SPECIFIC ALTERNATIVE MASS SPECTROMETRIC TECHNIQUES FOR THE TRACE ANALYSIS OF CHLORDANES AND POLYCHLORINATED *n*-ALKANES

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LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to by Roman numbers I to V in the text:

I. Z. Zencak, M. Oehme and S. Skopp "Detection of chlordanes by positive ion chemical ionization in an ion trap: a comparitive study of the non-conventional reagents acetonitrile, acrylonitrile and dichloromethane" (2001) *Rapid Commun. Mass Spectrom.* **15**, 1719-1725.

II. Z. Zencak, M. Reth and M. Oehme "Dichloromethane-Enhanced Negative Ion Chemical Ionization for the Determination of Polychlorinated *n*-Alkanes" (2003) *Anal. Chem.* **75**, 2487-2492.

III. Z. Zencak, M. Reth and M. Oehme "Determination of Total Polychlorinated *n*-Alkane Concentration in Biota by Electron Ionization-MS/MS" (2004) *Anal. Chem.* **76**, 1957-1962.

IV. Z. Zencak, A. Borgen, M. Reth and M. Oehme "Evaluation of four Mass Spectrometric Methods for the Analysis of Polychlorinated *n*-Alkanes" (2005) *J. Chromatogr.* A, *in press*.

V. Z. Zencak and M. Oehme "Chloride-Enhanced Atmospheric Pressure Chemical Ionization Mass Spectrometry of Polychlorinated *n*-Alkanes" (2004) *Rapid Commun. Mass Spectrom.* **18**, 2235-2240.

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ABBREVIATIONS

ac	Alternate current
APCI	Atmospheric pressure chemical ionization
CI	Chemical ionization
CID	Collision induced dissociation
dc	Direct current
ECNI	Electron capture negative ionization
EI	Electron ionization
ESI	Electrospray ionization
GC	Gas chromatography
i.d.	Inner diameter
HR	High resolution
HRGC	High resolution gas chromatography
HRMS	High resolution mass spectrometry
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
LR	Low resolution
LRMS	Low resolution mass spectrometry
m/z	Mass-to-charge ratio
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NICI	Negative ion chemical ionization
PCAs	Polychlorinated *n*-alkanes (sPCAs: short chain PCAs, mPCAs: medium chain PCAs, and lPCAs: long chain PCAs)
PICI	Positive ion chemical ionization
RF	Radio frequency
S/N	Signal-to-noise ratio
SIM	Selected ion monitoring

SUMMARY

Mass spectrometry is a well established technique in environmental analysis. However, not all of its possibilities are currently being exploited. In this work alternative mass spectrometric methods were developed and their possibilities evaluated for the analysis of chlordanes and polychlorinated *n*-alkanes (PCAs).

The use of non-conventional reagent gases was evaluated for ion trap positive ion chemical ionization of chlordanes. The application of acetonitrile, acrylonitrile and dichloromethane as reagent gases allowed limits of detection ranging from the low- to the mid-pg range. Ionization occurred by charge transfer reaction and led to HCl eliminations, losses of chlorine radicals, and to a retro-Diels-Alder fragmentation reaction. Furthermore, differences in the mass spectra of *cis*-/*trans*-stereoisomers were observed when acetonitrile and dichloromethane were applied as reagent.

Since ion trap positive ion chemical ionization did not allow the analysis of PCAs, a method was developed employing a CH_4/CH_2Cl_2 reagent gas mixture for the negative ion chemical ionization (NICI) of PCAs. This technique proved to be a real alternative to the conventionally applied electron capture negative ionization (ECNI). A nearly exclusive formation of $[M+Cl]^-$ adduct ions was observed and the formation of other fragment ions was suppressed. This enhanced selectivity and sensitivity and allowed limits of detection of 1 ng/µl technical PCA mixture. Compared to ECNI the new technique allowed the detection of lower chlorinated PCAs and lowered the interferences between different PCA congeners by reducing the ionization yield of other polychlorinated compounds present in the sample extracts (e.g. toxaphenes and chlordanes). Furthermore, CH_4/CH_2Cl_2-NICI allowed a reliable quantification independent from the degree of chlorination of the PCA

standard. Finally, the method was applied to the determination of PCAs in fish liver samples.

The use of electron ionization (EI) tandem mass spectrometry (MS/MS) was also evaluated for the analysis of PCAs. Three collision induced fragmentation reactions common to all or most PCAs could be identified and allowed limits of detection in the range 0.1-0.2 ng/µl, which is one order of magnitude lower than the limits of detection achieved by ECNI combined with low resolution mass spectrometry. Furthermore, this technique allowed the simultaneous detection of short, medium, and long chained PCAs. Since the ionization yield was neither dependent on the degree of chlorination nor on the chain length of the studied PCAs, the quantification was not influenced by the selected quantification standard. Due to the short time of analysis and data processing, as well as to the high sensitivity and selectivity, this technique was suited for fast screening of environmental samples. It allowed the determination of PCAs in various fish livers (total PCA concentrations in the range 50-600 ng/g wet weight) as well as in sediment samples (total PCA concentrations in the range 50-300 ng/g dry weight).

The high tendency of PCAs to form chloride adduct ions observed in the experiments with dichloromethane as reagent gas was exploited to develop a liquid chromatography-mass spectrometry method. The use of chloroform as mobile phase for normal phase liquid chromatography allowed the formation of chloride adduct ions of PCAs under atmospheric pressure chemical ionization (APCI). This technique achieved limits of detections of 1-2 ng/µl for technical PCA mixtures. Furthermore, response factors of PCA mixtures with different degrees of chlorination varied less than under electron capture negative ionization. The developed technique allowed the determination of medium chained PCAs in two paint samples. The determined concentrations were in the range 8-12%, and the results were in very good agreement with those obtained by electron ionization tandem mass spectrometry.

Finally, the developed methods as well as ECNI combined with low and high resolution mass spectrometry were compared in terms of suitability for the quantitative analysis of PCAs. All methods showed an accuracy of ±21% or less for the analysis of spiked fish samples. However, the analysis of fish liver samples showed deviations of up to 46%. Since the use of quantification standards with different degrees of chlorination can result in systematic errors of >100% when ECNI is applied, the influence of the selection of the quantification standard was investigated for all methods. The results obtained by CH_4/CH_2Cl_2-NICI and EI-MS/MS were not dependent on the degree of chlorination of the PCA standard used for quantification. However, chloride attachment APCI showed an influence of both degree of chlorination and chain length on the response factors of PCAs.

1 INTRODUCTION

1.1 Mass spectrometry and its role in environmental analysis

The tremendous success of mass spectrometry (MS) as analytical technique in the environmental sciences is based on a number of scientific and technical progresses occurred during the past 40 years. Important environmental pollutants were discovered for the first time during the late 1960s (Jensen, 1966; Lotspeich, 1969). Mass spectrometry combined with gas chromatography (GC) was early recognized as powerful tool to determine the nature and extent of environmental contamination. This early success of GC-MS also stimulated research to combine other separation techniques with MS. In the 1980s, the successful coupling of mass spectrometry with liquid chromatography (LC) allowed the detection of compounds not amenable to GC. Furthermore, new sample introduction and ionization techniques were developed and greatly expanded the possibilities of MS.

Currently, the analysis of important environmental contaminants such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, hexachlorocyclohexanes, and polychlorinated dibenzo-*p*-dioxins, is unthinkable without mass spectrometry (Richardson, 2002). In the late 1960s, when the detrimental environmental effects of pollutants became evident, there was a growing need for their reliable determination in environmental samples. Mass spectrometry rapidly became the method of choice in terms of sensitivity and selectivity. Detection of 10^{-12} to 10^{-15} g of single compounds were possible with a selectivity sufficient for accurate quantification in complex environmental matrices. Moreover, MS allowed structure identification and elucidation of environmental contaminants with ng or pg quantities of substance, which is not possible with other analytical techniques. Nowadays, MS is a well established analytical technique, which also plays an important role in many other fields such as food analyses, forensic sciences, and pharmaceutical analyses.

Besides routinely (conventionally) applied techniques such as electron ionization and chemical ionization using methane as reagent gas, a large number of ingenuous methods were developed (e.g. employing water or N_2O/CH_4-mixtures as reagent gases for chemical ionization). However, they have not found widespread application due to various reasons: Some techniques require instrument modifications by the user, others require to optimize and control too many variables. However, in some cases the conventional techniques do not provide results with the necessary sensitivity or selectivity. In such cases, less exploited techniques may be valid alternatives.

Although mass spectrometric instrumentation has undergone many technical improvements during the past 30 years, the basics of ionization, mass separation, and ion detection has remained unchanged. Currently, many techniques are available for both ionization and mass analysis. The following chapters will give a brief description of the methodologies relevant for this work.

1.2 Ionization techniques

1.2.1 Electron ionization

Electron ionization (EI) is widely used in organic mass spectrometry and has become the standard technique for GC-MS. Ion sources for EI consist of a heated filament emitting electrons, which are accelerated towards an anode. The interaction between the electron beam and the outer electrons of the analyte molecule introduced into the ion source leads to electronic excitation. If enough energy is transferred, an electron is expelled, which leads to the formation of a radical cation $[M]^{+\cdot}$. A maximal ionization yield is achieved with electrons of ca. 70 eV energy for organic molecules. The ionization process occurs within ca. 10^{-15} to 10^{-16} s. Consequently, the geometry of molecules does not change during ionization. Since the ionization potential for organic compounds is approximately 8-13 eV, the use of electrons of 70 eV transfers an excess of energy to the analyte molecules, which leads to bond cleavage and fragment formation. Some fragment ions provide information for structure identification or elucidation. The ionization yield of EI is about $1/10^5$, the residual sample molecules are removed by vacuum pumps (McLafferty and Tureček, 1993).

EI gives an excellent reproducibility and comparability of mass spectra. This allowed the establishment of spectra libraries, which are an important tool for compound identification. The high reproducibility of the EI process and the direct correlation between analyte amount and the resulting ion current make this ionization method very suited for quantitative analysis.

1.2.2 Chemical ionization and electron capture negative ionization

Chemical ionization (CI) bases on gas phase reactions between analyte molecules and reagent gas ions usually formed by EI (Harrison, 1992). At pressures of ca. 130 Pa (ca. 1 Torr) in the ionization chamber, a molecule will undergo hundreds of collisions (free path of ca. 0.05 mm), which is necessary for the intermolecular reactions needed for CI (De Hoffmann and Stroobant, 1999). Depending on the analyte and on the applied reagent gas, positive ions (positive ion chemical ionization, PICI) or negative ions (negative ion chemical ionization, NICI) are formed. Since the energy transferred to the analyte during CI is lower than in EI, CI gives mass spectra with less or no fragmentation compared to EI.

In contrast to EI sources, ion sources for CI have tighter ionization chambers with small apertures (Oehme, 1998). Additionally, an adequate pumping system must be available. In this way the ion source can be operated at the required reagent gas pressure and high vacuum can be maintained in the remaining parts of the mass spectrometer (McLafferty and Tureček, 1993).

Typical ion-molecule reactions observed in CI are: Proton transfer, adduct formation and charge transfer reactions (De Hoffmann and Stroobant, 1999). Proton transfer is probably the most common ionization reaction in CI. This chemical reaction can be described as acid-base reaction, where the tendency of a reagent ion $[RH]^+$ to protonate the analyte M may be assessed from their proton affinity values. The proton affinity is the negative of the enthalpy change of the protonation reactions (Harrison, 1992). If the analyte has a higher proton affinity than the reagent gas, $[M+H]^+$ ions will be formed. The formation of adduct ions between analyte molecules and reagent gas ions is often observed in CI. Finally, radical cations are formed by charge transfer reactions but with a smaller energy content than in EI. Therefore, less fragmentation is usually observed.

The mostly applied reagent gases for CI are methane, isobutane and ammonia. Methane forms $[CH_5]^+$, $[C_2H_5]^+$ and $[C_3H_5]^+$ as reagent ions, with proton affinities of 546, 684, and 736 kJ/mol, respectively (Oehme, 1998; Hunter and Lias, 1998). Isobutane has a slightly higher proton affinity than methane, which may enhance selectivity for certain compounds. The use of ammonia as reagent gas is suited for the selective ionization of amines, due to its high proton affinity. Besides these three reagent gases, several others have been successfully applied. However, despite some considerable advantages, they are seldom used in practice, thus these are assigned as non-conventional. Some examples of application of non-conventional CI reagents are: N_2O/CH_4-NICI for the differentiation of isomers of polycyclic aromatic hydrocarbons by MS (Oehme, 1983), CH_2Cl_2-NICI for the selective detection of organic acids, amides, and phenols (Tannenbaum *et al.*, 1975), and $(CH_3)_2O$-PICI for the differentiation of isomeric alkenes and cycloalkanes (Keogh, 1982).

The ionization of a reagent gas also forms a large number of secondary electrons. These are slowed down to thermal equilibrium with the environment and have energies of 0-15 eV. If the analyte molecules have a sufficient electron affinity and cross-section, they will “capture” the thermal electrons and form molecular anions or negative fragment ions (Dougherty, 1981). This ionization technique is called electron capture negative ionization (ECNI). The term “NICI” is often used to describe this process. However, this is not correct, since no chemical reaction is used for ionization. ECNI plays an important role in environmental analysis, due to its high sensitivity and selectivity for the detection of halogenated compounds, polynitro compounds and selected polycyclic aromatic hydrocarbons (Budde, 2001).

1.2.3 Atmospheric pressure chemical ionization

The logical extension of CI in an ion source designed to maintain a pressure of 25-260 Pa (0.2-2 Torr) is ionization at atmospheric pressure. The frequency of collisions at atmospheric pressure increases enormously and thermodynamic equilibrium is rapidly reached. Therefore, the ionization efficiency of APCI is very high (De Hoffmann and Stroobant, 1999).

Although the first APCI sources were operated on GC-MS instruments, it was rapidly recognized that APCI together with electrospray ionization was the ideal solution for coupling LC with MS (Munson, 2000). This allowed online detection of polar and/or high-molecular compounds not amenable to GC and represented an important step in the development of MS. A pneumatic nebulizer is used in the APCI source of a modern LC-MS instrument to create a fine spray that is directed into a heated desolvation chamber (200-500 °C). After vaporization the LC eluate is ionized by a corona discharge.

Chemical reactions between gaseous eluent ions and analyte molecules lead to ionization of the latter. Similar reactions to those observed for CI take place. Proton transfer is the most common ionization reaction in APCI. Protonation or deprotonation of the analyte molecules will take place depending on the difference between the gas phase proton affinity of the reagent gas and of the analyte. Additionally, the formation of adduct ions is often observed.

The application of conventional electrospray ionization (ESI) and APCI is still limited to medium polar to polar compounds. During the past few years, different strategies have been developed to ionize nonpolar compounds by ESI or APCI as well (Hayen and Karst, 2003). Such strategies include coordination ion spray, on-line electrochemical conversion (Van Berkel *et al.*, 1998), atmospheric pressure electron capture negative ionization (Singh *et al.*, 2000) as well as the selection of a suited mobile phase (Marvin *et al.*, 1999). In this way nonpolar compounds such as polycyclic aromatic compounds (Marvin *et al.*, 1999; Van Leeuwen *et al.*, 2004) and hexabromocyclododecane could be detected (Budakowsky and Tomy, 2003). The use of such non-conventional ionization techniques allows to include compounds usually analyzed by GC-MS.

1.3 Mass analysis

The ions formed during the ionization process are accelerated towards the mass analyzer by a repeller (or by a drow-out) potential and separated according to their mass-to-charge ratios (*m/z*) by a variety of techniques. Table 1.1 compares different techniques for mass separation used in this work.

Table 1.1: Characteristics of ion trap, quadrupole and electromagnetic mass analysers.

	Quadrupole	Triple quadrupole	Ion trap	Magnetic sector
Ion transmission	continuous	continuous	pulsed	continuous
Ion separation	rf and dc voltage	rf and dc voltage	rf voltage	magnetic and electric field
Resolution	low (ca. 1000)[a]	low (ca. 1000)[a]	low (ca. 1000)[b]	high (≥100000)[c]
Mass range	1-2000 *m/z*	1-2000 *m/z*	1-2000 *m/z*	1-20000 *m/z*
Scan speed	4000 u/s	4000 u/s	4000 u/s	ca. 1000 u/s (exponential)
Pressure requirement	$\leq 10^{-5}$ Torr	$\leq 10^{-5}$ Torr	$\leq 10^{-4}$ Torr	$\leq 10^{-6}$ Torr
Notes	higher sensitivity in selected ion monitoring mode	MS/MS, high sensitivity in selected reaction monitoring mode	MS^n, scan mode with high sensitivity	MS/MS with quadrupoles, HRMS, exact mass determination

[a] at *m/z* 1000
[b] at *m/z* 1000, higher resolution is possible
[c] using internal mass calibration

1.3.1 Quadrupole mass analyzers

The introduction of quadrupole mass spectrometers as GC detectors by Finnigan Corporation represented a breakthrough in analytical chemistry in the late 1960s. Since these instruments were more affordable than magnetic field mass spectrometers, the application of MS rapidly increased. Today, the linear quadrupole mass filter is probably the mostly applied mass analyzer (Niessen, 2003).

The linear quadrupole mass filter consists of four parallel rods. A combination of direct current voltage and radio frequency voltage is applied to each pair of diagonally situated rods. For each pair of rods the direct current potential is of opposite sign and the radio frequency voltage has a phase displacement of 2π (De Hoffmann and Stroobant, 1999). The combination of these potentials creates a mass filter, which allows only ions with a particular *m/z* to pass through the quadrupole. Ions with different *m/z* collide with the rods and are discharged. The motion of the ions through the electrostatic fields in the quadrupole can be described by the Mathieu equations. For the linear quadrupole the solutions of these equations are:

$$a_u = a_x = -a_y = \frac{8zeU}{m\omega^2 r_0^2} \qquad \text{and} \qquad q_u = q_x = -q_y = \frac{4zeV}{m\omega^2 r_0^2}$$

These parameters are only dependant on U (direct voltage), V (radio frequency voltage), and on the *m/z* of the ion. Therefore, knowing the potentials applied to the quadrupole it is possible to calculate a_u and q_u for each *m/z* value. Figure 1.1 shows the stability diagram for ions in a quadrupole field. If a_u and q_u of a certain ion are in the shaded area, the ion will pass through the quadrupole without colliding with the rods. The potentials applied allow only ions with a narrow range of *m/z* values to pass through the filter. During the mass scan a_u and q_u values of different ions move along a line (Figure 1.1). Higher resolution can be achieved with a higher a_u/q_u ratio (Oehme, 1998).

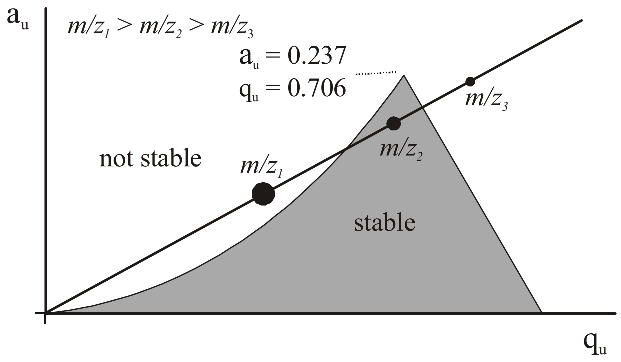


Figure 1.1: Stability diagram for ions in a linear quadrupole. Ions are represented by black circles.

1.3.2 Ion traps

After its invention in 1953, it took more than three decades before the ion trap mass spectrometer was introduced as GC detector by the Finnigan Corporation in 1985. Since then, many applications of these instruments for environmental analysis have been reported (March, 2000).

Conceptually, an ion trap can be imagined as a quadrupole bent to a ring. It consists of two end cap electrodes and a ring electrode to which a radio frequency voltage with variable amplitude and constant frequency is applied. This generates a three dimensional quadrupole field, which allows to store ions. Helium is used as damping gas to limit the energy distribution of the ions in the ion trap and to enhance resolution (Stafford *et al.*, 1984). The motion of the ions can be described by the Mathieu equations as well. For the ion trap, their solution is:

$$a_u = a_z = -2a_r = \frac{16zeU}{m\omega^2(r_0^2 + 2z_0^2)} \quad \text{and} \quad q_u = q_x = -2q_r = \frac{8zeV}{m\omega^2(r_0^2 + 2z_0^2)}$$

where U is the direct voltage and V the radio frequency voltage. These solutions can be graphically visualized by the stability diagram, which is shown in Figure 1.2.

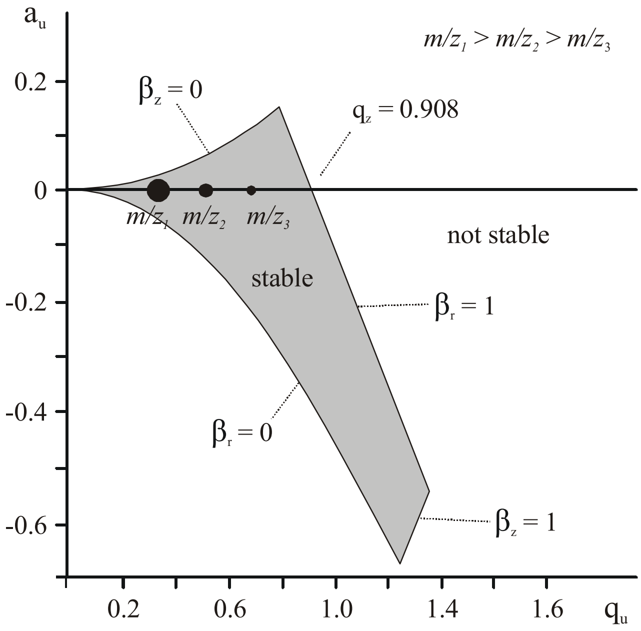


Figure 1.2: Stability diagram for ions in a quadrupole ion trap. Ions are represented by black circles. $\beta_z = 0$ and 1, and $\beta_r = 0$ and 1 are the stability boundaries.

