02$). This happens because congeners with chlorine atoms bound to the terminal carbons undergo a different fragmentation. Indeed, for congeners with monochlorine substituted carbon atoms $[M–Cl]^-$ ions are predominantly formed. Congeners with three chlorine substituents at terminal carbon atoms form both $[M]^-$ and $[M–Cl]^-$ as the most abundant ions. On the other hand, the probability for complete chlorination at the terminal carbon atoms in an uncontrolled synthesis of CP mixture is very low as the reactivity of hydrogen atoms is higher on the secondary carbon atoms than on a terminal carbon atom [23]. As was shown by Korytar *et al.* [12] among eight individual PCA congeners spiked to polychlorinated decane mixtures those ones having three chlorine substituents were hardly seen in the technical mixture. Furthermore, in free radical reactions with low positional selectivity the probability of hydrogen substitution at a carbon with already bound chlorine atom is low. This denotes that technical SCCP mixtures do not contain any isomers with more than one chlorine atom bound to any carbon atom in relevant amounts. This needs to be considered when selecting individual isomers for calibration purposes. Use of isomers fully chlorinated at one or both terminal carbon atoms are, thus, inappropriate. Therefore, only PCA individual standards with monochlorine substituted carbon atoms were used for quantification.

Comparing the RRFs of isomers of the same congener with monochlorine substituted carbon atoms (C_{10} congeners) showed maximum deviation of 25%. The averaged RRFs were used for quantification of congener groups with the same number of chlorine atoms as in the molecules of the PCA standard. Thus, a $C_{10}H_{17}Cl_5$ standard was used to quantify congeners $C_{10}H_{17}Cl_5$, $C_{11}H_{19}Cl_5$, etc., a $C_{10}H_{16}Cl_6$ standard-for $C_{10}H_{16}Cl_6$, $C_{11}H_{18}Cl_6$, $C_{12}H_{20}Cl_6$, etc. Unfortunately, the possible effect of the carbon chain lengths of individual PCAs on RRF was not possible to estimate as not all of them with monochlorine substituted carbon atoms were commercially available. As shown by Reth *et al.* [18] the contribution of the carbon chain length on the total response factor could be ±50% while the influence of the chlorine content is 250–570% for SCCP mixtures with 50 and 60% Cl.

4.5 *Quantification of fish samples*

For comparison of the four presented quantification methods three fish species and IRM (eel tissue) were quantified by all methods (Table 2). The results are presented as the sum of C_{10}–C_{13} SCCPs. For estimation of the accuracy of the quantification the reference SCCP mixture with 63% chlorine content was processed according to the four described

Table 2. Quantification of SCCPs in four fish samples using the four methods.

Sample	Calculated Cl content %	Fat content %	Tomy (Method 1) ng g^{-1} ww	Reth (Method 2) ng g^{-1} ww	Combined Coelhan-Reth (Method 3) ng g^{-1} ww	Individual PCA std (Method 4) ng g^{-1} ww	Spiked concentration ng g^{-1} ww
Reference SCCP 63%[c]	63.6	–	27780[a](8.5)	27830[a](8.7)	24575[a] (−4.0)	16710[a] (−35)	25590[a]
Spiked cod[c] liver	63.2	50	530[b] (−15)	520[b] (−17)	520[b] (−17)	310[b] (−50)	625[b]
Cod liver[c]	60.2	50	106	180	110	195	–
Hake liver	63.4	50	299	320	275	220	–
Salmon tissue	60.3	17	21	32	36	29	–
IRM (eel)[c]	63.6	20	88	65	90	125	–

Notes: The relative deviation from nominal value is given in brackets.
[a]The value is expressed as amount, ng.
[b]Concentration of the spike.
[c]Averaged concentrations of duplicates.

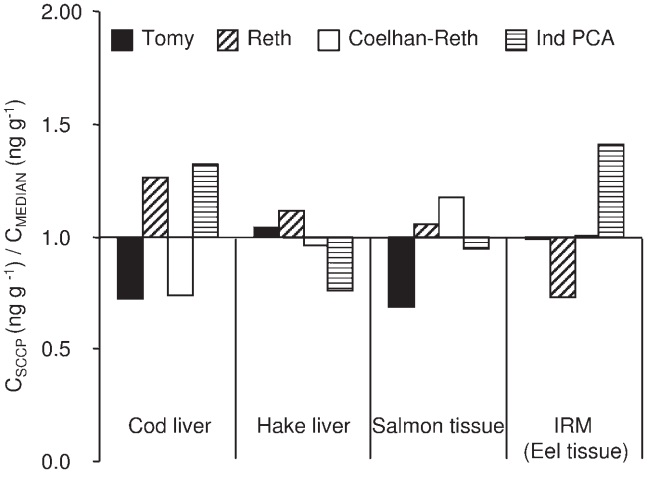

Figure 4. Estimated SCCP concentrations in fish relative to the median concentration of four quantification methods.

