COMPARISON OF QUANTIFICATION METHODS FOR ANALYSIS OF POLYCHLORINATED ALKANES MEASURED WITH ELECTRONE CAPTURE NEGATIVE IONIZATION

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Introduction

Chlorinated paraffins (CPs) are complex technical mixtures of polychlorinated alkanes (PCA) with carbon lengths of C10–C13 (short-chain CPs), C14–C17 (medium-chain CPs) or >C17 (long-chain CPs) and a chlorination degree of 30-70%. They are produced by chlorination of n-alkanes using molecular chlorine at temperatures between 50 and 150 °C, at elevated pressures and/or in the presence of UV light (1). PCAs are ubiquitous persistent environmental pollutants. Therefore, several countries have imposed regulations on short-chain CPs (sCCPs) use and singled them out as priority pollutants (e.g. European Union in the Water Frame Directive) (2). The 25th Adaptation to Technical Progress to the Dangerous Substances (Directive 67/548/EEC) has formally classified C10-13 PCAs as Category 3 carcinogens (R40) and as Dangerous for the Environment (R50/53). They are also under consideration to be included in Stockholm Convention on Persistent Organic Pollutants (POPs). All the applied regulations create needs for monitoring of these compounds in environmental samples. The analysis and quantification of PCAs is extremely difficult task due to the complex composition (<10.000 congeners) of CP products and compared to other chlorinated POPs, such as polychlorinated biphenyls (PCBs), limited information is available about the environmental concentrations and toxicity of CPs. Therefore, there is a growing need to establish quality assured analytical methods for the determination of CPs in the environment.

GC-MS with electrone capture negative ionization (ECNI) is one the most used analytical technique for determination of PCAs, because it provides sufficient sensitivity and enables to distinguish between congener groups. As response of ECNI-MS is very much dependent on chlorination degree the most critical point in the analysis of is the proper selection of a PCA external standard for quantification, since composition and in particular chlorine content of the PCAs in environmental samples and in the external standards can be very different. In the last decade, various quantification methods have been reported for GC-ECNI-MS technique (3)(4)(5). In the present study, several quantification methods were considered, and compared including two new approaches. The standard mixtures and individual standards were measured by GC-ECNI-LRMS instrument and relative errors of quantification methods were estimated.

Materials and methods

Solvents for residue analysis grade were purchased from Promochem (Wesel, Germany). PCA standard mixtures C10-C13 51.5%, 55.5% and 63% chlo+rine (100 ng/ul solutions in cyclohexane), C10 44.82%, 55.00%, C10 65.02% (10 ng/ul in cyclohexane) were obtained from Dr. Ehrenstorfer, Augsburg, Germany and PCA-60 mixture from Dover Chemical corporation, Dover, USA. Additionally, control PCA standard solution was prepared by mixing C10 44.82% with C10 65.02% 1+2 (PCA check standard mixture with 60.25% chlorine content). The individual PCA standards were obtained from Chiron AS, Norway and from Dr. Ehrenstorfer, Augsburg, Germany. PCB112 (Dr. Ehrenstorfer, Augsburg, Germany) was employed as an injection standard.

Instrumentation

All the measurements were performed on HP 6890 GC-ECNI-LRMS equipped with capillary column (DB-5, 50m, 0.25mm i.d., 0.25μm film) and split/splitless injector (splitless time 1.5 min). The carrier gas was He at constant flow

rate of 1.2 ml/min and initial column pressure 140 kPa. A volume of 1 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 20min. The transfer line temperature was set 280°C. The HP5973 mass spectrometer was operated in negative chemical ionization (ECNI) mode using methane with 10% ammonia (99.99% purity) as a reagent gas (1.5 ml/min). Calibration of the mass spectrometer was done at m/z 185, 351, 449 using perflouro-5,8-dimethyl-3,6,9-trioxidodecane (PFDTD). The ion source temperature was 200°C and quadrupole temperature 100°C. The electron energy was 60eV and emission electron current 50 µA. For each group of PCA congeners the signal of two the most abundant isotopes of the [M-Cl]⁻ ions (6) were recorded in selected ion monitoring mode with dwell times of 30ms. Quantification was done using MS-Chemstation software.