Since most commercial instruments do not apply a direct current potential to the electrodes, a_z is equal to zero (March, 1997). Therefore, ions stored in the ion trap can be displayed on the q_z axis of the stability diagram and ions with low *m/z* will have higher q_z values. In the “mass-selective axial instability mode of operation” the amplitude of the radio frequency is increased. Consequently, the q_z of the stored ions increase. When this value reaches the stability boundary ($q_z = 0.908$) the ion is ejected from the ion trap and can be detected. In this way ions with increasing *m/z* can be detected consecutively and mass spectra recorded. Other, more sophisticated, operations applying additional voltages to the end cap electrodes are also possible (March, 1998).

The ability of ion trap mass spectrometers to store selected ions and fragment them by collision induced dissociation (CID) is of great interest for structure elucidation as well as for quantitative applications demanding high selectivity (Jonscher and Yates, 1997). Additionally, ionization can be performed inside the ion trap. CI reagent gas ions can be stabilized in the ion trap so that longer reaction times can be achieved. Therefore, lower partial pressures of reagent gas are necessary (ca. 10^{-3} Pa corresponding to ca. 10^{-5} Torr), which opens the way to the use of non-conventional (e.g. corrosive) reagent gases. Some examples of such non-conventional ion trap CI reagents are: H_2O-PICI for the ionization of polycyclic aromatic hydrocarbons (Landrock *et al.*, 1995) and acetonitrile-PICI to locate double bonds in unsaturated fatty acids (Van Pelt and Brenna, 1999).

1.3.3 Double focusing electromagnetic mass spectrometers

Double focusing mass analyzers with a magnetic and an electrostatic sector have been available since the 1950s. These instruments can now achieve mass resolution of 100’000 or more. This allows highly selective detection of target compounds and an exact mass determination for the calculation of the elemental composition of unknown compounds.

The mass separation is usually performed by the magnetic sector. The ions formed in the ion source are accelerated by a potential difference (4-12 kV) and pass through a perpendicular magnetic field. Ions in motion through a magnetic field are subject to a force which is perpendicular to both their trajectory and the magnetic field (Lorenz force law). This force depends only on the charge and velocity of the ions. Therefore, ions with different momentum follow different trajectories and are separated. An electrostatic sector, which focuses ions according to their kinetic energy is used to achieve a better mass resolution. The mass spectrum can be acquired varying the strength of the magnetic field and keeping the accelerating voltage and the potential of the electric sector at constant values. Another way of operation is to hold the magnetic field constant and scan the accelerating potential. In this case, the electric sector potential tracks the accelerating voltage. This mode of operation is usually used for selected ion monitoring, since it has the advantage that the electric field is not subject to hysteresis (De Hoffmann and Stroobant, 1999).

Double focusing mass analyzers offer several advantages. Only the possibility to avoid interferences from other contaminants operating at high resolution (HR) is of relevance for this work. HRMS has been recognized as necessary for determination of compounds such as polychlorodibenzo-*p*-dioxins and dibenzofurans in complex matrices (Oehme, 1998). However, HRMS instrumentation is expensive and not available at many laboratories. Therefore, the development of alternative methods basing on low resolution (LR) instrumentation is necessary to allow more extensive and affordable measurements of environmental contaminants.

1.3.4 Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) consists of the selection of a precursor ion, its fragmentation by collision with an inert gas (collision induced dissociation, CID), and the acquisition of the mass spectrum of the product ions. MS/MS techniques are widely used in quantitative analysis as well as for structure identification. Among the four different MS/MS scan modes, selected reaction monitoring is usually used for quantitative applications due to the high selectivity and sensitivity achieved.

MS/MS can be performed in space by coupling two mass analyzers or in time using an ion storage instrument (Johnson *et al.*, 1990). Triple quadrupoles are mostly used for MS/MS in space. The precursor ions are selected in the first quadrupole and fragmented by CID in a second quadrupole operated in RF-only mode. Finally, the formed product ions are analyzed in the third quadrupole. MS/MS in time is usually performed in ion traps. Here, the precursor ions are isolated in the ion trap and then fragmented by CID so that the product ion mass spectrum can be acquired. Whereas quadrupole instruments usually have only two stages of mass analysis, the MS/MS process in the ion trap can be repeated several times: Multiple mass spectrometry (MS^n).

Ion trap and triple quadrupoles do not give exactly identical MS/MS spectra. Fragment ions formed by CID in triple quadrupoles undergo further collisions so that further fragmentation can be observed. In ion traps only the selected parent ions are excited and fragmented by CID and further fragmentation of the product ions is avoided. Non-resonant (also called broad-band activation) can be used to obtain MS/MS spectra similar to triple quadrupoles. However, the mass range of the product ions, which can be detected by the ion trap is limited. Since excitation is performed keeping the precursor ion at $q_u = 0.4$, only product ions with *m/z* higher than approximatively one third of the parent ion *m/z* can be stored. This effect is known as low mass cut off. As consequence, fragment ions with low *m/z* are not present in ion trap MS/MS spectra, whereas they are normally detected by triple quadrupole instruments (Johnson *et al.*, 1990). Although MS/MS is well established in the field of LC-MS, it is not so frequently used in GC-MS where single quadrupole instruments dominate the market.

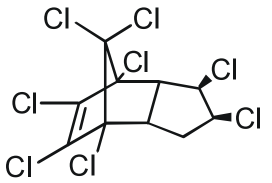
1.4 Polychlorinated environmental contaminants: Two examples

In the past 50 years, many polychlorinated compounds used for technical applications or as pesticides have caused environmental concern (Budde, 2001). Some examples are polychlorinated biphenyls, bornanes, and cyclohexanes. Nowadays, environmental levels of these compounds are mostly determined by mass spectrometry. However, the usually applied methods do not always make benefit of the possibilities of modern mass spectrometry. A brief discussion of the application of MS to the analysis of two classes of polychlorinated contaminants used in this work will demonstrate this.

1.4.1 Chlordane

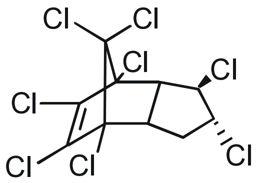
The insecticide Chlordane is produced by Diels-Alder reaction from pentachlorocyclopentadiene and cyclopentadiene with subsequent chlorination. The result is a complex mixture of structurally similar compounds consisting of predominantly hexa- to decachlorinated congeners and isomers, of which 147 could be identified in the technical mixture. Figure 1.3 shows the structures of selected chlordane compounds, that were investigated in this work. Chlordane was mainly used as soil and non-food plant insecticide since its development in 1945 and was banned in the United States in 1983 .

The technical mixture has a vapor pressure of 10^{-3} Pa at 25 °C and a water solubility of 32 µg/l, which increases to 400 µg/l in the presence of humic substances. The octanol-water partition coefficients log K_{OW} are in the range 5.4-6.4 for the main congeners. Due to their physico-chemical properties, to the prolonged unrestricted use, and their bioaccumulation and persistence several chlordanes and their metabolites are ubiquitously present in the environment, especially in aquatic biota. Residues of chlordanes have also been detected in air, in biota from remote areas such as polar regions and in human adipose tissue. The detection of chlordanes in biota from remote areas points to a global dispersion. Long-range atmospheric transport has been identified as the main distribution mechanism. Typical environmental levels of chlordanes in marine biota are in the range 100-400 ng/g lipid weight (Karlsson, 1998).



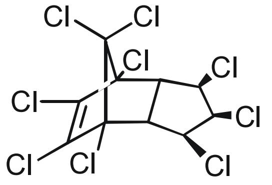
1-exo-2-exo-4,5,6,7,8,8-
octachloro-3a,4,7,7a-tetrahydro-
4,7-methanoindane

cis-chlordane



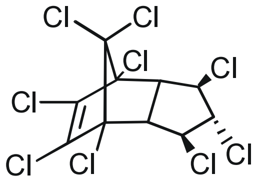
1-exo-2-endo-4,5,6,7,8,8-
octachloro-3a,4,7,7a-tetrahydro-
4,7-methanoindane

trans-chlordane



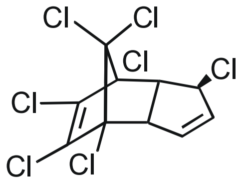
1-exo-2-exo-3-exo-4,5,6,7,8,8-
nonachloro-3a,4,7,7a-tetrahydro-
4,7-methanoindane

cis-nonachlor



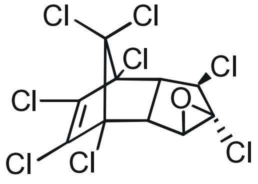
1-exo-2-endo-3-exo-4,5,6,7,8,8-
nonachloro-3a,4,7,7a-tetrahydro-
4,7-methanoindane

trans-nonachlor



1-exo-4,5,6,7,8,8-
heptachloro-3a,4,7,7a-tetrahydro-
4,7-methanoindene

heptachlor



1-exo-2-eexo-4,5,6,7,8,8-octachloro-
2,3-exo-epoxy-3a,4,7,7a-tetrahydro-
4,7-methanoindane

oxychlordane

Figure 1.3: Chemical structures of selected components of the pesticide chlordane and of the metabolite oxychlordane. IUPAC and trivial names are given.

Cis- and *trans*-chlordane have an acute toxicity of LD_{50} = 320-400 mg/kg body weight for rats. Some metabolites have even lower LD_{50} values (e.g. oxychlordane is about twenty times more toxic). Additionally, chlordane has been classified by the International Agency for cancer research as potential human carcinogen (Abadin *et al.*, 1994).

1.4.2 State of the art of chlordane analysis

The determination of chlordanes in environmental samples is usually performed by HRGC combined with ECNI-MS after an appropriate sample clean up. The use of ECNI allows to monitor the molecular ion providing good selectivity and instrumental detection limits in the range 1-10 pg for different chlordanes (Dearth and Hites, 1991). EI gives fragment ions formed by losses of Cl and HCl or by retro Diels-Alder reaction, and the molecular ion is often not observed. However, the retro Diels-Alder fragmentation can be used to determine the number of chlorine atoms attached to each of the two rings. Beside this, no further information about the position of the chlorine atoms can be obtained by conventional mass spectrometry (Karlsson, 1998).

Since many components of the technical mixture and their metabolites are chiral and their enantiomers show different metabolization rates and toxicity (Abadin *et al.*, 1994), enantioselective analysis of these compounds is of importance. Enantiomers of different chlordanes could be separated on chiral columns using alkylated α- or β-cyclodextrins as chiral selector (Buser and Müller, 1993; Karlsson, 1998).

1.4.3 Polychlorinated *n*-alkanes

Polychlorinated *n*-alkanes (PCAs, also called chlorinated paraffins or CPs) are complex technical mixtures containing thousands of different isomers, congeners, diastereomers and enantiomers. These mixtures are produced by radical chlorination of *n*-alkanes, in presence of UV light or heating. The chlorine content of the products varies between 30 and 70%. PCAs are divided into short chain PCAs (C_{10-13}, sPCAs), medium chain PCAs (C_{14-17}, mPCAs) and long chain PCAs ($C_{>17}$, lPCAs) depending on the length of the carbon chain. Since their first large scale usage in 1932 as extreme pressure additives, the purity of PCA products was improved increasing the purity of the *n*-alkane feedstocks (Muir *et al.*, 2000). Modern commercial products contain additives added to inhibit decomposition of PCAs by loss of HCl at elevated temperatures and to increase thermal stability when used as flame retardants (e.g. antimony oxide). Other common stabilizers include epoxides and organotin compounds (GDCh, 1996).

Depending on chain length and chlorine content, chlorinated paraffins are colorless or yellowish, low to highly viscous liquids or glassy to waxy solids. PCAs are chemically stable up to 200-300 °C. The environmentally important physico-chemical properties of PCAs are summarized in Table 1.2.

As consequence of the different physical and chemical properties, miscellaneous PCA formulations are used for a wide range of applications. Some examples are: Additives in metal working fluids (short, medium, and long chain PCAs, 50-60% Cl content), as secondary plasticizers in polyvinyl chloride and in other plastics (usually mPCAs, 40-60% Cl content) and as fire retardants in plastics (short, medium, and long chain PCAs, 50-70%

Cl content). The world wide production of PCAs in 1993 has been estimated to be 300 kt/year (Muir *et al.*, 2000).

Table 1.2: Physico-chemical properties of selected PCAs.

Compound	Vapor pressure [Pa][a, b]	Water solubility [µg/l][a, c]	log K_{OW}[a, d]
$C_{10}H_{18}Cl_4$	66	1260	5.93
$C_{10}H_{17}Cl_5$	4-66	678-994	6.04-6.20
$C_{10}H_{13}Cl_9$	0.24	n.a.	n.a.
$C_{11}H_{20}Cl_4$	10	575	5.93
$C_{11}H_{19}Cl_5$	1-2	546-962	6.04-6.40
$C_{11}H_{18}Cl_6$	0.5-2	37	6.4
$C_{12}H_{20}Cl_6$	n.a.	n.a.	6.40-6.77
$C_{12}H_{18}Cl_8$	n.a.	n.a.	7.0
$C_{13}H_{23}Cl_5$	0.032	30	6.61
$C_{13}H_{21}Cl_7$	n.a.	n.a.	7.14
$C_{17}H_{32}Cl_4$	$4.0\ 10^{-3}$	$2.9\ 10^{-2}$	n.a.
$C_{17}H_{27}Cl_9$	$1.7\ 10^{-5}$	$6.6\ 10^{-1}$	n.a.
$C_{20}H_{38}Cl_4$	$4.5\ 10^{-5}$	n.a.	n.a.
$C_{20}H_{33}Cl_8$	$1.9\ 10^{-7}$	$5.3\ 10^{-3}$	n.a.

log K_{OW}: octanol-water partition coefficient, n.a.: not available
Data from: [a] (Muir *et al.*, 2000), [b] (Drouillard *et al.*, 1998), [c] (Drouillard *et al.*, 1998), [d] (Sijm and Sinnige, 1995)

In comparison to other chlorinated persistent organic pollutants, limited information is available about the toxicity of PCAs. PCAs have low acute toxicity (Farrar, 2000), but liver, thyroid, and kidney carcinomas were also observed (Bucher *et al.*, 1997). Additionally, sPCAs showed chronic toxicity to aquatic biota, whereas mPCAs and lPCAs did not (Thompson, 2000). The manufacture and use of sPCAs have been banned in the European Community, due to their higher bioaccumulation factors (WHO, 1996) and to the higher toxicity. Additionally, sPCAs have been included in the list of substances for priority action of the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2001), in the list of priority dangerous substances of the

European water framework directive (European Community, 2001) and in that of selected substances for immediate priority action of the Helsinki Commission (HELCOM, 2002).

As consequence of their widespread and unrestricted use and of the properties mentioned above, PCAs are present in aquatic and terrestrial food webs in rural and remote areas. Typical PCA concentrations in the environment are summarized in Table 1.3. Due to the very different physical properties of PCA congeners and homologues, partial fractionation of the original technical composition may occur during phase transition and atmospheric transport. Compared to technical PCAs, a higher amount of the more volatile low chlorinates sPCAs has been observed in samples from the Arctic (Tomy *et al.*, 1999; Reth, 2004).

Table 1.3: Overview of sPCA levels in different environmental matrices.

Sample	Sampling location	Sampling date	sPCA concentration	Reference
Air	Spitsbergen, Norway	1999	9-57 pg/m^3	(Borgen *et al.*, 2000)
Air	Egbert, Canada	1990	65-924 pg/m^3	(Stern and Tomy, 2000)
Freshwater biota	different locations, Norway	not specified	108-3700 ng/g fat	(Borgen *et al.*, 2001)
Marine biota	North Sea	2002	19-286 ng/g wet weight	(Reth *et al.*, 2004)
Freshwater particulate matter	different locations, Germany	not specified	69-860 ng/g dry weight	(Maulshagen *et al.*, 2003)
Marine sediments	different locations, Canada	not specified	4.52-135 ng/g dry weight	(Tomy *et al.*, 1999)

1.4.4 State of the art of PCA analysis

Analysis of PCAs in environmental samples is far from being well established. Quantification of PCAs is extremely difficult due to the complex composition of PCA products, and only a small number of laboratories worldwide currently has the necessary expertise (UNECE, 2003). Nevertheless, reliable analytical methods will be needed in the near future, since sPCAs have been included in the regulatory programs of the European Community and environmental levels of PCAs have to be monitored more extensively.

Currently, the standard method for the determination of PCAs is HRGC combined with ECNI-MS after an appropriate sample clean up (Schmid and Müller, 1985). The methodology and required quantification procedure have been described extensively by Tomy *et al.* (1997). The authors stated, that the use of HRMS is necessary to avoid interferences by other polychlorinated compounds and between PCA isotope signals with the same nominal *m/z*. However, it could be demonstrated that LRMS can be used despite some limitations (Reth and Oehme, 2004).

Seven laboratories participated in the only interlaboratory study on sPCA analysis, which has been performed until now (Tomy *et al.*, 1999). This work clearly showed the major limitation of the current methods: Quantification of PCA solutions with a different chlorine content than the quantification standards led to errors of up to 300%. This problem has also been described extensively by Coelhan *et al.* (2000). Additionally, the analysis of PCAs by HRGC-ECNI-MS and the related data processing are very time consuming. Therefore, more accurate and/or screening methods are needed.

2 AIM OF THE WORK

The aim of this work was to develop, adapt, and evaluate new mass spectrometric methods for the analysis of chlordanes and polychlorinated *n*-alkanes employing non-conventional and less used techniques. Methodologies with a potential for an improved mass spectrometric performance should be evaluated and applied, including ion trap, single- and triple-quadrupole mass spectrometers in combination with different ionization techniques. Besides general method development and validation, the following problems and applications should be addressed.

As first task, it should be investigated, if specific ion molecule reactions can be used to differentiate between stereoisomers of chlordane compounds by ion trap PICI using unusual reagent gases.

Another major goal was to explore, if dichloromethane can be used as reagent gas for negative ion chemical ionization of PCAs and if this technique can overcome some of the major problems of the usually applied ECNI. Furthermore, the tendency of PCAs to form chloride adduct ions should be exploited to develop an analytical method using liquid chromatography combined with mass spectrometry.

Another objective was to explore the possibilities of electron ionization combined with tandem mass spectrometry. Since the ECNI method usually employed for PCA analysis and the consequent data processing are very time consuming, the aim was to develop a fast semi-quantitative screening method.

Finally, the comparability of results obtained by different mass spectrometric methods should be investigated and their advantages and limitations determined. Special attention was given to the applicability of the studied techniques to the quantification of PCAs in environmental samples.

3 GENERAL DISCUSSION

3.1 Chemical ionization with non-conventional reagent gases

The most widely used reagent gases for CI are methane, isobutane, and ammonia, although many different reagent gases are theoretically suited for CI. Ionization by proton transfer is the mostly used reaction in CI. The use of reagent gases with lower proton affinities than the analyte will lead to formation of $[M+H]^+$ ions. The three reagent gases mentioned above cover the range of available proton affinities for CI of organic molecules (Table 3.1).

Table 3.1: Gas phase proton affinities (at 25 °C) of some CI reagent gases and comparison with different classes of organic molecules. Data from literature (Harrison, 1992; Oehme, 1998; Hunter and Lias, 1998; De Hoffmann and Stroobant, 1999).

Reagent gas	Proton affinity [kJ/mol]	Compound class	Proton affinity [kJ/mol]
N_2O	580		
CH_4	**546**[a], **684**[b], **736**[c]	alkanes and chloroalkanes	550-650
CH_2Cl_2	585		
$(CH_3)_3CH$	**677**	alkenes	670-840
H_2O	696		
		acids	710-800
		aldehydes	720-810
CH_3OH	761	alcohols	750-815
		aromatic compounds	750-880
CH_3CN	787	esters	780-840
		ethers	790-840
		ketones	820-840
NH_3	**858**	amines	855-950

[a] for $[CH_5]^+$, [b] for $[C_2H_5]^+$, [c] for $[C_3H_5]^+$

However, proton transfer reactions are not the only possibility in CI. Ionization by charge transfer or adduct formation are alternatives, which often provide structure specific information. Additionally, they may allow to differentiate between isomers or stereoisomers. Since a reagent gas pressure of ca. 100-200 Pa is necessary in the reaction volume of conventional ion sources, the boiling point of a reagent gas is a limiting factor. Additionally, some compounds interesting for CI are corrosive and may damage components of the mass spectrometer (e.g. filament).

3.1.1 Ion trap chemical ionization

The ability of ion traps to store ions over prolonged periods can be exploited for CI. Reaction times of 10-200 ms allow a sufficient number of collisions between analyte molecules and reagent gas ions at low partial pressures of reagent gas (ca. $1.3\ 10^{-3}$ Pa). This allows the use of reagent gases with low vapor pressures and requires only small quantities of reagent gas, which is important for studies involving isotope labeled reagents (Moneti *et al.*, 1998). Additionally, the corrosion by aggressive reagent gases such as water does not occur (Landrock *et al.*, 1995).