methods. To see the effect of matrix on the analysis cod liver was spiked with the SCCP 63% mixture (the concentration of the spike is shown in Table 2). In Method 3 the values of individual carbon chain length SCCPs were summed to obtain the total ($C_{10}+C_{11}+C_{12}+C_{13}$) concentration. For a quantification based on individual standards (Method 4) the values of each individual congener group were summed to obtain the sum C_{10}–C_{13}. The first three methods agreed well with the concentration in the reference SCCP 63% mixture and in the spiked cod liver with a deviation from nominal values of less than 20%. Methods that used individual PCA standards resulted in an underestimation of SCCP concentrations of $\leq$50% from the nominal value. This is probably explained by using only individual PCA standards of C_{10} carbon chain length with different number of chlorines in the molecules. PCA standards of C_{11}–C_{13} carbon chain lengths with monochlorine substituted carbon atoms were not commercially available. Therefore, it was not possible to include the effect of carbon chain length on the RRF in the quantification.

No reference concentration of SCCPs in the fish samples was known. Therefore, for comparison of the obtained results by the four quantification methods the median value of data was used. In Figure 4 the estimated concentration of SCCPs in fish samples (ng g^{-1}) by each method was related to the median value. The results of the three methods agreed with median value within 30%. Taking into account quantification errors in SCCP analysis of 100% or more the deviation found in this study is relatively low. In spite of an underestimation of the SCCP concentration in standard mixture application of individual PCA standards did not result in systematic underestimation of SCCP concentrations in the fish samples and resulted in a deviation from median value of $\leq$40%. The average concentrations found in tested fish species were 30–290 ng g^{-1} wet weight or 180–570 ng g^{-1} lipid weight which corresponds to literature data [4,19,24,25]. Application of LRMS instead of HRMS could result in some overestimation of the quantity of SCCPs in the fish samples. Reth *et al.* [14] reported that quantification of major congener groups (C_{10} with 5–7 chlorine atoms, C_{11}, C_{12} and C_{13} SCCPs with 5–8 chlorine atoms) are not affected by any other congener groups while the presence of minor

congener groups with 9–10 chlorine atoms and C_{10} with 8 chlorine atoms could be disturbed by the presence of MCCPs.

The full validation of the methods obviously requires more replicate measurements, spiking levels and samples to be analysed. Uncertainly of the methods applied should preferably not exceed 50%, for example, for analysis of surface water according to the requirements of the Water Frame Directive [26]. Concerning the four tested quantification methods the advantage of the Methods 2 and 3 is that they compensate for the differences of chlorine content in standards and environmental samples. For those methods no matching of the patterns is necessary. Therefore, the Reth method (Method 2) and the combined Coelhan-Reth method (Method 3) can be recommended for a routine quantification by GC-ECNI-MS in monitoring of SCCPs. The additional advantage of the combined Coelhan-Reth method (Method 3) is the quantification of the SCCPs by individual carbon chain length (e.g. $C_{10}, C_{11}, C_{12}, C_{13}$) and in that way the characterisation of CP mixtures. The Tomy method (Method 1) requires close resemblance of congener group patterns of the used external standard to that of the sample to have reliable results. Therefore, the result of the method is strongly depending on which standard is selected for quantification. The method that uses individual standards for the quantification of SCCPs (Method 4) resulted in a larger deviation probably because not all the necessary standards presently are available. However, the clear advantage of the method is that standards are clearly defined. This makes the quantification results comparable between different laboratories. Such an approach has been applied, for example, in the European legislation for toxaphene [27]. In addition, the method allows a detailed quantification per congener group of SCCP mixtures. Provided all necessary individual congeners would be available as pure standards and not as isomer mixtures, improvement of the method could be expected.

Acknowledgements

We would like to thank Judith van Hesselingen, Quy Dao and Alwin Kruijt from IMARES, The Netherlands, for practical assistance. The authors would also like to thank Foppe Smedes from Deltares, The Netherlands, for valuable comments and editorial contribution.

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