Results and discussion

Quantification of PCAs using G. Tomy approach
The basic assumption used for the calculations is that the response a of congener group would be proportional to the number of chlorine atoms as well as to its molar concentration (7). Several PCA standard mixtures were measured by GC-ECNII-LRMS and processed according to G. Tomy procedure. Bar graph plots of the ion signal profiles for the different standards (C10-C13 51.5%, 55.5%, PCA-60% and 63% chlorine content) were generated and compared. The average molecular mass and the fractional relative abundance of the molecular formula were used in quantitative calculations as a correction factor for difference of the patterns. The results of calculations of the standards against each other are presented in Table 1. For PCA C10-C13 51.5% and 55.5% standards that have a very similar composition the relative deviation was within 50%, but for the standards with very different congener patterns and chlorination degree the deviation could be >500%. The differences in composition of the standards were the major contribution to the quantification error.

Quantification approach applied by M.Reth
This method was based on the determination of the relation between the relative total response factor of a PCA mixture and the degree of chlorination (5). Standard PCA mixtures (C10-C13 51.5, 55.5, 60 and 63%) were analyzed to compose a regression line. A reasonably linear correlation (R^2>0.9) was found and used for quantification (Fig.1). The deviation of the control standards with a different chlorination degree was within 30%. This method of quantification does not depend on the congener group patterns of the standard and the sample and accounts for the total chlorination of the measured sample in the relation with the total relative response factor. This reduces significantly the quantification errors compared to the method of Tomy (7).

Combined quantification approach of Coelhan and Reth
Coelhan (4) has suggested to quantify each group of different alkane chain length (i.e. C10, C11, C12, C13) applying several individual PCA standard mixtures with different chlorination degrees. Application of the individual PCA standard mixtures with several chlorination degrees and homolog group distributions that differ from those in the sample leads to quantification errors 90-1100% (4). The disadvantage of Coelhan method is that it is difficult to select a standard with the same PCA composition to that of the sample. In the present work we therefore propose to use M. Reth approach (5) for determination of the correlation between the relative total response factor of individual PCA mixture (i.e. individual standard C10, C11, C12, C13) and the degree of chlorination. Presently, only C10 individual standard mixtures with 44.82%, 55%, 65.02% chlorine content were used to compose the correlation curve (Fig.2). The deviation of the prepared control standard C10 with 60.25% chlorine content was 54.4%. These first results indicates that combination of Coelhan and Reth method allows to report concentrations of individual carbon-chain-length groups while quantification error is reduced to the level comparable with the Reth method.

Study of the response factors of the individual PCA congeners

An interesting issue for quantification of PCAs is the exploration of the response factors of the individual PCA congeners with different chlorine content. If different congeners of the same congener group would have identical response factors (a difference of 20-30 % is acceptable), this would allow to use one congener as an external standard for quantification of an entire congener group. The response factors of available individual congeners were studied. As is shown in Table 2, relative response factors (RRF) for the congeners with maximum one chlorine per carbon is increasing with increased number of chlorine atoms in the molecule. For instance, RRF for pentachlorodecane $(1,2,5,6,9-C_{10}H_{17}Cl_5)$ is 0.022 as against 1.8 for nonachlorodecane $(1,2,3,4,5,6,7,8,9-C_{10}H_{13}Cl_9)$. Such observation is not unexpected. However, it is rather surprising that RRF for congeners with 8 chlorines atoms but with three Cl atoms on the first and on the last carbon of the chain (*e.g.* $1,1,1,3,6,10,10,10-C_{10}H_{14}Cl_8$) is in the same order of magnitude as RRF of pentachlorinated congeners. It seems that addition of chlorine atoms on the carbon which already contains chlorine does not increase response of the ECNI method. From these observations it is obvious that the RRF is very much dependent on the chlorine pattern in the molecule. In addition, the difference in RRF for two isomers of the same congener was also observed (*c.f.* $1,2,5,6,9,10-C10H16Cl6$ congener in standards CP-4 and CP-5). Therefore, it is hard to imagine that the quantification of congener groups based on single congener using ECNI source could be more precise than quantification based on technical mixtures.

References

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Table 1: Obtained amounts and relative deviations for different PCA standards (C10-C13 51.5%, 55.5%, 60%, 63%) against each other.