The chemical ionization process in an ion trap is summarized in Figure 3.1. First, electrons are introduced into the ion trap and ionize the reagent gas by EI. During this time all ions with *m/z* higher than the reagent gas ions are ejected to prevent EI of the analyte. Then, the reagent gas ions are allowed to react with the analyte ions. Finally, the reagent gas ions are ejected from the ion trap and the CI mass spectrum of the analyte is acquired. An advantage of the ion trap is that specific ions can be selected for chemical ionization. In this way, the reactivity of different ions formed by the reagent gas can be evaluated. This allows a better control of charge transfer and proton transfer reactions (Berberich *et al.*, 1989). The use of CI with non-conventional reagent gases for the differentiation of stereoisomers has been reported by Mancel and Sellier (2000). The authors applied acetone and dimethylether PICI to differentiate *cis*- and *trans*-cyclopentanediols, as well as *cis*- and *trans*-cyclohexanediols.

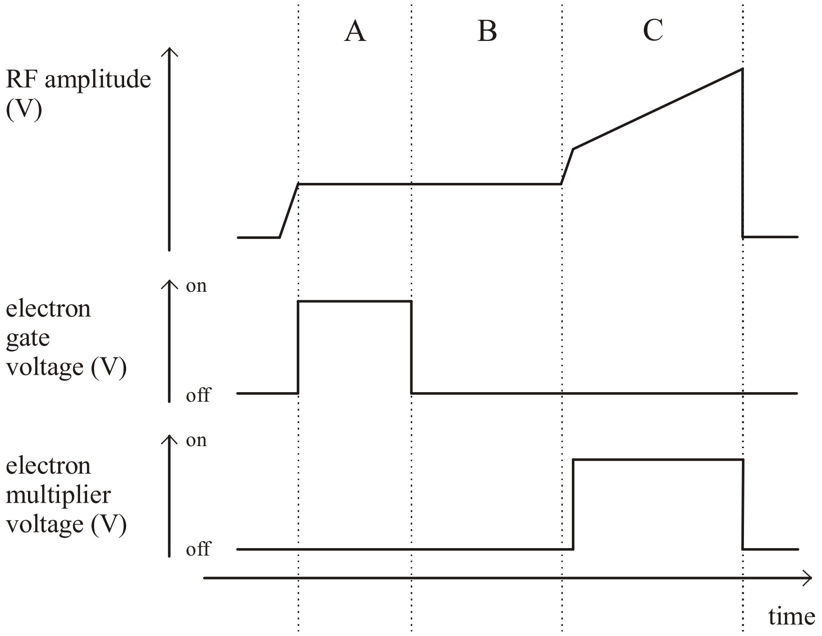


Figure 3.1: Operation of the ion trap in the CI mode. A: Ionization of the reagent gas. B: Reaction between analyte and reagent gas ions. C: Mass scan of the analyte ions.

3.1.1.1 Structure specific gas phase reactions for the analysis of chlordanes

The applicability of acetonitrile, acrylonitrile and dichloromethane as reagent gases for the detection of chlordanes by PICI was evaluated in Paper I. Acetonitrile was chosen since its suitability for stereoselective gas phase reactions has been reported by Mancel and Sellier (2000). In addition, its gas phase chemistry has been extensively studied (Heerma *et al.*, 1986; Wincel, 1998; Oldham, 1999). Besides the protonated molecule ion, acetonitrile forms $[C_3H_4N]^+$, which can react with unsaturated hydrocarbons (Oldham and Svatos, 1999). Acrylonitrile probably forms a corresponding ion (Heerma *et al.*, 1986; Wincel *et al.*, 1990). Dichloromethane was selected due to its low proton affinity.

According to Table 3.1, the proton affinity of *cis-*/*trans*-chlordane and *cis-*/*trans*-nonachlor can be estimated to 550-700 kJ/mol, so that protonation cannot be expected by acetonitrile (787 kJ/mol) and acrylonitrile (estimated proton affinity of ca. 790 kJ/mol). Dichloromethane has a similar proton affinity as chlordanes, so that proton transfer reactions may take place. The PICI mass spectra of *cis*-chlordane obtained using the three reagent gases are shown in Figure 3.2. Neither the protonated molecular ions nor adduct ions were formed by all reagent gases. Only ionization by charge transfer reaction was observed. The only exception was CH_2Cl_2-PICI of heptachlor, where $[M+H]^+$ of very low relative abundance could be detected.

The use of all three reagent gases led to a retro-Diels-Alder fragmentation reaction (see Figure 3 in Paper I), resulting in abundant fragment ions. The only exception was acrylonitrile PICI of heptachlor. Dearth and Hites also observed retro-Diels-Alder fragmentation in the ECNI mass spectra of chlordanes (Dearth and Hites, 1991). In contrast to ECNI, where retro-Diels-Alder fragmentation forms pentachloro-cyclopentadiene anions, the positive charge was stabilized in PICI on the other cyclopentene ring formed by retro-Diels-Alder fragmentation. Moreover, this fragmentation led to different relative abundances for *cis-*/*trans*-stereoisomers, when acetonitrile and dichloromethane PICI were applied. Ovcharenko *et al.* (2001) also reported stereoselectity of the retro-Diels-Alder fragmentation for norbornene derivates using PICI.

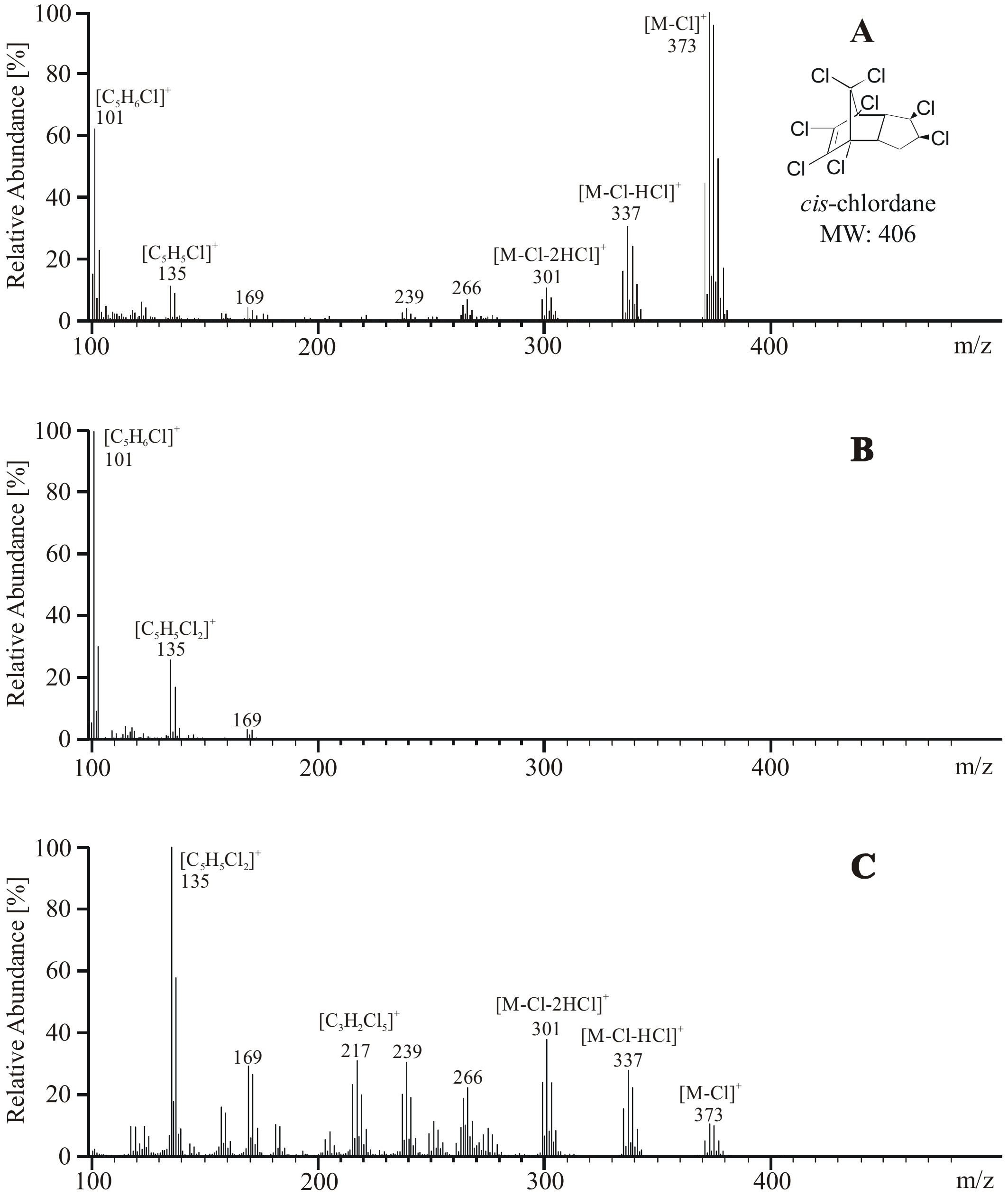


Figure 3.2: Ion trap PICI mass spectra of *cis*-chlordane obtained using acetonitrile (A), acrylonitrile (B) and dichloromethane (C) as reagent gases.

The most interesting feature of ion trap PICI of chlordanes is the stereoselectivity of the fragmentation process. Since the use of ECNI-MS is well established for the analysis of chlordanes the ion trap PICI technique was not further developed for quantification.

3.1.1.2 Ion trap PICI of polychlorinated *n*-alkanes

Ion trap PICI of polychlorinated *n*-alkanes was evaluated using acetonitrile, acrylonitrile, dichloromethane, methanol, and water as reagent gas. The most promising results were achieved by H_2O-PICI, which allowed the ionization of these compounds by a charge transfer reaction. In comparison to EI, the most intensive fragment ions were formed by loss of chlorine or by elimination of HCl and the fragmentation of the carbon skeleton was strongly reduced. Figure 3.3 shows the mass spectra of a sPCA congener obtained by EI and H_2O-PICI.

However, H_2O-PICI of sPCAs showed no linear relationship between sample quantity and signal area as can be seen from Figure 3.4. Since the main aim was to develop a quantification alternative to ECNI, H_2O-PICI was not further investigated and other possibilities were studied (see chapter 3.1.2.1).

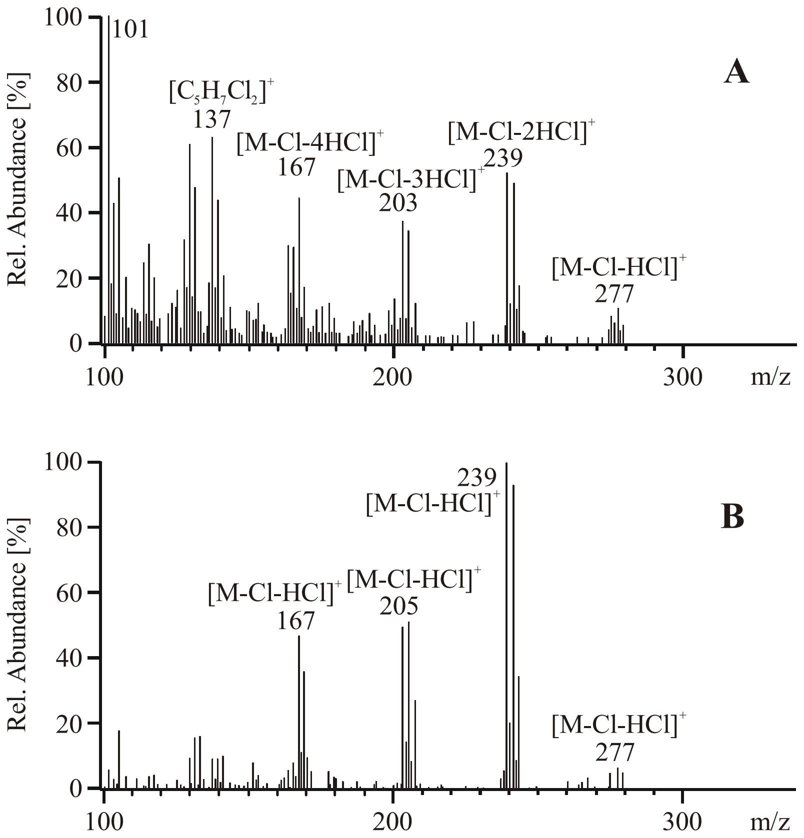


Figure 3.3: Mass spectra of 1,2,5,6,9,10-hexachlorodecane obtained by ion trap EI (A) and H_2O-PICI (B). The scan range was *m/z* 100-400.

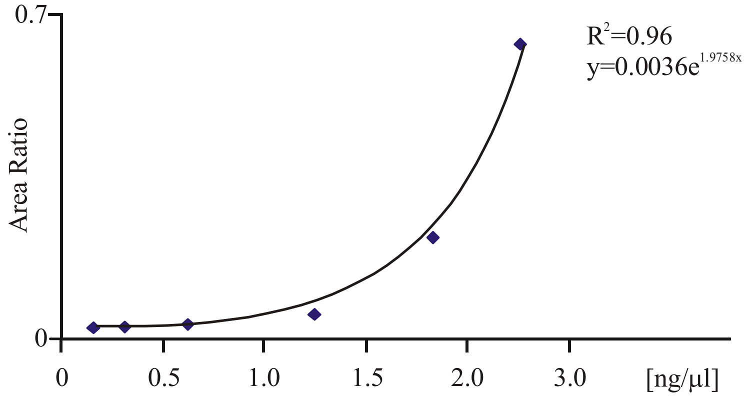


Figure 3.4: Exponential relationship between amount and relative signal area of 1,2,5,6,9,10-hexachlorodecane obtained by ion trap H_2O-PICI. Tetrachloronaphthalene was used as internal standard. $[M\text{-}Cl\text{-}2HCl]^+$ and $[M+H]^+$ were selected for 1,2,5,6,9,10-hexachlorodecane and for tetrachloronaphthalene, respectively.

3.1.2 Non-conventional reagent gases for CI in conventional ion sources

CI with other reagent gases than methane, isobutane and ammonia has been also performed in conventional ion sources. Mixtures of different gases have also been applied. The superoxide reagent ion O_2^- formed by O_2/N_2 mixtures has been used for the ionization of alcohols and polycyclic aromatic compounds (Hunt *et al.*, 1975). However, the use of oxygen in higher concentrations is limited due to significantly shortened filament lifetime. Hunt *et al.* avoided this using a discharge ionization source (Hunt *et al.*, 1975). Another possible reagent is the hydroxide ion, which can be formed by a N_2O/CH_4 mixture (Budde, 2001). It has been successfully applied for the ionization of alcohols (Bruins, 1979), polycyclic aromatic compounds (Oehme, 1983), and polychlorinated dibenzo-*p*-dioxins (Oehme and Kirschmer, 1984).

Reagents, which are liquid at room temperature, were also successfully applied for CI in conventional ion sources. One example is the use of dichloromethane for NICI detection of aliphatic and aromatic carboxylic acids, amides, amines and phenols by chloride adduct formation (Tannenbaum *et al.*, 1975). Stemmler and Hites (1985) applied a CH_4/CH_2Cl_2 mixture for NICI of chlordanes and reported chloride attachment for only a few compounds. Both research groups did not specify in their publications how dichloromethane was introduced into the ion source. Dougherty (1981) recommended the use of a reagent gas mixture of 93% methane (or isobutane), 5% dichloromethane and 2% oxygen as reagent gas for chloride attachment NICI without giving any indication about its preparation.

3.1.2.1 CH_4/CH_2Cl_2-NICI: An alternative to ECNI of polychlorinated *n*-alkanes

The tendency of some sPCA congeners to form chloride adduct ions under ECNI conditions has been previously reported (Tomy *et al.*, 1998). The authors observed chloride adducts at relative abundances of 25% or less, depending on the temperature of the ion source, the injected amount of analyte and the sPCA congener studied. In this work, ECNI formed exclusively chloride adducts of tetrachlorodecanes. For other congeners the relative abundance of $[M+Cl]^-$ decreased with increasing degree of chlorination (see Paper II). The mass spectrometer was modified to allow the use of CH_4/CH_2Cl_2 mixtures (Figure 3.5). Further details are given in Paper II.

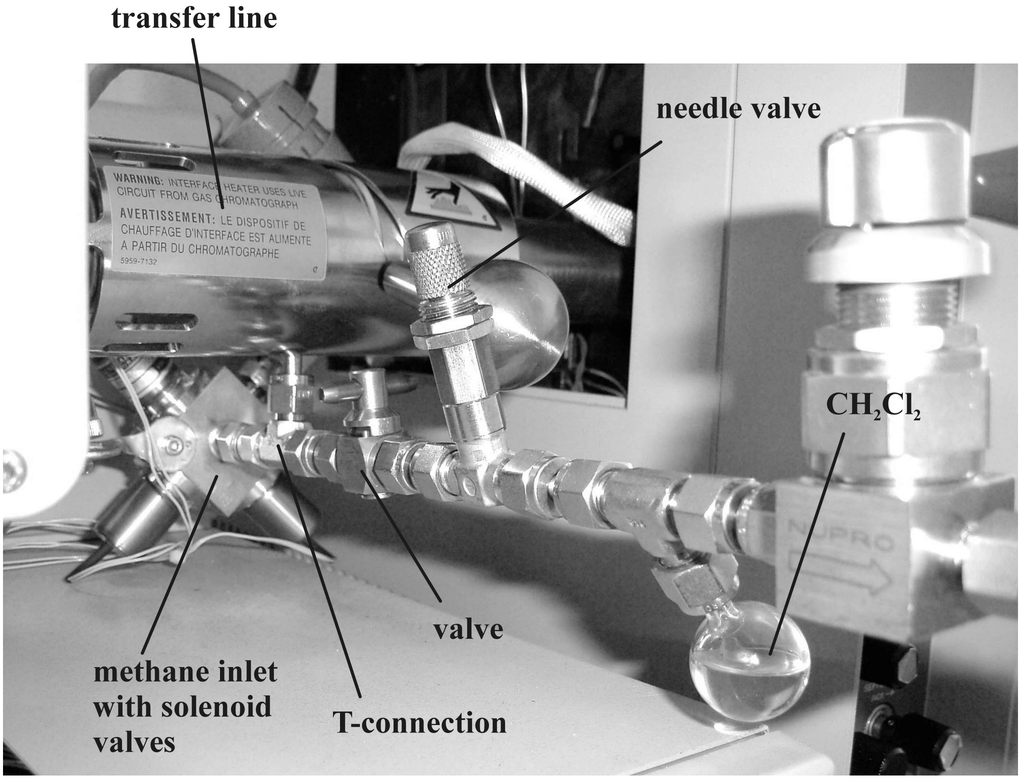


Figure 3.5: Modified transfer line of a HP 5989B mass spectrometer for the use of CH_4/CH_2Cl_2 reagent gas mixtures (further details are given in Paper II).

The addition of CH_2Cl_2 to the reagent gas favored the almost exclusive formation of $[M+Cl]^-$ ions. The optimal composition of the reagent gas mixture was 80% CH_4 and 20% CH_2Cl_2 (percentages referred to the ion source pressure) and was similar to that applied by Stemmler and Hites (1985). Dougherty (1981) summarized the reactions which take place as follows:

$$\begin{array}{lcl} CH_2Cl_2 + e_s^- & \rightarrow & CH_2Cl^{\cdot} + Cl^- \\ CH_2Cl_2 + Cl^- & \rightarrow & [CH_2Cl_3]^- \\ M + Cl^- & \rightarrow & [M+Cl]^- \end{array}$$

Besides these reactions, the formation of $[HCl_2]^-$ and of $[2CH_2Cl_2+Cl]^-$ was also observed (see Figure 2 in Paper II). The possible geometries of chloride adducts of methylchloride in the gas phase have been studied by Glukhovtsev *et al.* (1995) and are summarized in Figure 3.6.

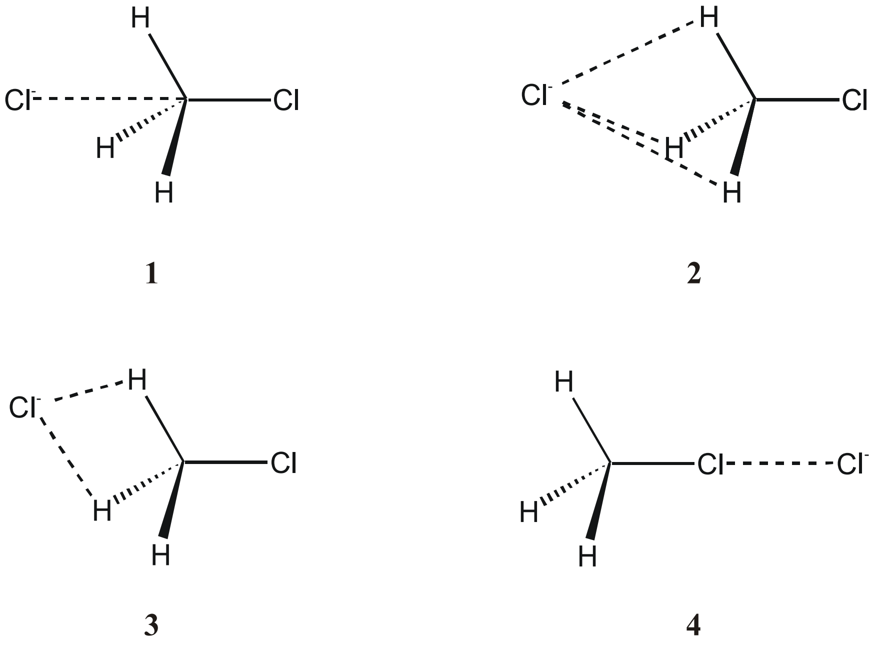


Figure 3.6: Possible structures of chloride-chloromethane complexes (Glukhovtsev *et al.*, 1995).

Originally, chloride attachment to a carbon atom (structure **1**) was proposed as mechanism for the formation of $[M+Cl]^-$ (Tannenbaum *et al.*, 1975; Dougherty, 1981). However, later studies showed that this structure is less stable than the hydrogen bonded structures **2** and **3** (Cremer and Kraka, 1986). This seems to be confirmed by the tendency of lower chlorinated *n*-alkanes to form higher quantities of $[M+Cl]^-$ ions when ECNI is applied (Paper II). Tomy *et al.* (1998) suggested that PCAs are able to assume a chelate conformation with the chloride anion as shown in Figure 3.7.

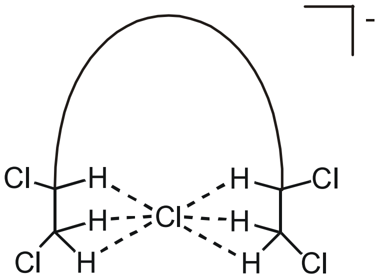


Figure 3.7: Chelate conformation of PCAs with a chloride anion as proposed by Tomy *et al.* (1998).

Electron capture reactions can also take place between the analyte and the low energy secondary electrons present in the reagent gas plasma (Dougherty, 1981). This was also observed in this work. However, the ionization yield of the ECNI process was strongly reduced by the CH_2Cl_2. Therefore, interferences of other polychlorinated compounds such as chlordanes, toxaphenes, and polychlorinated biphenyls in the PCA quantification were also strongly reduced. This is a considerable advantage, since current sample clean up techniques do not allow a complete separation of PCAs from these compounds (Reth *et al.*, 2004).

3.1.2.2 Analysis of technical PCA mixtures

The developed technique is a real alternative for the analysis of technical sPCA (Paper II), and mPCA mixtures (Paper IV). The use of CH_4/CH_2Cl_2-NICI led to the almost exclusive formation of $[M+Cl]^-$ ions. This reduced the interferences between different PCA congeners compared to CH_4-ECNI forming simultaneously $[M-Cl]^-$, $[M-HCl]^-$ and $[M+Cl]^-$ ions. For example, both $C_{11}H_{18}Cl_6$ and $C_{11}H_{20}Cl_4$ had isotope mass signals of *m/z* 327 in ECNI, $[M-Cl]^-$ for the first congener and $[M+Cl]^-$ for the second one (Reth and Oehme, 2004). Such interferences could be avoided by CH_4/CH_2Cl_2-NICI. The analysis of PCA mixtures by CH_4/CH_2Cl_2-NICI showed considerable differences in the congener and homologue patterns compared to ECNI (see Figure 3.8 and Paper II).

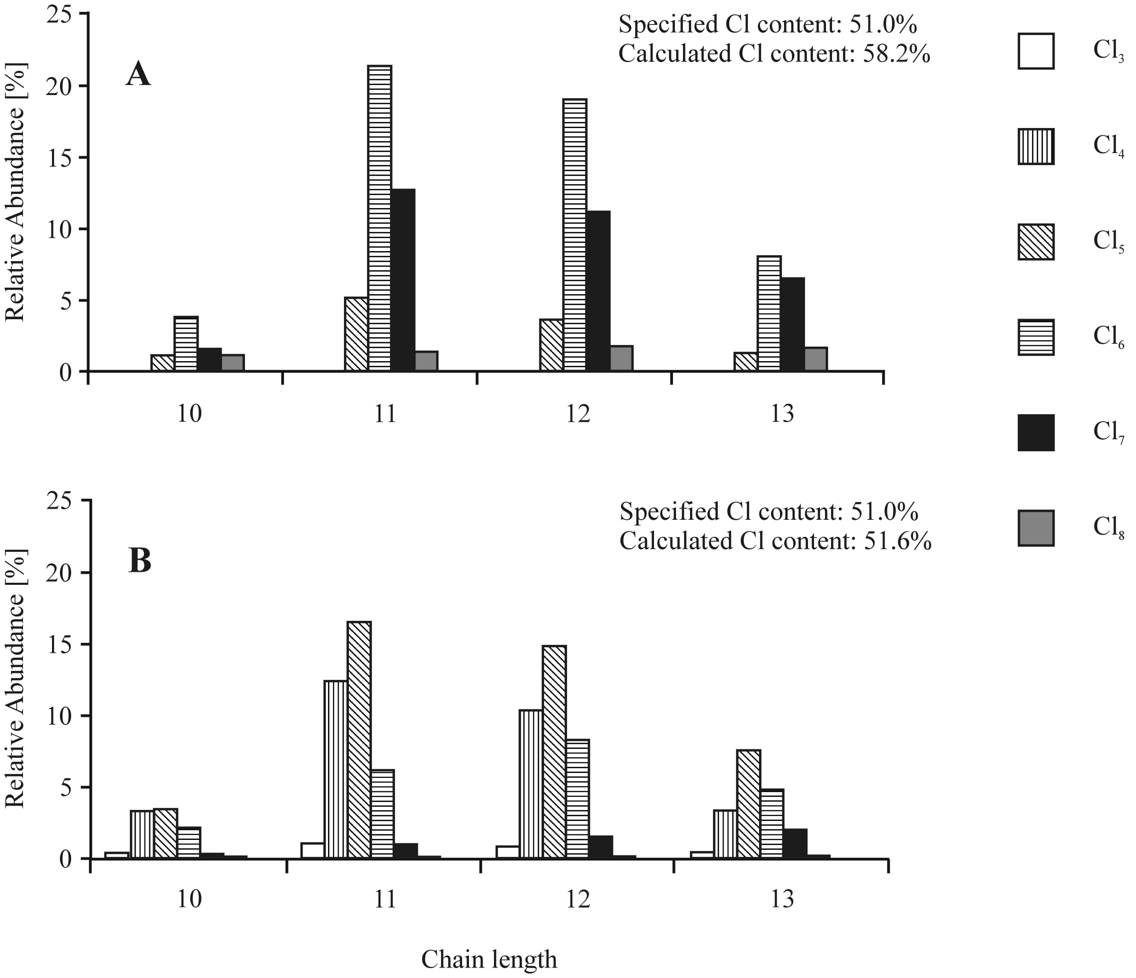


Figure 3.8: Congener and homologue patterns of a technical sPCA mixture with 51.0% Cl content recorded with CH_4-ECNI (A) and CH_4/CH_2Cl_2-NICI (B).

The differences between the two ionization methods were considerable: Cl_5 PCA congeners were the most abundant when CH_4/CH_2Cl_2-NICI was applied and Cl_6 congeners for ECNI. Moreover, CH_4/CH_2Cl_2-NICI enabled the detection of Cl_3 and Cl_4 PCA congeners.

The response factors of PCAs in the ECNI mode depended strongly on the degree of chlorination. Therefore, ECNI results overestimate the relative concentrations of the higher chlorinated congeners (see also chapter 3.3.2 and Paper IV). The response factors of PCAs in the CH_4/CH_2Cl_2-NICI mode were less dependant on the degree of chlorination. The calculated degree of chlorination of the technical mixture confirmed this. ECNI led to an overestimation, whereas the agreement was good between the value specified by the manufacturer and that obtained by CH_4/CH_2Cl_2-NICI (Figure 3.8 and Table 2 in Paper II). This has considerable consequences for the quantification of PCAs using technical mixtures as standard (see chapter 3.3.2).

3.1.2.3 Analysis of environmental samples

The applicability of CH_4/CH_2Cl_2-NICI to the analysis of sPCAs in fish livers was demonstrated in Paper II. The major advantage of this technique was the suppression of the ionization of other polychlorinated compounds, not completely separable from PCAs by sample clean up. sPCA concentrations of 213 and 619 ng/g (wet weight) were determined by CH_4/CH_2Cl_2-NICI in two pooled liver samples of North Sea dab (*Limanda limanda*, capture location: North Sea, 54° 15.64’ N, 7° 29.79’ E). These concentrations were comparable to those reported in the literature (100-1700 ng/g wet weight) and determined mainly by ECNI-HRMS (Borgen *et al.*, 2001). Figure 3.9 shows the congener and homologue patterns determined in the fish livers.

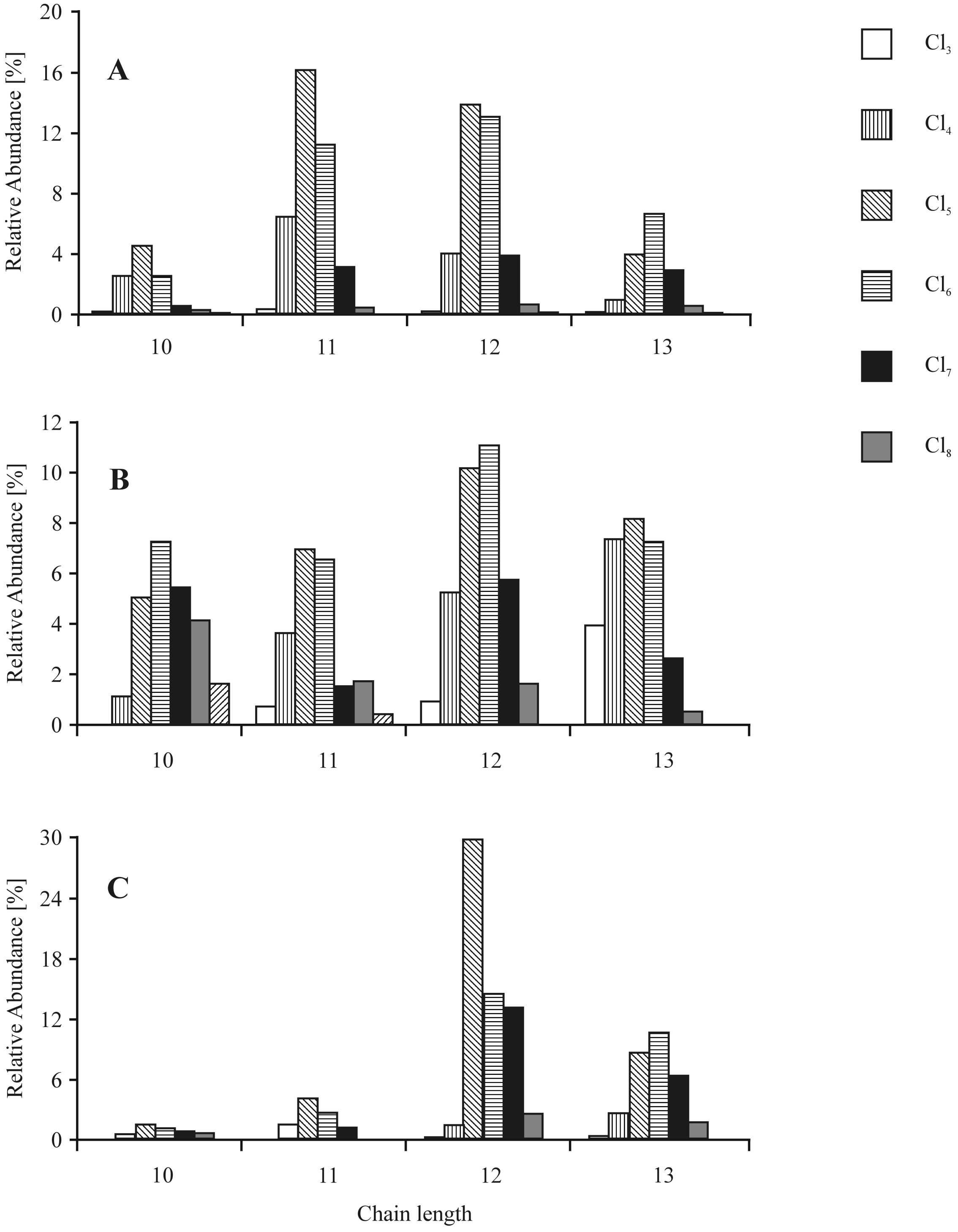


Figure 3.9: PCA congener and homologue patterns obtained by CH_4/CH_2Cl_2-NICI of a technical sPCA mixture with 55.5% Cl (A), and of two extracts of North Sea dab livers (B,C).

The congener and homologue distribution was different between the two fish liver samples although they were from the same species captured at the same location. Similar differences were also observed by Reth *et al.* (2004) using ECNI. Additionally, sPCA and mPCA concentrations in fish liver samples from the Baltic Sea were determined and the results compared with those obtained by other analytical methods (Table 3 in Paper IV).

CH_4/CH_2Cl_2-NICI was also applied to the analysis of sPCAs and mPCAs in two sediments from the Baltic Sea. Here, the determination of PCAs by conventional ECNI was not possible, since the final sample extracts contained considerable amounts of other interfering halogenated compounds. The suppression of the ionization of these compounds by CH_4/CH_2Cl_2-NICI allowed the quantification of sPCAs and mPCAs. sPCA concentrations were 48 and 98 ng/g (dry weight) and mPCA concentrations 199 and 91 ng/g (dry weight) (Hüttig, 2004).

3.1.3 Atmospheric pressure chemical ionization

High performance liquid chromatography (HPLC) coupled to atmospheric pressure chemical ionization mass spectrometry is often applied to the analysis of semipolar to polar compounds (Willoughby *et al.*, 1998). In APCI the ionization of the analyte molecules is mostly achieved by proton transfer reaction with solvent molecules ionized by a corona discharge. Nonpolar compounds such as PCAs usually have low proton affinity and low gas phase acidity. Therefore, the formation of $[M+H]^+$ or $[M-H]^-$ is not favored and such compounds are rarely analyzed by LC-MS. However, all reactions observed in CI such as proton transfer, charge transfer reactions, electron capture, and adduct formation take also place in APCI (De Hoffmann and Stroobant, 1999). In this work, the formation of chloride adduct ions by APCI was particularly studied. Zhao and Yinon (2002) reported the use of chloride adduct formation for the detection of nitrate ester explosives. The authors simply added ammonium chloride to the mobile phase to enhance the formation of chloride adducts. Evans *et al.* (2002) used chloride enhanced APCI (Cl^--APCI) to develop a screening method for explosives. The authors introduced dichloromethane into the APCI source by a supplementary gas feed passing air through dichloromethane.

3.1.3.1 Chloride enhanced APCI-MS for the analysis of polychlorinated *n*-alkanes

CH_4/CH_2Cl_2-NICI of PCAs let expect that $[M+Cl]^-$ ions of PCAs can also be formed by APCI if a chloride source is provided. Therefore, $CHCl_3$ was used as mobile phase for normal phase liquid chromatography of PCAs (Paper V). $[CHCl_3+Cl]^-$, Cl^-, and $[H_2O+Cl]^-$ ions were formed from the mobile phase (see mass spectrum in Figure 3.10). In contrast to CH_4/CH_2Cl_2-NICI, $[HCl_2]^-$ ions were not observed and dimers of the reagent had a relative abundance <5%.

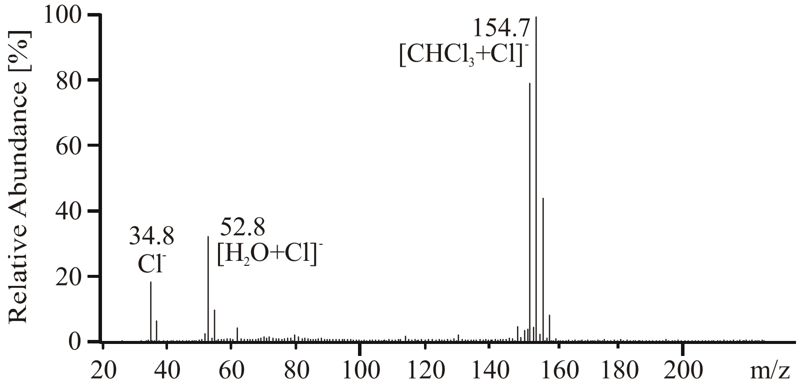


Figure 3.10: APCI negative ion mass spectrum of $CHCl_3$.

Cl^--APCI ionized PCAs forming exclusively $[M+Cl]^-$ ions. The application of this ionization technique to the analysis of technical mixtures gave well reproducible and comparable results (see Figure 2 in Paper V). The response factors of three sPCA mixtures with degrees of chlorination of 51, 55.5, and 63% varied by a factor <3, which was considerably less than for HRGC-ECNI-MS (factor of 9). The variation of the response factors of different PCA mixtures will be discussed more extensively in chapter 3.3.2. The use of high performance liquid chromatography combined with Cl^--APCI-MS is of particular interest for the analysis of lPCA mixtures, which may be difficult by gas chromatography due to their low volatility. Figure 3.11 shows the mass spectrum of a lPCA mixture with a degree of chlorination of 36%.

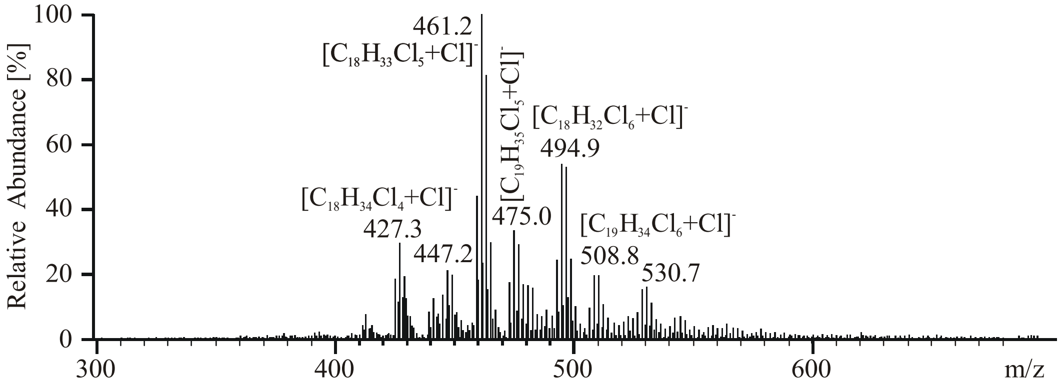


Figure 3.11: Cl^--APCI negative ion mass spectrum of a lPCA mixture with 36% Cl content. The main congeners are indicated.

3.1.3.2 Analysis of household commodities.

The developed method was applied to the detection of PCAs in household commodities (Paper V). mPCAs were detected in two paint samples at concentrations around 10%. The results were in good agreement with the results obtained by HRGC-EI-MS/MS. Furthermore, they were in the range of typical concentrations of additives in paints, which is 2-20% (Bolliger and Randegger-Vollrath, 2003; Ineos Chlor, 2003). Compared to HRGC-ECNI-MS and to HRGC-CH_4/CH_2Cl_2-NICI-MS, the use of HPLC-Cl^--APCI-MS allowed to reduce the analysis time to ca. 10 minutes per sample. This is only comparable to HRGC-EI-MS/MS, which does not provide any information about the congener and homologue composition (see chapter 3.2).

Due to the encouraging results obtained for the analysis of household commodities, the applicability of this method to environmental samples should be investigated in the future. However, the concomitant presence of short, medium, and long chained PCAs can be expected. Therefore, the interpretation of the PCA mass spectra, which is an important step in the quantification method, could result difficult or even impossible. Figure 3.12 compares the mass spectrum of mPCAs and of a mixture of short, medium, and long chained PCAs. As can be seen the isotope patterns are not easily recognized due to the presence of different congeners forming ions with similar *m/z*.

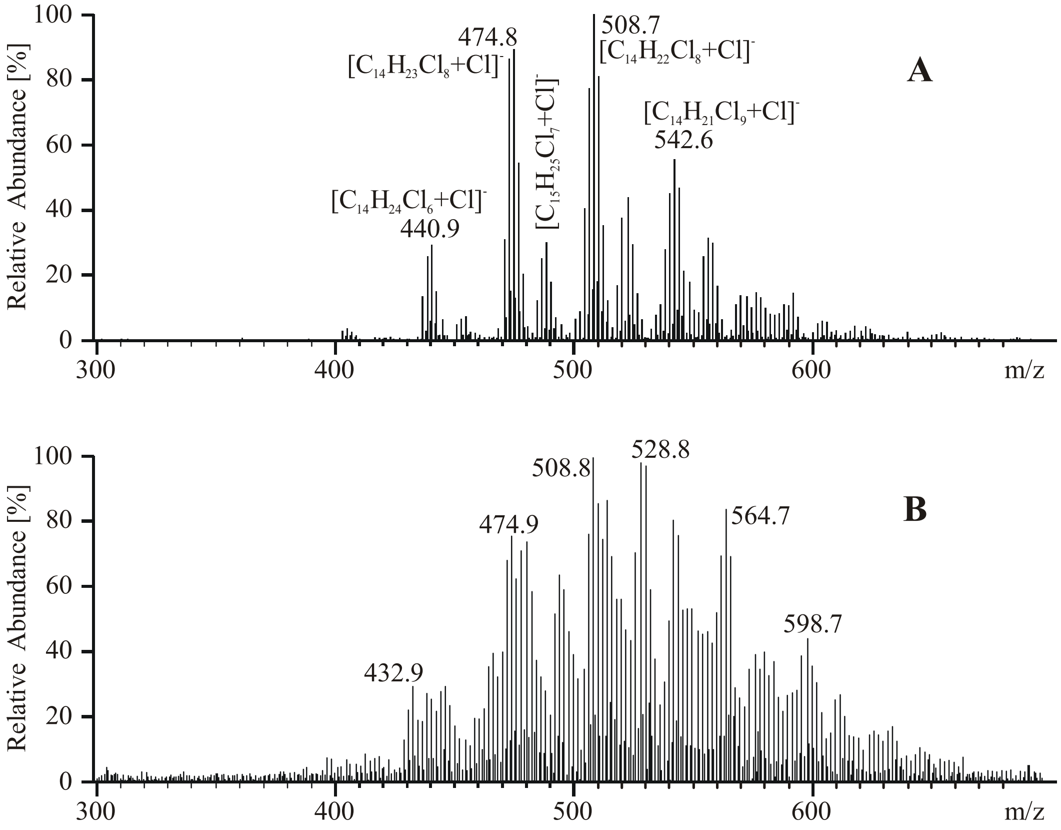


Figure 3.12: Cl^--APCI negative ion mass spectrum of mPCAs with a Cl content of 57% (A) and of a 1+1+1 mixture (v/v) of sPCAs of 63% Cl content, mPCAs of 57% Cl content, and lPCAs of 59% Cl content (B). The main congeners are indicated.