Sample	Amounts obtained (ng) using calculation based on				Relative deviation (%) using quantification based on			
	PCA 51.5%	PCA 55.5%	PCA 60%	PCA 63%	PCA 51.5%	PCA 55.5%	PCA 60%	PCA 63%
PCA 51.5%	**12326**	8261	3865	1977	0	33	69	84
PCA 55.5%	19800	**13270**	6209	3176	49	0	53	76
PCA 60%	1921	1287	**599**	309	221	115	0	48
PCA 63%	73034	48949	22904	**11715**	523	318	96	0

Figure 1: Dependence of the total relative response factor on the degree of chlorination for four C10-C13 mixtures (51.5 (57.4)%, 55.5 (59.02)%, 60 (60.84)% and 63 (63.24)%) chlorine using the Reth method. In the brackets the calculated degree of chlorination is represented for each used standard PCA mixture which is different from the reported by the producer.
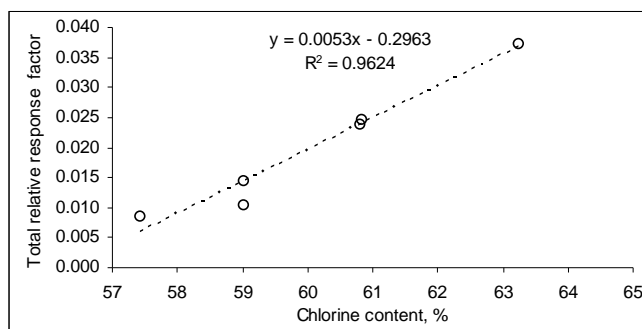
Figure 2: The plot of the total relative response factor versus the chlorine content of C10 standard mixture with 44.82 (61.1)%, 55 (61.3)%, 65 (65.4)% chlorine using combined Coelhan-Reth method. In the brackets the calculated degree of chlorination is represented for each individual PCA standard mixture used which is different from the reported by the producer.
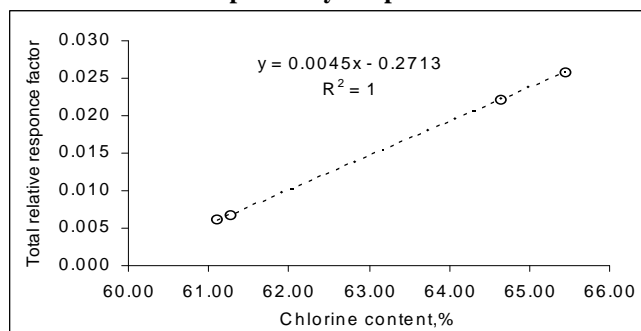
Table 2: Relative response factors (RRF) of individual PCA congeners

Nr	Individual isomer		Batch nr	Producer	RRF
1	1,1,1,3,8,9	$C_9H_{14}Cl_6$	3925	Chiron	0.024
2	1,1,1,3,9,10	$C_{10}H_{16}Cl_6$	3744	Chiron	0.014
3	1,1,1,3,10,11	$C_{11}H_{18}Cl_6$	3745	Chiron	0.014
4	1,1,1,3,11,12	$C_{12}H_{20}Cl_6$	3746	Chiron	0.009
5	1,1,1,3,12,13	$C_{13}H_{22}Cl_6$	3747	Chiron	0.010
6	1,1,1,3,6,8,8,8	$C_8H_{10}Cl_8$	3748	Chiron	0.004
7	1,1,1,3,8,10,10,10	$C_{10}H_{14}Cl_8$	3857	Chiron	0.024
8	1,1,1,3,9,11,11,11	$C_{11}H_{16}Cl_8$	3749	Chiron	0.012
9	1,1,1,3,10,12,12,12	$C_{12}H_{18}Cl_8$	3761	Chiron	0.007
10	1,1,1,3,11,13,13,13	$C_{13}H_{20}Cl_8$	3762	Chiron	0.006
11	1,1,1,3,12,14,14,14	$C_{14}H_{22}Cl_8$	3763	Chiron	0.013
12	1,2,5,6,9	$C_{10}H_{17}Cl_5$	CP-3	Dr. Ehrenstorfer	0.022
13	1,2,4,5,9,10	$C_{10}H_{16}Cl_6$	CP-6	Dr. Ehrenstorfer	0.181
14	1,2,5,6,9,10	$C_{10}H_{16}Cl_6$	CP-4	Dr. Ehrenstorfer	0.169
15	1,2,5,6,9,10	$C_{10}H_{16}Cl_6$	CP-5	Dr. Ehrenstorfer	0.037
16	1,2,4,5,6,9,10	$C_{10}H_{15}Cl_7$	CP-7	Dr. Ehrenstorfer	1.1
17	2,3,4,5,6,7,8,9	$C_{10}H_{14}Cl_8$	CP-9	Dr. Ehrenstorfer	1.4
18	1,2,3,4,5,6,7,8,9	$C_{10}H_{13}Cl_9$	CP-10	Dr. Ehrenstorfer	1.8