This problem would be avoided if a separation method able to separate PCAs according to their chain lengths could be developed. Coelhan (2003) reported the preparative separation of sPCA and mPCA mixtures using gelpermeation chromatography. Therefore, analytical gelpermeation chromatography was applied instead of normal phase chromatography. Two columns (300 mm length and 7.8 mm i.d.) connected in series and filled with Plgel (5 µm particle size and 50 Å pore size; Polymer Laboratories, UK) were employed. Tetrahydrofurane and chloroform were used as mobile phase at flow rates between 0.4 and 0.8 ml/min. However, a separation of the PCA congeners, which are critical when low resolution mass spectrometry is applied (e.g. $C_{11}H_{16}Cl_8$ and $C_{16}H_{28}Cl_6$), could not be achieved.

3.2 Electron ionization combined with tandem mass spectrometry

Electron ionization of PCAs leads to a strong fragmentation and to the formation of unspecific ions and was considered not suitable for the detection of PCAs in environmental samples (Gjøs and Gustavsen, 1982). However, Junk and Meisch (1993) reported a method based on the use of EI for the detection of PCAs. The authors observed that all PCAs form $[C_5H_{10}Cl]^+$ at *m/z* 105 and applied this for the detection of PCAs in paving stones from a metal-working industrial plant, which were known to be highly contaminated with cutting fluids. The PCA concentration determined in this sample was 582 mg/kg. Noteworthy was the small discrimination effect for different degrees of chlorination observed by the authors. Despite these encouraging results the method was never applied for the detection of low concentrations of PCAs in more complex matrices.

3.2.1 Electron ionization of PCAs

EI mass spectra of PCAs are characterized by the absence of the molecular ion and by low abundances of fragment ions with high masses (>250 u). Figure 3.13 shows the ion trap EI mass spectra of a technical PCA mixture and of a single PCA congener.

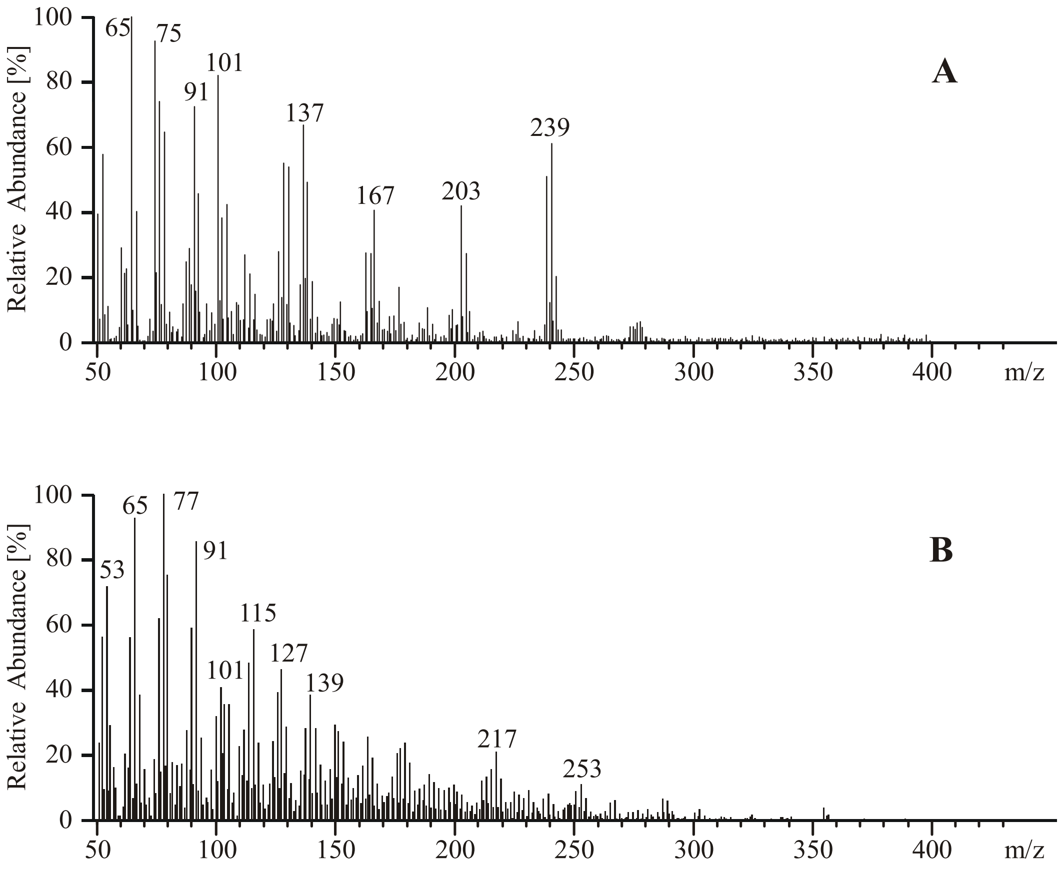


Figure 3.13: Ion trap EI mass spectra of 1,2,5,6,9,10-hexachlorodecane (A) and of a technical sPCA mixture with a chlorination degree of 63% (B). Scan range *m/z* 50-400.

The fragmentation of C_{10}-PCA congeners under EI conditions was extensively studied by Castells *et al.* (2004) applying ion trap MS^n. The authors reported two main fragmentation pathways. The first consisted of the successive losses of HCl and $Cl^\bullet$ from the molecular ion. In the second, a bond cleavage between the two central carbon atoms of the molecular ion was observed. Successive dehydrochlorination of the C_5-cations formed then $[C_5H_8Cl]^+$ and $[C_5H_6Cl]^+$. $[C_5H_{10}Cl]^+$ ions previously used for the determination of PCAs by Junk and Meisch (1993) were observed in PCA mixtures with chlorine contents lower than 30%.

In this work EI was also applied for the analysis of PCAs (Paper III). Similarly to the work of Junk and Meisch (1993) several fragment ions were observed in the mass spectra of all technical mixtures (see Table 1 in Paper III). Furthermore, the extracted mass chromatograms of these fragment ions showed signals which were very similar to the signal obtained by full scan acquisition (see Figure 2 in Paper III). Therefore, it was concluded that some of the fragment ions formed by EI are common to most PCAs and may be used for PCA determination. In Paper III an elemental composition was proposed for each of these fragment ions (Table 1 in Paper III). It was later verified by exact mass determination applying high resolution mass spectrometry. A MAT95Q double focusing electromagnetic mass spectrometer (Finnigan MAT, Palo Alto, USA) was used. The experimental conditions were as follows: Direct insertion of a technical sPCA mixture (Hordalub 500, degree of chlorination of 62%, Höchst AG, Germany), probe heated from 40 °C to 260 °C at 40 °C/min, filament emission current 0.4 mA, accelerating voltage 5 kV, and resolution 10’000. The results are summarized in Table 3.2. As can be seen, the proposed elemental compositions could be confirmed for all investigated fragment ions and the deviation from the theoretical value was around 5 ppm or less.

Table 3.2: Determination of the exact mass of the EI fragment ions common to most PCAs. Uncertainties of the measured *m/z* (standard deviation, n=10) are given.

Proposed chemical composition	Theoretical *m/z*	Measured *m/z*	Δppm	Substance and ion used for lock mass
$[C_4H_3]^+$	51.02348	51.02325 ± 0.00011	-4.4	CH_2Cl_2, $[CH_2Cl]^+$
$[C_4H_5]^+$	53.03913	53.03895 ± 0.00013	-3.3	perfluorokerosene, $[CF_2H]^+$
$[C_5H_5]^+$	65.03913	65.03926 ± 0.00009	2.1	PCAs, $[C_5H_7]^+$
$[C_5H_7]^+$	67.05478	67.05515 ± 0.00010	5.6	perfluorokerosene, $[CF_3]^+$
$[C_6H_5]^+$	77.03913	77.03926 ± 0.00012	1.7	pyridine, $[C_5H_5N]^+$
$[C_6H_7]^+$	79.05478	79.05519 ± 0.00015	5.2	PCAs, $[C_6H_9]^+$
$[C_6H_9]^+$	81.07043	81.07097 ± 0.00010	6.7	CH_2Cl_2, $[CF_2H]^+$
$[C_7H_7]^+$	91.05478	91.05523 ± 0.00020	5.0	perfluorokerosene, $[C_3F_3]^+$
$[C_5H_5Cl]^+$	102.02363	102.02405 ± 0.00083	4.1	perfluorokerosene, $[C_2F_4]^+$

3.2.2 Use of tandem mass spectrometry

EI combined with tandem mass spectrometry has been successfully applied for the analysis of polychlorinated compounds such as toxaphenes in environmental samples (Skopp *et al.*, 2002). In many cases, the use of tandem mass spectrometry allowed to improve both sensitivity and selectivity (Buser and Müller, 1994; Skopp *et al.*, 2002). Despite these considerable advantages, EI-MS/MS has been applied for the analysis of such compounds only occasionally compared to EI-MS and ECNI-MS.

The fragment ions common to all PCAs had rather low mass-to-charge ratios and were not very specific for PCAs, so that interferences from other sample components were possible eluting within the retention time range of PCAs. Therefore, ion trap and triple quadrupole EI-MS/MS methods were developed (Paper III). Despite the increase in selectivity, only two ion trap fragmentation reactions (respectively, three for the triple quadrupole mass spectrometer) proved to be suited for a quantitative determination of PCAs. A detailed comparison between the performance of both instruments is given in Paper III. In general, the use of the triple quadrupole resulted in a higher sensitivity so that this instrument was preferred for further analysis of environmental samples (Hüttig *et al.*, 2004; Reth, 2004). Therefore, ion trap EI-MS/MS was not included in the comparison of different mass spectrometric methods for PCA analysis, which is presented in Paper IV. Since the applied fragmentation reactions were common to all PCAs, this method allowed the simultaneous detection of short, medium, and long chained PCAs giving the total PCA concentration in the sample. Due to the short analytical time the method was very suited for screening. However, a congener and homologue specific analysis is not possible.

3.2.3 Analysis of environmental samples

Fish livers from the North and Baltic Sea were analyzed by EI-MS/MS to demonstrate the applicability of the new method (Paper III). Further fish liver samples from the Baltic Sea were investigated by EI-MS/MS and the results compared to those obtained by ECNI-LRMS, ECNI-HRMS and CH_4/CH_2Cl_2-NICI-LRMS (see Table 4 in Paper IV). Total PCA concentrations in samples from the North and Baltic Sea were in the range of 50-600 ng/g (wet weight). EI-MS/MS was also applied to the analysis of cod liver samples from the European Arctic (Iceland and Norway). Total PCA concentrations of 50-100 ng/g (wet weight) were found (Reth, 2004). Furthermore, EI-MS/MS was applied to the analysis of sediments collected in the North and Baltic Sea. For two samples the results were compared to those obtained by CH_4/CH_2Cl_2-NICI-LRMS (Hüttig, 2004). In the first sample, a total PCA concentration of 262 ng/g (dry weight) was found by EI-MS/MS and the result obtained by CH_4/CH_2Cl_2-NICI-LRMS was 297 ng/g (dry weight). The PCA concentration in the second samples obtained by the two methods was 116 and 139 ng/g (dry weight), respectively. These results showed a good agreement between the two methods. Due to its rapidity, EI-MS/MS is currently used as screening method for the determination of PCAs. Homologue and congener specific analysis is then performed by ECNI-MS for selected samples.

3.3 Comparison of the different mass spectrometric methods for PCA analysis

A detailed comparison of the performance and suitability of different mass spectrometric methods for the determination of PCAs in environmental samples is presented in Paper IV. ECNI-LRMS, ECNI-HRMS, CH_4/CH_2Cl_2-NICI-LRMS, and EI-MS/MS were compared analyzing technical PCA mixtures, spiked fish samples as well as fish liver samples. Cl^--APCI-MS was not included in this comparison, since its suitability for the analysis of environmental samples has not been proven yet. All methods were suited for the analysis of PCA solutions and of spiked samples as long as the same PCA mixture was used as quantification standard (Table 1 and 2 in Paper IV). Additionally, no considerable differences between the results obtained by ECNI-HRMS and ECNI-LRMS were observed, when interferences between different PCAs in the LRMS mode were reduced as described by Reth and Oehme (2004). However, the analysis of fish samples showed higher deviations between the results obtained by the four methods (Table 3 in Paper IV). A possible explanation is that the composition of the PCAs in the quantification standard did not match well the composition of the PCAs in the sample. Especially for ECNI, the choice of the PCA standard used for quantification has a strong influence on the results (see also Table 4 in Paper IV). In the following chapters the different mass spectrometric methods used in this work are further compared with special emphasis on quantification.

3.3.1 Features, advantages, and drawbacks of the different methods

The advantages and limitations of the five mass spectrometric methods for PCA analysis are summarized in Table 3.3. ECNI is normally employed for PCA analysis. However, its main drawback is the already discussed influence of the degree of chlorination of the PCA standard used for quantification, if quantification is performed according to Tomy *et al.* (1997). Recently, an alternative approach has been reported by Reth *et al.* (2004). Here, a linear correlation was found between the response factor of a PCA mixture and its degree of chlorination. This allowed to calculate the response factor of the PCAs in the sample and to correct the difference between the degree of chlorination of PCAs in the sample and in the quantification standard.

The use of the CH_4/CH_2Cl_2 reagent gas mixture for NICI of PCAs allowed to overcome the main limitation of ECNI: The response factors of congeners with a different degree of chlorination showed a lower variation than for ECNI (Paper II and chapter 3.3.2.1). Consequently, the degree of chlorination of the PCA standard did not significantly influence the quantification (Table 4 in Paper IV). Finally, the use of this reagent gas mixture allowed the detection of lower chlorinated PCAs and suppressed the ionization of other polychlorinated compounds. This enhanced selectivity and allowed to achieve a sensitivity similar to ECNI. The main drawback of this method is a rapid contamination of the ion source by a black residue. Cleaning of the instrument was necessary after ca. 48 hours of operation. This makes this method not suitable for routine analysis. A mixture of argon and dichloromethane was also applied to try to minimize this problem. Comparable results could be achieved setting the ion source pressure at 60 Pa (40 Pa Ar + 20 Pa CH_2Cl_2), all other instrument parameters were as described in Paper II. However, a similar

Table 3.3: Advantages and drawbacks of five mass spectrometric methods applied for PCA analysis.

Method	Advantages	Drawbacks
HRGC-ECNI-LRMS	provides congener and homologue specific information available at many laboratories	quantification dependent on the selected PCA standard [a] no detection of lower chlorinated PCAs higher risk of interferences between different PCA congeners and from other polychlorinated compounds[b]
HRGC-ECNI-HRMS	provides congener and homologue specific information no interferences between different PCA congeners and from other polychlorinated compounds	quantification dependent on the selected PCA standard [a] no detection of lower chlorinated PCAs expensive and not available at many laboratories
HRGC-CH_4/CH_2Cl_2-NICI-LRMS	detection of lower chlorinated PCAs response factors of different congeners are not dependent on their degree of chlorination, thus quantification is not influenced by the selected PCA standard suppression of ionization of other polychlorinated compounds enhances selectivity for PCAs	instrument modifications are necessary not suited for routine analysis due to limited instrument robustness
HRGC-EI-MS/MS	very short analysis time (ca. 10 min/sample) quantification is not dependent on the selected PCA standard suited as screening method high selectivity and sensitivity	no congener and homologue specific information no possibility to distinguish between sPCAs, mPCAs, and lPCAs
HPLC-Cl^--APCI-MS	very short analytical time (ca. 10 min/sample) provides congener and homologue specific information suppression of ionization of other polychlorinated compounds	suitability for the analysis of environmental samples not yet proven no chromatographic separation of different PCA congeners and homologues and risk of interferences between congeners with same nominal mass

[a] if quantification is performed according to Tomy *et al.* (1997), but can be eliminated with the quantification method proposed by Reth *et al.* (2004)

[b] can be partly avoided by a careful sample clean up and applying the procedure described by Reth and Oehme (2004)

black residue was formed in the ion source after ca. 48 hours of operation. Therefore, the use of Ar/CH_2Cl_2 mixtures was not further investigated.

EI-MS/MS has demonstrated its suitability for the determination of PCAs in environmental samples as well (Table 4 in Paper IV). The response factors of different sPCA and mPCA mixtures did not vary by more than 20% (Paper II), and quantification was not dependent on the choice of the quantification standard. The speed of analysis makes EI-MS/MS very suited for screening purposes. However, congener and homologue specific information cannot be provided by this method and further analysis by ECNI-MS or CH_4/CH_2Cl_2-NICI-MS may be necessary.

Finally, the use of HPLC-Cl^--APCI-MS also allowed to analyze PCAs. One of the advantages of the use of liquid chromatography is the low risk of discrimination of less volatile PCAs. Additionally, the variation of the response factors of different PCA congeners was lower than under ECNI conditions. However, no chromatographic separation of PCA congener groups could be achieved, and it was not possible to distinguish congeners which form $[M+Cl]^-$ ions with the same nominal *m/z*. The applicability for the analysis of environmental samples has still to be proven.

3.3.2 Response factors and quantification

As already discussed the quantification of PCAs by ECNI is strongly dependent on the selected PCA standard. This can be explained by different response factors of different congeners. Congeners with a higher degree of chlorination have higher response factors. Consequently, a bias of >100% can be observed, if PCAs are quantified using a PCA standard of different degree of chlorination (Table 4 in Paper IV). This can be partly avoided by a quantification standard with a composition as similar as possible to the PCAs in the sample. However, this time-consuming solution is not satisfactory for routine analysis. In the following two chapters the response factors of different PCA mixtures are compared for the different mass spectrometric methods.

3.3.2.1 Influence of the degree of chlorination

The relative response factors of C_{10}-, and C_{11}-PCAs with different degrees of chlorination are shown in Figure 3.14. As observed by Reth (2004), the response factors obtained for ECNI-MS measurements strongly increased with increasing degree of chlorination. The consequences for quantitative analysis of PCAs are obvious: if the PCAs in the sample have a higher chlorination degree than the standard, their concentration will be overestimated, and in the opposite case their concentration will be underestimated. Additionally, the relative concentration of higher chlorinated congeners will be systematically too high in the congener patterns (see also Figure 3.8 in chapter 3.1.2.2).

On contrary, almost no variation of the response factors was observed, when CH_4/CH_2Cl_2-NICI was applied. Similarly to CH_4/CH_2Cl_2-NICI, the use of EI-MS/MS showed a low variation of the response factors. Identical results were obtained for all three fragmentation reactions. This makes CH_4/CH_2Cl_2-NICI and EI-MS/MS very interesting for quantitation. Finally, the response factors obtained by Cl^--APCI showed a considerable variation. They increased quite linearly with increasing degree of chlorination. This is surprising, since the formation of chloride adducts is not depending on the chlorine contents of the PCAs when CH_4/CH_2Cl_2-NICI is applied. No explanation can be given for this phenomenon so far.

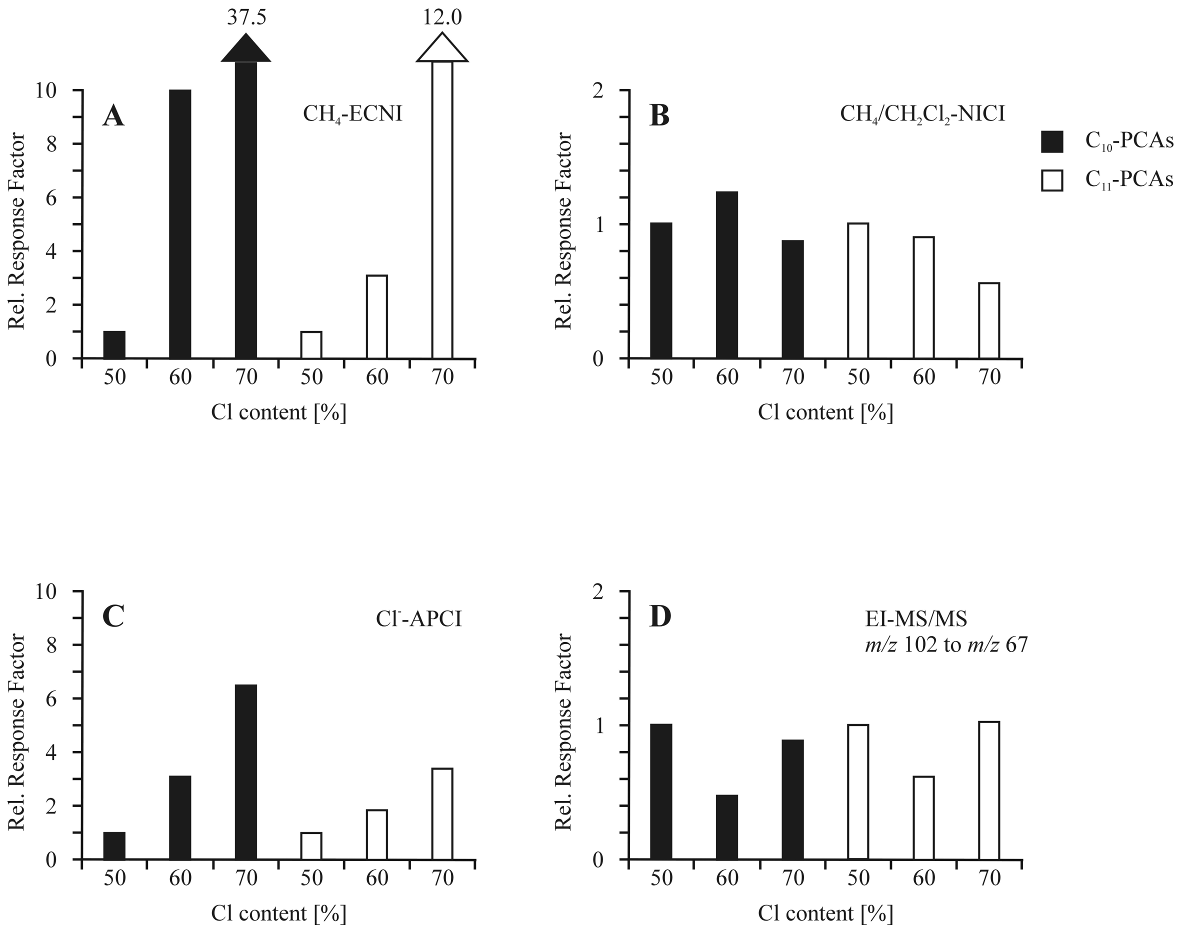


Figure 3.14: Relative response factors of C_{10}- and C_{11}-PCAs (normalized to the mixtures with a Cl content of 50%). Four different ionization techniques are compared: CH_4-ECNI (A), CH_4/CH_2Cl_2-NICI (B), Cl^--APCI (C), and EI-MS/MS with the fragmentation *m/z* 102 to 67 (D). Response factors were calculated on mass basis using the sum of the signals of all congeners. ECNI data obtained from Reth (2004).

3.3.2.2 Influence of the carbon chain length

The relative response factors of different sPCA homologues with the same Cl content but with different chain lengths (normalized to the C_{10}-mixtures) are shown in Figure 3.15. The response factors obtained for ECNI-MS showed relatively little variation for PCAs with a degree of chlorination of 50 and 60%. EI-MS/MS and CH_4/CH_2Cl_2-NICI let to similar response factors for all studied mixtures and all three fragmentation reactions. Finally, the response factors obtained by Cl^--APCI increased with increasing chain length of the studied PCAs, which again was not the case when CH_4/CH_2Cl_2-NICI was applied.

In general, the response factors determined for HRGC-MS methods showed a quite low variation, when the chain length of the studied PCAs was varied. However, only a small range of chain lengths could be investigated and discrimination of long chained PCAs cannot be excluded.

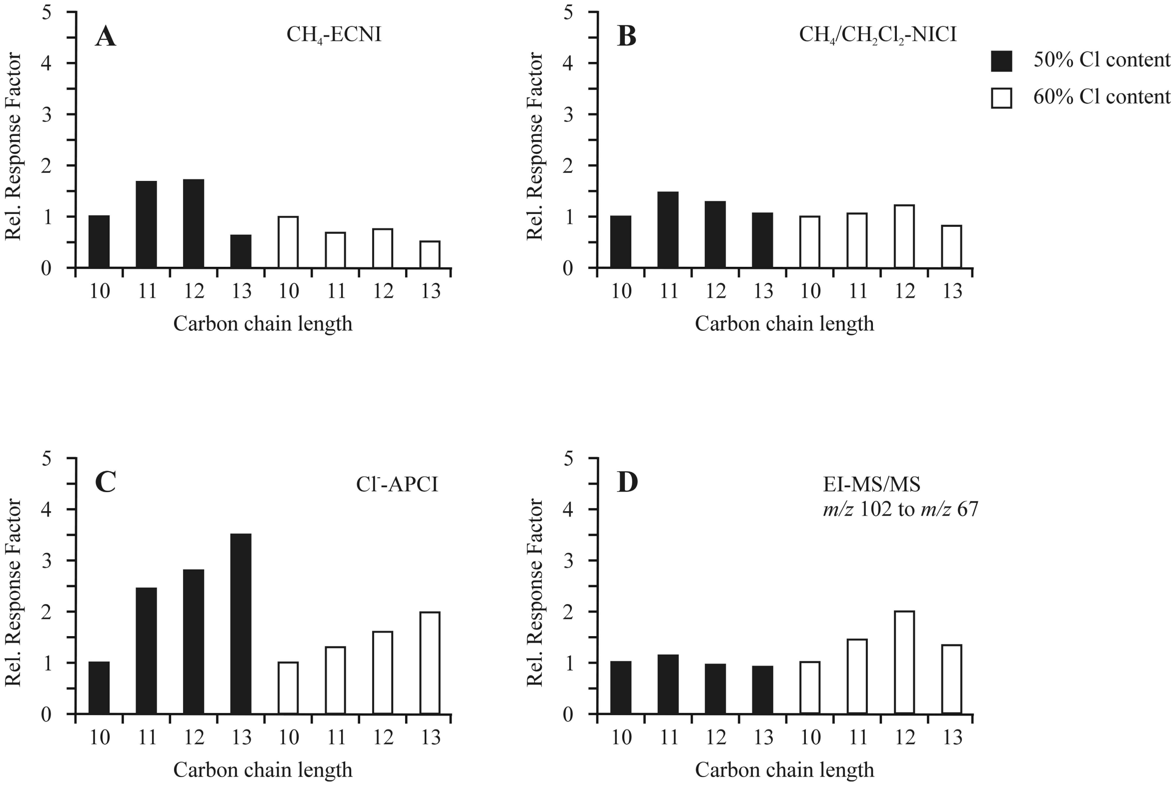


Figure 3.15: Relative response factors of sPCA homologues with a degree of chlorination of 50 and 60% (normalized to the mixtures of C_{10}-homologues). Four different ionization techniques are compared: CH_4-ECNI (A), CH_4/CH_2Cl_2-NICI (B), Cl^--APCI (C), and EI-MS/MS with the fragmentation *m/z* 102 to 67 (D). Response factors were calculated on mass basis using the sum of the signals of all congeners. ECNI data obtained from Reth (2004).

3.3.3 Applicability to the analysis of environmental samples

The development of new mass spectrometric methods for the analysis of PCAs in environmental samples was one of the main aims of the present work. The suitability of ECNI-LRMS, EI-MS/MS, and CH_4/CH_2Cl_2-LRMS could be demonstrated. The first two techniques are also suitable for routine analysis, whereas the latter requires frequent instrument maintenance. Cl^--APCI was not applied for the analysis of environmental samples yet. Preliminary studies on the ionization of other polychlorinated compounds such as toxaphenes, chlordanes, polychlorinated biphenyls, and hexachlorocyclohexanes have shown that these compounds are not ionized by this technique. This would provide a very high selectivity for PCAs and make this method an interesting option for the analysis of environmental samples, if sufficient sensitivity can be achieved.

4 CONCLUSIONS AND OUTLOOK

Alternatives to the usually applied conventional mass spectrometric methods were developed and investigated for the analysis of chlordanes and polychlorinated *n*-alkanes. The possibilities of the use of non-conventional reagent gases for ion trap chemical ionization were demonstrated for chlordanes as test compounds. The differentiation of *cis-/trans*-stereoisomers by mass spectrometry was achieved. The potentialities of the use of non-conventional reagent gases and the possible stereoselectivity of the ionization process should be studied with more interest in the future.

Three new mass spectrometric methods were developed for the analysis of PCAs. Although limited attention has been paid to the analysis of these compounds during the last decades, the environmental relevance of PCAs was finally recognized and sPCAs have been recently included in the regulatory program of the European community. Consequently, these substances should be monitored by European countries. The methods developed in this work are useful analytical tools using different technical approaches and are capable to allow the very difficult determination of PCAs in the environment.

Moreover, the systematic comparison between the conventionally applied ECNI-MS and the newly developed techniques allowed to identify several gaps in the analysis of PCAs. The difficulty of quantification using PCA mixtures as standards is probably the most important one. If higher attention is not paid to this problem in the future, the comparability of the data reported by different groups will be questionable or even impossible.

Finally, additional data about toxicology, environmental distribution, and fate of PCAs are still needed. Reliable analytical methods will play a key role in this research. The coupling of the mass spectrometric methods presented in this work with a separation method of better capabilities than HRGC may open new possibilities. The emerging technique of comprehensive two dimensional gas chromatography has a substantial potential to separate at least congener groups.

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Paper I:

DETECTION OF CHLORDANES BY POSITIVE ION CHEMICAL IONIZATION IN AN ION TRAP: A COMPARITIVE STUDY OF THE NON-CONVENTIONAL REAGENTS ACETONITRILE, ACRYLONITRILE AND DICHLOROMETHANE

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RCM

Detection of chlordanes by positive ion chemical ionization in an ion trap: a comparitive study of the non-conventional reagents acetonitrile, acrylonitrile and dichloromethane

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The detection of some chlordane compounds (heptachlor, cis-/trans-chlordane and cis-/trans-nonachlor) by positive ion chemical ionization (PICI) in an ion trap was studied using acetonitrile, acrylonitrile and dichloromethane as non-conventional reagent gases. These reagent gases initiated specific fragmentation reactions and resulted in different response factors. All reagent gases enabled detection limits in the low-pg range for heptachlor, whereas the detection limits of cis-/trans-chlordane and cis-/trans-nonachlor were in the mid-pg range. Additionally, the acetonitrile and dichloromethane PICI mass spectra of the cis- and trans-stereoisomers of chlordane and nonachlor were different. Copyright © 2001 John Wiley & Sons, Ltd.

Compared to electron ionization (EI), positive ion chemical ionization (PICI) mass spectrometry (MS) is a more powerful tool for structure identification.[1] It often allows differentiation among isomers.[2,3] In addition, selectivity and/or sensitivity can be increased for selected compound classes by using reagent gases with different proton affinities and ion-molecule formation properties. However, a conventional CI ion source does not allow choice of corrosive reagent gases. Furthermore, a relatively high pressure of reagent gas is required to achieve a good ionization yield. Therefore, only compounds with a boiling point only slightly above room temperature (or lower) can be used.

Ion trap mass spectrometry enables the trapping of ions over relatively long periods (ms), thus increasing tremendously the possibility of collisions between analyte molecules and reagent gas ions. This allows one to obtain PICI conditions with only ppm concentrations of reagent gas, so that reagents with a rather low vapour pressure can be applied.[4,5] The successful use of such so-called non-conventional reagent gases has been reported in the literature for acetonitrile,[2,6] dimethyl ether,[3] acetone,[7] water[8] and (*S*)-2-pyrrolydinemethanol.[3] Structure-specific reactions were observed, allowing discrimination among structural isomers[2,9] or diastereomers.[3]

Chlordane compounds were selected as model structures for this work for the following reasons. Chlordane is a technical insecticide that consists of a complex mixture of various structurally related compounds, predominantly hexa- to decachlorinated cyclodienes and isomers. It was mainly used as a soil and non-food plant insecticide. The use of chlordane in the United States was limited by the US Environmental Protection Agency in 1978 and 1987.[10] Chlordane is toxic and considered to be a possible human carcinogen.[10] It shows a strong synergistic effect on other artificial estrogens and/or endocrine disrupters.[11] Several components of technical chlordane and their metabolites are persistent environmental contaminants which strongly accumulate in the food chain. Figure 1 shows the structures of the most abundant components of technical chlordane that were selected for this study. Since concentrations at the lowest trophic levels are often in the pg range, sensitive analytical methods are required allowing both quantification and/or unequivocal structure identification. Quantification is usually performed by high-resolution gas chromatography (HRGC) combined with electron capture ion detection or negative ion chemical ionization mass spectrometry (NICI-MS).[12,13]

To our best knowledge, ion trap PICI has not been applied before for chlordane detection in the trace (ng) or ultra-trace range (pg). In this work, the potential of PICI with non-conventional reagent gases for structure-selective chlordane determination in the trace range was evaluated.

EXPERIMENTAL

Chemicals and solvents

cis-Chlordane (crystalline, 99.4%), trans-chlordane (crystalline, 99.4%), cis-nonachlor (crystalline, 98.0%), trans-nonachlor (crystalline, 99.3%) and heptachlor (crystalline, 99.7%) were purchased from Promochem (Wesel, Germany). A standard solution was prepared in cyclohexane (pesticide

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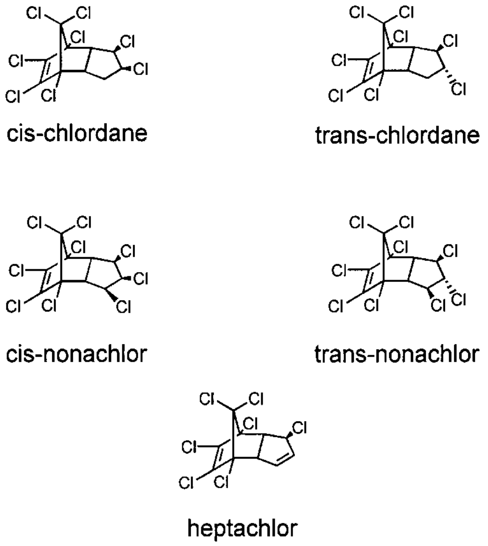

Figure 1. Structures of chlordane compounds selected for this study.

analysis quality; Scharlau, Barcelona, Spain). The concentrations of the chlordane standard were as follows: 45.6 ng/µL cis-chlordane, 52.0 ng/µL trans-chlordane, 35.7 ng/µL cis-nonachlor, 45.7 ng/µL trans-nonachlor and 227 ng/µL heptachlor. Acetonitrile (>99.9%; Romil, Cambridge, GB), acrylonitrile (>99.5%; Fluka, Buochs, Switzerland) and dichloromethane (99.8%; Scharlau, Barcelona, Spain) were used as reagent gases for the PICI experiments.

Instrumentation

The chromatographic separation was performed using a Star 3400 CX (Varian, Walnut Creek, USA) gas chromatograph equipped with a split/splitless injector. A fused silica capillary column (30 m long, 0.25 mm i.d.) coated with a 0.2 µm thick film of DB-5MS (J&W Scientific, USA) was used. 1 µL of the standard solution was injected in the splitless mode. The injector temperature was 200 °C and the splitless time was set to 2 min. Helium (99.999%; Carbagas, Basel, Switzerland) was used as carrier gas at a pressure of 68.9 kPa (10 psi). The temperature program was: 100 °C isothermal for 2 min, then 10 °C/min to 260 °C, and isothermal until the last compound eluted.

A Saturn 2000 (Varian, Walnut Creek, USA) was used as ion trap MS detector. It was tuned regularly with perfluorotributylamine using the fragment ions at *m/z* 69, 131, 264, 414, 464 and 502. For all experiments the transfer line temperature was set to 270 °C, the manifold temperature to 40 °C and the ion trap temperature to 220 °C. The EI mass spectra were acquired with a filament emission current of 10 µA (at 70 eV electron energy). PICI mass spectra were recorded using the automatic gain control with a target total ion current (TIC) of 25 000 counts and a pre-scan ionization time of 100 µs. The instrument was operated in the selected ejection chemical ionization mode. PICI ion trap parameters used for the different reagent gases are summarized in Table 1. The reagent gases were introduced into the ion trap through the standard inlet and their pressure regulated so that an ionization time of 100 µs produced an ion current of ca. 12 000 counts at a filament current of 10 µA. If this target value could not be reached by adjusting the pressure, the filament emission current was changed.

RESULTS AND DISCUSSION

Acetonitrile PICI-MS

The mass spectrum of the reagent acetonitrile is shown in Fig. 2. Besides the presence of $[M - H]^+$ and $[M + H]^+$ ions at *m/z* 40 and 42, respectively, adducts at *m/z* 54 and 59 were also visible corresponding to the elemental composition $[C_3H_4N]^+$ and $[C_2H_5NO]^{+\cdot}$. The formation and reactivity of the ions at *m/z* 54 have already been described by Wincel *et al.*[14,15] as well as its ability to form adducts at the double bond of unsaturated hydrocarbons leading to bond cleavage.[1,9] The ion at *m/z* 59 is probably generated by reaction in the ion trap with residual oxygen or water.

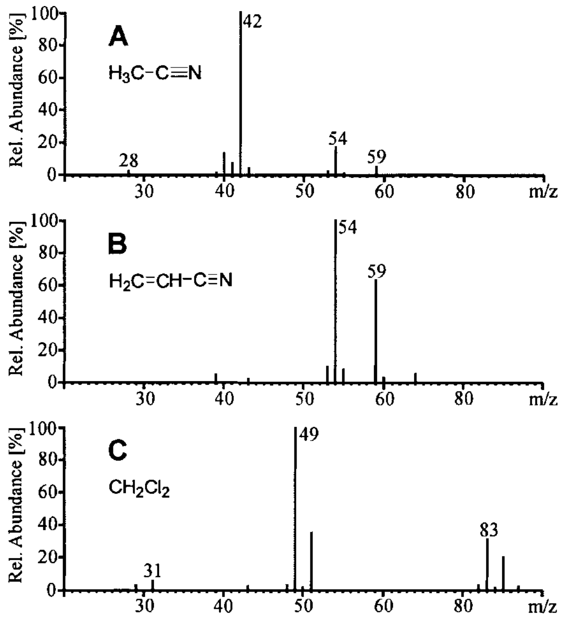

Figure 2. Mass spectra of the reagent gases (A) acetonitrile, (B) acrylonitrile, and (C) dichloromethane used for the PICI experiments.

Table 1. Ion trap parameters for the applied reagent gases

Reagent gas	CI storage level (*m/z*)	Ejection amplitude (V)	Background mass (*m/z*)	Max. ionization time (ms)	Max. reaction time (ms)	Emission current (µA)
Acetonitrile	25	20	65	2	40	20
Dichloromethane	15	12.5	120	2	40	20
Acrylonitrile	25	20	80	2	40	5

Table 2. Relative abundances and standard deviations (n = 5) of the main ions present in the PICI mass spectra of heptachlor, cis-/trans-chlordane and cis-/trans-nonachlor obtained with different reagent gases

Compound	Isomer	Reagent gas	$[C_5H_5Cl]^+$ ($m/z = 100$)	$[C_5H_3Cl_5]^+$ ($m/z = 236$)	$[M\text{-}2Cl\text{-}HCl]^+$ ($m/z = 264$)	$[M\text{-}Cl\text{-}HCl]^+$ ($m/z = 299$)	$[M\text{-}Cl]^+$ ($m/z = 335$)	$[M]^+$ ($m/z = 370$)
Heptachlor		CH_3CN	84 ± 6	5.1 ± 1.2	10 ± 1	7.1 ± 1.3	100	1.1 ± 0.4
		CH_2Cl_2	n.m.	n.d.	16 ± 1	21 ± 1	100	n.d.
		C_2H_4CN	2.3 ± 1.1	n.d.	8.8 ± 0.9	4.2 ± 1.2	100	n.d.

Compound	Isomer	Reagent gas	$[C_5H_6Cl]^+$ ($m/z = 101$)	$[C_5H_5Cl_2]^+$ ($m/z = 135$)	$[C_2H_3Cl_4]^+$ ($m/z = 169$)	$[C_3H_2Cl_5]^+$ ($m/z = 215$)	$[C_7H_2Cl_5]^+$ ($m/z = 261$)	$[M\text{-}Cl\text{-}2HCl]^+$ ($m/z = 299$)	$[M\text{-}Cl\text{-}HCl]^+$ ($m/z = 335$)	$[M\text{-}Cl]^+$ ($m/z = 371$)
Chlordane	cis	CH3CN	60 ± 4	12 ± 2	2.0 ± 0.2	n.d.	2.1 ± 0.8	10 ± 1	29 ± 2	100
	trans		49 ± 5	10 ± 1	4.2 ± 1.1	n.d.	9.0 ± 1.1	10 ± 1	21 ± 2	100
	cis	CH_2Cl_2	n.m.	100	30 ± 1	34 ± 1	10 ± 2	43 ± 4	30 ± 4	11 ± 2
	trans		n.m.	100	21 ± 1	26 ± 1	56 ± 5	47 ± 6	31 ± 4	13 ± 2
	cis	C_2H_4CN	100	18 ± 7	2.2 ± 0.8	n.d.	n.d.	n.d.	n.d.	n.d.
	trans		100	15 ± 3	2.1 ± 0.4	n.d.	n.d.	n.d.	n.d.	n.d.

Compound	Isomer	Reagent gas	$[C_5H_5Cl]^+$ ($m/z = 100$)	$[C_5H_5Cl_2]^+$ ($m/z = 135$)	$[C_3H_2Cl_3]^+$ ($m/z = 143$)	$[C_5H_4Cl_3]^+$ ($m/z = 169$)	$[C_7H_2Cl_5]^+$ ($m/z = 261$)	$[M\text{-}2Cl\text{-}2HCl]^+$ ($m/z = 298$)	$[M\text{-}Cl\text{-}2HCl]^+$ ($m/z = 333$)	$[M\text{-}Cl\text{-}HCl]^+$ ($m/z = 369$)	$[M\text{-}Cl]^+$ ($m/z = 405$)
Nonachlor	cis	CH_3CN	14 ± 4	73 ± 9	5.8 ± 0.5	18 ± 2	1.3 ± 0.2	4.2 ± 1.1	14 ± 1	14 ± 2	100
	trans		16 ± 5	46 ± 9	6.2 ± 1.1	9.2 ± 1.3	4.9 ± 1.0	4.3 ± 1.3	3.8 ± 0.8	7.1 ± 1.2	100
	cis	CH_2Cl_2	n.m.	100	7.4 ± 0.5	22 ± 1	1.2 ± 0.3	3.8 ± 0.9	4.1 ± 0.8	10 ± 1	9.2 ± 0.2
	trans		n.m.	100	16 ± 1	21 ± 1	8.1 ± 1.4	8.2 ± 0.8	7.9 ± 0.7	12 ± 1	20 ± 1
	cis	C_2H_4CN	n.d.	100	n.d.	3.7 ± 2.2	n.d.	n.d.	n.d.	n.d.	n.d.
	trans		n.d.	100	n.d.	4.1 ± 1.9	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected (<1%).
n.m.: not measured.

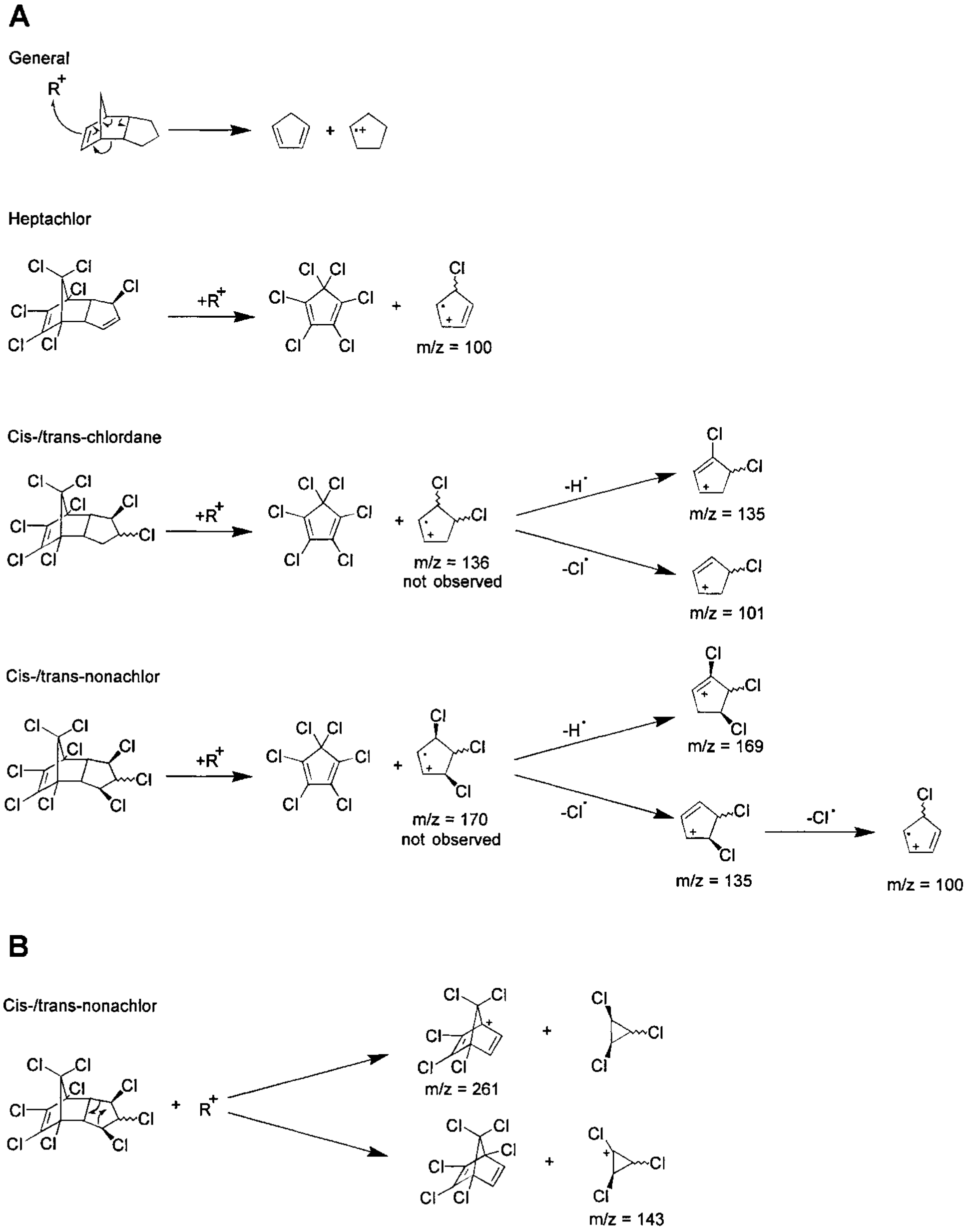

Figure 3. (A) 'Retro-Diels-Alder' fragmentation mechanisms for heptachlor, cis- and trans-chlordane and cis- and trans-nonachlor (R^+: reagent gas ion) and (B) fragmentation of the chlorinated five ring leading to the *m/z* 261 ion mainly observed in the mass spectra of the trans-stereoisomers.

The relative abundances of the main ions present in the acetonitrile PICI mass spectra of heptachlor, chlordane and nonachlor are summarized in Table 2. In contrast to methane NICI,[16] no molecular ions were present in the acetonitrile PICI mass spectra of cis-/trans-chlordane and cis-/trans-nonachor. In all cases, $[M - Cl]^+$ was the base peak ion. Other fragments resulted from further HCl eliminations or loss of chlorine atoms, but their relative abundances were lower than 20%. In addition a retro-Diels-Alder fragmentation was observed, as previously reported by Dearth and Hites[16] for methane NICI. The proposed reaction mechanism is shown in Fig. 3. In contrast to methane NICI, the charge was not stabilized on the cyclopentadiene moiety but on the cyclopentene fragment, generating mono- to trichlorocyclopentene cations depending on the original number of chlorine atoms present in the five-membered ring. Additional loss of $H^{\cdot}$ or $Cl^{\cdot}$ led to ions at *m/z* 135 and 101 for cis-/trans-chlordane, and to ions at *m/z* 169, 135 and 100 for cis-/trans-nonachlor. Since the residence time in the ion trap is relatively long (ca. 25–30 ms), it is not possible to evaluate whether a labile radical ion intermediate is formed (ions at *m/z* 136 for cis-/trans-chlordane and *m/z* 170 for cis-/trans-nonachlor), or whether the retro-Diels-Alder reaction plus the loss of the hydrogen or chlorine radical form a one-step process. Furthermore, it is interesting to note that heptachlor and cis-/trans-nonachlor formed a radical ion at *m/z* 100, whereas cis-/trans-chlordane did not.

A cyclic fragmentation reaction of the partially chlorinated

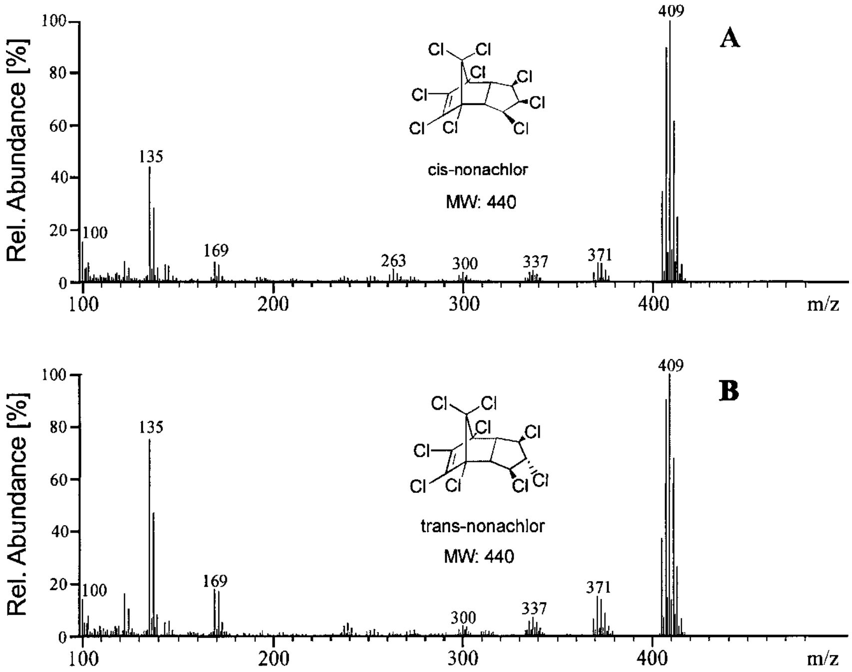

Figure 4. Acetonitrile PICI mass spectra of (A) cis- and (B) trans-nonachlor.

five-membered ring could also be observed, leading to a fragment at *m/z* 261 for cis-/trans-chlordane and cis-/trans-nonachlor and to a trichlorocyclopropene cation (*m/z* 143) only for cis-/trans-nonachlor (see proposed reaction mechanism in Fig. 3). The relative abundance of the species at *m/z* 261 was five times higher for the trans-compounds, which indicates that the stereochemical configuration of the chlorine atoms plays an important role in this fragmentation mechanism.

Since the retro-Diels-Alder reaction described above involves the trans/cis region of the studied chlordanes, the PICI mass spectra of cis- and trans-chlordane should be different. The relative abundances of the fragment at *m/z* 101 for cis- and trans-chlordanes differed by 11% with a precision of 5%, which is not enough for a clear differentiation of the two stereoisomers. However, significantly different intensities for the cis/trans-stereoisomers were observed for the ions at *m/z* 261 and 335. The former ion was hardly visible in the mass spectrum of cis-chlordane while the latter was much more abundant for cis- than for trans-chlordane. This also indicates that HCl elimination from $[M - Cl]^+$ involves the cis/trans region of the molecule.

Similarly, cis- and trans-nonachlor could be distinguished by differences in the relative abundances of the ions at *m/z* 135, 169, 261, 333 and 369. Here again the species at *m/z* 261 was practically absent in the mass spectrum of cis-nonachlor but clearly present in that of trans-nonachlor. The ions formed by the retro-Diels-Alder reaction (*m/z* 135 and 169) had higher relative abundances in the mass spectrum of cis-nonachlor. Moreover, the relative abundances of $[M - Cl - HCl]^+$ (*m/z* 369) and $[M - Cl - 2HCl]^+$ (*m/z* 333) also differed and were more intense for the cis-compound. This confirms the involvement of the stereospecific center in HCl elimination, as mentioned above. The acetonitrile PICI mass spectra of cis- and trans-chlordane are shown in Fig. 4.

Acrylonitrile PICI-MS

The mass spectrum of the reagent acrylonitrile is shown in Fig. 2. Acrylonitrile mainly formed two ions, protonated acrylonitrile molecules at *m/z* 54 and $[C_2H_5NO]^{+\cdot}$ ions (*m/z* 59). Both were also observed in the acetonitrile mass spectra at a relative abundance below 10%. $[C_3H_5N]^+$ ions (*m/z* 54) are known to react with high selectivity with alkenes.[8]

The relative abundances of the main ions present in the acrylonitrile PICI mass spectra of heptachlor, chlordane and nonachlor are summarized in Table 2. The acrylonitrile PICI mass spectrum of heptachlor shows the $[M - Cl]^+$ ion at *m/z* 335 as base peak ion. The ions $[M - Cl - HCl]^+$ at *m/z* 299 and $[M - 2Cl - HCl]^+$ at *m/z* 264 had relative abundances below 10%. The retro-Diels-Alder product at *m/z* 100 showed a very weak intensity of only 2%. The missing retro-Diels-Alder reaction of heptachlor is probably a further indication of the reactivity of the *m/z* 54 ion with double bonds, as this ion probably reacts faster with the non-chlorinated double bond of heptachlor than with the other double bond common to all the chlordanes, leading to the absence of the retro-Diels-Alder products.

In contrast to heptachlor, the acrylonitrile PICI mass spectra of cis-/trans-chlordane and cis-/trans-nonachlor contained only ions formed by the retro-Diels-Alder reaction, and did not allow differentiation between cis/trans-stereoisomers. For cis-/trans-chlordane, the chlorocyclopentene radical ion (*m/z* 101) and the dichloropentene cation (*m/z* 135) were observed. Additionally, a fragment ion with *m/z* 169 corresponding to $[C_5H_4Cl_3]^+$ was detected with a relative abundance of 2%. For cis-/trans-nonachlor, the fragment ions formed by the retro-Diels-Alder reaction were detected at *m/z* 169 (trichlorocyclopentene cation) and *m/z* 135 (dichlorocyclopentene cation). In contrast to acetonitrile PICI, the chlorocyclopentene radical ions (*m/z* 100) had a relative abundance below 1%.

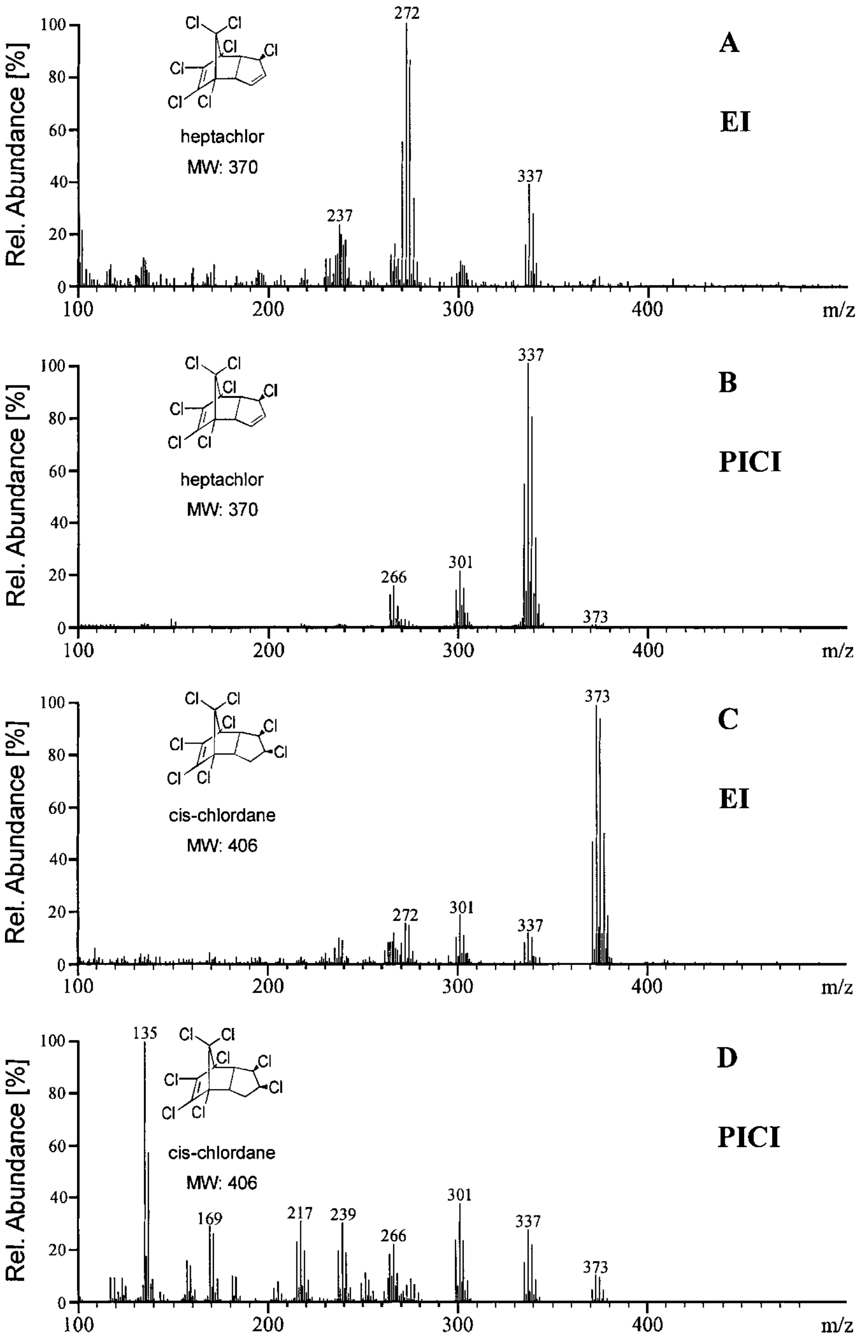

Figure 5. Mass spectra of heptachlor (A, B) and cis-chlordane (C, D) obtained by ion trap MS in the EI and PICI (CH_2Cl_2) modes.

Dichloromethane PICI-MS

The mass spectrum of the dichloromethane reagent gas ions is shown in Fig. 2. Dichloromethane mainly formed *m/z* 83 by hydride abstraction from the dichloromethane molecules, and fragments at *m/z* 49 resulting from the loss of a chloride anion. After longer reaction times (80–100 ms), ions at *m/z* 97 corresponding to $[C_2H_3Cl_2]^+$ were detected, so that the background mass parameter had to be set to *m/z* 120. This means that all ions with *m/z* below 120 were ejected from the ion trap prior to the analytical scan. Consequently, no ions at *m/z* values lower than *m/z* 120 could be detected.

The relative abundances of the main ions present in the dichloromethane PICI mass spectra of heptachlor, chlordane and nonachlor are summarized in Table 2. The retro-Diels-Alder reaction was the dominating fragmentation. For heptachlor its product ion at *m/z* 100 could not be observed due to the mass range limited to *m/z* 120. Therefore, $[M - Cl]^+$ (*m/z* 335) was the base peak ion. The other observed ions at *m/z* 299 and 264 were formed by subsequent HCl elimination and loss of a chlorine radical. Additionally, the protonated molecule, $[M + H]^+$, could be detected with a relative abundance of 1%.

For cis-/trans-chlordane the product of the retro-Diels-Alder reaction at *m/z* 135 was the base peak ion. Additionally, dichloromethane PICI of cis-/trans-chlordane produced several ions formed by loss of chlorine followed by

HCl eliminations, for example, those at *m/z* 371 and 335, or by cleavage of the carbon skeleton. No cis- or trans-chlordane $[M+H]^+$ ions could be detected. Table 2 shows that the two stereoisomers can be distinguished by the relative abundance differences of most of the ions generated by dichloromethane PICI (e.g., *m/z* 157, 169, 215 and 261). Particularly, the ionic species at *m/z* 261 shows a remarkable intensity difference for the two stereoisomers. Dichloromethane PICI mass spectra of cis- and trans-chlordane show more fragmentation than the ion trap (or quadrupole) EI mass spectra, whereas the opposite is the case for heptachlor (see Fig. 5).

cis-/trans-Nonachlor showed less fragmentation by dichloromethane PICI than cis-/trans-chlordane. The most abundant ion at *m/z* 135 was formed by the retro-Diels-Alder reaction as well as the ion at *m/z* 169. The other fragments were mainly generated from the molecular ion by loss of chlorine, followed by several HCl eliminations. The differences in the relative abundances of most ions not formed by the retro-Diels-Alder reaction allowed differentiation between these two stereoisomers.

Comparison of the different PICI reagent gases

As can be seen from Table 2, the nature and intensities of the fragment ions depend on the reagent gas used. Dichloromethane and acetonitrile, but not acrylonitrile, allowed differentiation between cis- and trans-stereoisomers. Furthermore, both acetonitrile and dichloromethane initiated the retro-Diels-Alder reaction plus fragmentation by loss of chlorine and HCl elimination for cis-/trans-chlordane and cis-/trans-nonachlor, whereas acrylonitrile PICI generated only ions formed by the retro-Diels-Alder process.

To estimate the suitability of the studied PICI techniques for trace analysis of chlordanes in environmental samples, limits of detection (LOD) at a signal-to-noise ratio of 3:1 were estimated assuming a linear response. Heptachlor was thus estimated to be detectable at *m/z* 337 down to ca. 2 pg for all three reagent gases in the selected ion monitoring mode, which is comparable to NICI. cis-/trans-Chlordane had a much lower LOD with acetonitrile PICI (ca. 10 pg at *m/z* 373) than with dichloromethane PICI (ca. 250 pg at *m/z* 135) or with acrylonitrile PICI (ca. 500 pg at *m/z* 101). LODs not satisfactory for trace analysis were found for cis-/trans-nonachlor (ca. 40 pg for dichloromethane PICI at *m/z* 135, ca. 180 pg for acetonitrile PICI at *m/z* 409, and ca. 2000 pg for acrylonitrile PICI at *m/z* 135).

Acknowledgement

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Paper II:

DICHLOROMETHANE-ENHANCED NEGATIVE ION CHEMICAL IONIZATION FOR THE DETERMINATION OF POLYCHLORINATED *n*-ALKANES

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Dichloromethane-Enhanced Negative Ion Chemical Ionization for the Determination of Polychlorinated *n*-Alkanes

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The use of dichloromethane/methane reagent gas mixtures is described as an alternative to conventional electron capture negative ionization for the determination of polychlorinated *n*-alkanes (PCAs). A nearly exclusive formation of $[M + Cl]^-$ adduct ions was observed suppressing the generation of other fragment ions. The resulting enhanced selectivity and sensitivity lowered quantification limits to 3 ng for a technical PCA mixture and 10.5−13.5 pg for single congeners. Response factors for congeners of different degrees of chlorination varied by not more than a factor of 2. Interferences from other polychlorinated compounds present in environmental samples such as toxaphene or chlordanes were suppressed by a factor of 5 or more. The technique was applied for the determination of the composition of technical PCA mixtures as well as for the analysis of PCAs in North Sea dab liver.

Chemical ionization mass spectrometry (MS) is a powerful tool for steric structure identification[1] that often allows differentiation between isomers.[2] In addition, compound selectivity or sensitivity can be increased by choice of reagent gases with different proton affinities and ion−molecule reaction properties. However, in practice, only few reagent gases are used such as methane or isobutane. Occasionally, ammonia with a higher proton affinity than methane is employed to enhance selectivity. Methane and argon are most frequently utilized in electron capture negative ionization (ECNI) MS.

The use of less frequently applied reagent gases (often called nonconventional reagent gases) can result in gas-phase ion−molecule reactions that lead to increased sensitivity, selectivity, or both for certain compounds.[2−4] Such reactions can be observed in ion traps at reagent gas levels of a few hundreds of ppm due to the longer residence time of the ions. Chemical ionization in conventional ion sources requires a higher reagent gas pressure in the range of 0.7−2.0 mbar (0.5−1.5 Torr). This does not allow the use of corrosive reagent gases (e.g., water) or those leading to contamination by excessive polymerization reactions.

Chlorinated paraffins are complex technical mixtures consisting of polychlorinated *n*-alkanes (PCAs) with a chlorination degree between 30 and 70% and a linear alkane chain with length of C_{10}−C_{13} (short-chain PCAs), C_{14}−C_{17} (medium-chain PCAs), or $>C_{17}$ (long-chain PCAs).[5] The number of theoretically possible congeners, homologues, diastereomers, and enantiomers is huge within these limits (far beyond 10 000 compounds[6]), which explains the complexity of the resulting technical products. Due to their physical properties, PCAs found application as fire retardants, plasticizers in PVC, adhesives, and as extreme pressure additives in lubricants and cutting oils.[5] They are also replacements for other persistent chemicals such as polychlorinated biphenyls.[5, 7]

Due to a slow migration in the environment, high lipophilicity, and persistence,[7] PCAs can be found in aquatic and terrestrial food webs in rural and remote areas.[5] To date there is limited information about environmental levels of PCAs.[8−10] The main reason is the difficult analysis of PCAs due to their complex composition in environmental samples. Most PCA analyses have been performed by high-resolution gas chromatography (HRGC) coupled to ECNI-MS. High-resolution mass spectrometry (HRMS) was necessary to achieve a sufficient selectivity and sensitivity,[10] since it is difficult to separate completely PCAs from other polychlorinated pollutants such as chlordanes or toxaphenes during sample cleanup. Methane or argon were mostly used as reagent gases. Under these conditions, the mass spectra of PCAs contain the three main ions $[M - HCl]^-$, $[M - Cl]^-$, and $[M + Cl]^-$ with a structure-dependent relative abundance. The molecular ion $[M]^-$ has generally an intensity of <10%. Tomy et al. could show that ion source temperature and congener concentration have an influence on the relative abundance ratios.[11] Additionally,

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the ECNI response factors of single PCA congeners depend strongly on the number of chlorine atoms and their position at the carbon chain and vary by more than a factor of 20. Currently, a precise quantification is impossible due to compound overlap and the commercial availability of only a few single congeners. Therefore, technical mixtures with variable composition are usually used as reference standards.

This work had the aim of obtaining a more uniform ionization response as well as improved selectivity and sensitivity by addition of dichloromethane to the methane reagent gas. This should favor the formation of $[M + Cl]^-$ ions only by negative ion chemical ionization (NICI). The applicability of this technique for routine analysis was evaluated, and detection limits as well as linear range were determined. Details are given about instrument modification and method optimization, and complementary aspects are discussed and compared to CH_4-ECNI-MS. The composition of three technical mixtures and PCA levels in North Sea dab liver were determined to demonstrate its applicability.

EXPERIMENTAL SECTION

Chemicals and Solvents. 2,5,6,9-Tetrachlorodecane, 1,2,9,-10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane, and 1,2,5,6,9,-10-hexachlorodecane (10 ng/μL each in cyclohexane), the only single PCA congeners available on the market, as well as PCA C_{10-13} technical mixtures with chlorine contents of 51.0, 55.5, and 63.0% (100 ng/μL in cyclohexane) were purchased from Ehrenstorfer (Augsburg, Germany). Dichloromethane (99.8%, Scharlau, Barcelona, Spain) was used as reagent gas for the NICI experiments. α-Hexachlorocyclohexane, PCB 153, *trans*-chlordane, *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), and toxaphene 62 (Parlar nomenclature) were all purchased as crystalline solids (purity >99%) from LGC-Promochem (Wesel, Germany) and applied in concentrations of 8−18 ng/μL. A 10-ng aliquot of $[^{13}C_{10}]$-*trans*-chlordane (Cambridge Isotope Laboratories, Mandover, MA) was added as internal standard for quantification and 10 ng of octachloronaphthalene (Ehrenstorfer, Augsburg, Germany) as recovery standard.

Environmental Sample. A pooled sample from four North Sea dab (*Limanda limanda*) livers (5.151 g) caught in the North Sea (54°15.64′ N, 7°29.79′ E) was cleaned as described elsewhere.[12] Recoveries of $[^{13}C_{10}]$-*trans*-chlordane (internal standard) were 85−95%. Quantification was carried out by both CH_4-ECNI and CH_4/CH_2Cl_2-NICI using the technical PCA mixture with 55.5% chlorine content as reference.

Instrumentation. Separations were performed on a HP 5890II (Hewlett-Packard, Palo Alto, CA) gas chromatograph equipped with a split/splitless injector and a fused-silica capillary column (15-m length, 0.25-mm i.d.) coated with a 0.25-μm-thick film of DB35-MS (35% cross-linked phenyl-methylpolysiloxane, J&W Scientific, Folsom, CA). A 1.5-μL sample was injected in the splitless mode. The injector temperature was 250 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at an inlet pressure of 68.9 kPa (10 psi). The temperature program was 100 °C isothermal for 2 min, then 10 °C/min to 260 °C, and isothermal for 10 min.

A MS Engine HP 5989B (Hewlett-Packard) was employed in the NICI mode. The mass spectrometer was tuned to optimal performance using the fragment ions of perfluorotributylamine at m/z 283, 414, and 452. The transfer line temperature was kept at 270 °C, the ion source temperature at 200 °C, and the quadrupole temperature at 100 °C. Compounds were detected both in the scan mode (scan range m/z 50−550, 0.8 scan/s for CH_4-ECNI and m/z 210−710, 0.8 scan/s for CH_4/CH_2Cl_2-NICI) and by selected ion monitoring (SIM) mode of the two most intense isotope masses at dwell times of 100 ms each.

Methane (99.999%, Carbagas) ECNI was carried out at a reagent gas pressure of 1.0−1.6 mbar (0.8−1.2 Torr) measured with the standard thermocouple gauge. Dichloromethane was introduced through a modified transfer line. A Swagelok $^1/_4$-in. T-piece was placed in the methane inlet line. Gaseous dichloromethane was introduced via a diaphragm shut-off valve (SS-DLS4, Nupro) and a needle valve (SS-SS4, Nupro) allowing us to meter the dichloromethane pressure. A small glass bulb of 6-mL volume filled with 5 mL of dichloromethane was connected to the needle valve using a Swagelok connection with Teflon seal. This additional inlet line was evacuated by a further foreline pump before opening the Nupro valve to minimize the amount of air entering the mass spectrometer. To enable the best possible reproducibility of the reagent gas composition in the ion source, the pressure was determined at three different positions, with a thermocouple gauge in the transfer line before the T-piece, with a Bayard-Alpert ionization gauge in the ion source manifold as provided by the instrument manufacturer, and, additionally, in the reagent volume using a further thermocouple gauge connected to a 25-cm-long tube with an inner diameter of 9 mm fitting to the direct insertion probe port.

RESULTS AND DISCUSSION

ECNI of Single PCA Congeners. The four commercially available single PCA congeners were selected to study structure-related differences in the formation of chlorine adducts. Their CH_4-ECNI mass spectra are shown in Figure 1. The $[M + Cl]^-$ ion was the most intensive ion for 2,5,6,9- and 1,2,9,10-tetrachlorodecane. It had a relative abundance of 63% for 1,2,5,6,9-pentachlorodecane and only 12% for 1,2,5,6,9,10-hexachlorodecane. Neither $[M - HCl]^-$ nor $[M - Cl]^-$ was present in the mass spectra of 2,5,6,9-tetrachlorodecane and 1,2,9,10-tetrachlorodecane. $[M - HCl]^-$ was the most intensive ion for 1,2,5,6,9-pentachlorodecane and 1,2,5,6,9,10-hexachlorodecane, which showed a relative abundance of 47 and 68% for the $[M - Cl]^-$ fragment ion, respectively.

Furthermore, $[HCl_2]^-$ at m/z 71 had relative abundances of 67% for 2,5,6,9-tetrachlorodecane, 9% for 1,2,9,10-tetrachlorodecane, 38% for 1,2,5,6,9-pentachlorodecane, and 6% for 1,2,5,6,9,10-hexachlorodecane. These values were determined at an ion source temperature of 200 °C. This is in contrast to Tomy et al.[11] reporting $[HCl_2]^-$ as most intensive ion and much lower intensities for $[M - Cl]^-$, $[M - HCl]^-$, and $[M + Cl]^-$ at this ion source temperature. In general, no strong influence of the ion source temperature on the mass spectra of these compounds could be observed in this study. Higher chlorinated congeners showed a very low tendency of $[HCl_2]^-$ formation. The differences from the results obtained by Tomy et al. may probably be explained by the different construction and geometry of the ions sources: The influence of source geometry on fragment formation is a well-

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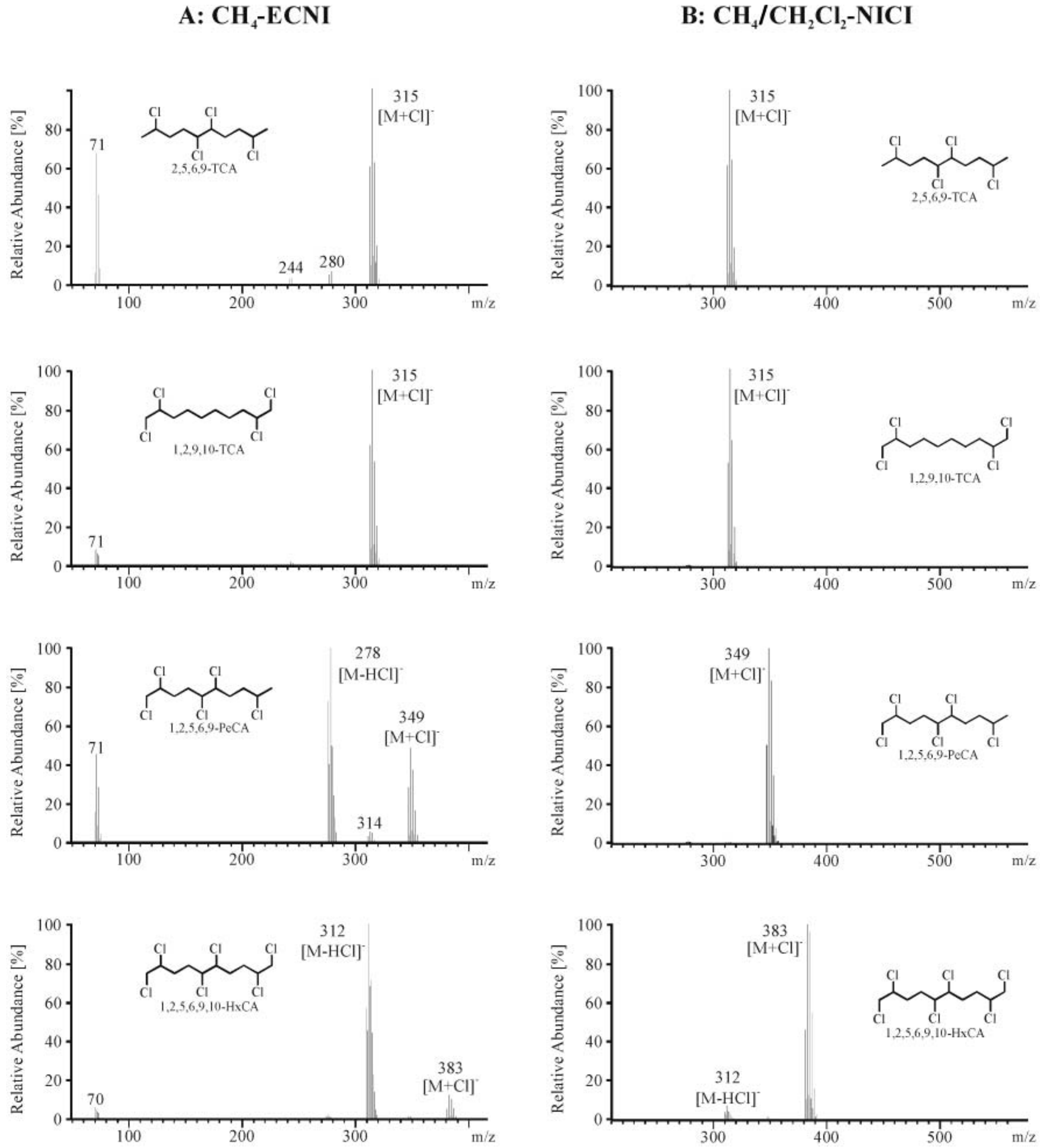

Figure 1. Mass spectra of single PCA congeners recorded with CH$_4$-ECNI (A) and CH$_4$/CH$_2$Cl$_2$-NICI (B). TCA, tetrachloroalkane; PeCA, pentachloroalkane; HxCA, hexachloroalkane.

known problem in chemical ionization.[1] Additionally, the relative response factors of the selected PCAs varied strongly for CH$_4$-ECNI as can be seen from Table 1. They increased from Cl$_4$ to Cl$_6$ by a factor of 22.5, which cannot be explained by the higher number of Cl atoms only.

CH$_2$Cl$_2$ as Reagent Gas. Different pressures and composition ratios of the CH$_4$/CH$_2$Cl$_2$ reagent gas mixture were evaluated. An almost exclusive formation of chlorine adducts and the highest signal-to-noise ratio were observed at a total reagent gas pressure of 2.0 mbar (1.5 Torr) measured at the transfer line inlet with the standard thermocouple gauge and a CH$_4$/CH$_2$Cl$_2$ ratio of 80:20. This corresponded to a pressure in the ion source manifold of 5.6 × 10^{-4} (4.2 × 10^{-4} Torr) and 7.5 mbar (5.6 × 10^{-1} Torr) in the reaction volume determined by the additional thermocouple gauge. Both ratio and pressure are very similar to those applied for the ionization of hexachlorocyclopentadiene derivatives by Stemmler and Hites using a CH$_4$/CH$_3$Cl mixture as reagent gas.[13] The negative ion mass spectrum of the reagent gas mixture is

Table 1. Response Factors of the Available Single Congeners Relative to 1,2,9,10-Tetrachlorodecane (Normalized to 1) Using CH$_4$-ECNI and CH$_4$/CH$_2$Cl$_2$-NICI[a]

	1,5,6,9-TCA	1,2,9,10-TCA	1,2,5,6,9-PeCA	1,2,5,6,9,10-HxCA
CH$_4$-ECNI	1.4	1.0	17.2	22.5
CH$_4$/CH$_2$Cl$_2$-NICI	2.4	1.0	2.3	1.6

[a] SIM detection of the most abundant isotope was carried out. TCA, tetrachlorodecane; PeCA, pentachlorodecane; HxCA, hexachlorodecane.

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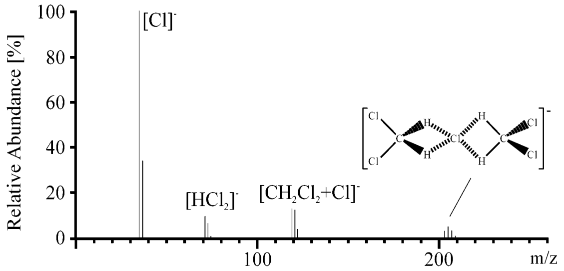

Figure 2. Negative ion mass spectrum of the CH_4/CH_2Cl_2 80:20 reagent gas mixture. A structure proposal of the ion at *m/z* 203 is also shown.

presented in Figure 2 and is dominated by the Cl^-. The highest mass is an adduct ion at *m/z* 203 corresponding to $[2CH_2Cl_2 + Cl]^-$. The structure of this ion was proposed according to the gas-phase stability data published by Glukhovtsev et al.[14] (see Figure 2). Consequently, the lowest mass for acquisition of mass spectra was set to *m/z* 210.

2,5,6,9-Tetrachlorodecane, 1,2,9,10-tetrachlorodecane, and 1,2,5,6,9-pentachlorodecane formed only the $[M + Cl]^-$ adducts with the CH_4/CH_2Cl_2 reagent gas mixture (see Figure 1). $[M + Cl]^-$ was also most intensive ion for 1,2,5,6,9,10-hexachlorodecane, and the relative abundance of $[M - HCl]^-$ dropped below 8% (Figure 1). The response factors of these four single congeners differed by not more than a factor of 2.4, which is nearly 10 times less than for CH_4-ECNI (see Table 1). This was due to much higher response factors for the Cl_4/Cl_5 congeners, which were similar to the Cl_6 compound. Limits of quantification of the single congeners were in the range 7−9 pg/μL.

Ionization of Interfering Compounds. CH_4-ECNI forms simultaneously $[M - Cl]^-$, $[M - HCl]^-$, and $[M + Cl]^-$ ions. Therefore, interferences between congeners of different degrees of chlorination are possible. Since a complete separation by HRGC is impossible, quantification by low-resolution MS is questionable. For example, mass *m/z* 327 can be $[C_{11}H_{18}{}^{37}Cl^{35}Cl_5]^-$ formed by loss of chlorine from a $C_{11}H_{18}Cl_6$ congener or $[C_{11}H_{20}{}^{35}Cl_5]^-$, the chlorine adduct of a $C_{11}H_{20}Cl_4$ congener. Such interferences can be overcome by HRMS at a resolution of 12 000.[10] However, the availability of such expensive instrumentation is limited. Ionization by the CH_4/CH_2Cl_2 reagent gas mixture formed nearly exclusively the chlorine adducts, eliminating almost completely such interferences.

Moreover, the sample cleanup procedures usually applied for the analysis of environmental samples do not separate PCAs entirely from other polychlorinated pollutants such as chlordanes or toxaphenes. Coelution of such compounds with the broad PCA signals interferes with the quantification by low-resolution MS. Ionization by CH_4-ECNI and CH_4/CH_2Cl_2-NICI was compared for α-hexachlorocyclohexane, *trans*-chlordane, PCB 153, *′p,p′*-DDT, and toxaphene 62 (Parlar nomenclature). All compounds except PCB 153 formed chloride adducts. However, in contrast to PCAs, their relative abundance was below 50%. Furthermore, the response of the possible interferences decreased ~5-fold with CH_4/CH_2Cl_2-NICI compared to CH_4-ECNI. Together with the increased sensitivity for PCAs, this resulted in an overall gain in selectivity by more than 1 order of magnitude.

Table 2. Relative Congener and Homologue Compositions of Three Technical PCA Mixtures with a Declared Chlorine Content of 51.0, 55.5, and 61.0%, Respectively[a]

	rel amt (%) for PCA technical mixture		
PCA isomer	51.0% Cl	55.5% Cl	61.0% Cl
$C_{10}H_{19}Cl_3$	0.34	0.13	0
$C_{10}H_{18}Cl_4$	2.90	2.48	0.14
$C_{10}H_{17}Cl_5$	2.87	4.45	1.50
$C_{10}H_{16}Cl_6$	1.99	2.47	3.45
$C_{10}H_{15}Cl_7$	0.27	0.50	2.36
$C_{10}H_{14}Cl_8$	0.08	0.23	1.31
$C_{10}H_{13}Cl_9$	<0.02	0.04	0.29
$C_{10}H_{12}Cl_{10}$	<0.02	<0.02	0.48
$C_{11}H_{21}Cl_3$	1.17	0.29	<0.02
$C_{11}H_{20}Cl_4$	12.61	6.36	0.08
$C_{11}H_{19}Cl_5$	16.95	16.01	2.11
$C_{11}H_{18}Cl_6$	6.37	11.10	13.09
$C_{11}H_{17}Cl_7$	0.87	3.06	12.98
$C_{11}H_{16}Cl_8$	0.10	0.40	4.90
$C_{11}H_{15}Cl_9$	0	0.08	1.36
$C_{11}H_{14}Cl_{10}$	0	0	0.22
$C_{12}H_{23}Cl_3$	0.84	0.14	<0.02
$C_{12}H_{22}Cl_4$	9.47	3.95	<0.02
$C_{12}H_{21}Cl_5$	15.84	13.73	1.80
$C_{12}H_{20}Cl_6$	7.75	12.94	9.17
$C_{12}H_{19}Cl_7$	1.45	3.81	14.01
$C_{12}H_{18}Cl_8$	0.10	0.60	9.23
$C_{12}H_{17}Cl_9$	<0.02	0.08	3.65
$C_{12}H_{16}Cl_{10}$	<0.02	<0.02	0.16
$C_{13}H_{25}Cl_3$	0.30	0.10	<0.02
$C_{13}H_{24}Cl_4$	3.89	0.90	<0.02
$C_{13}H_{23}Cl_5$	7.42	6.19	0.21
$C_{13}H_{22}Cl_6$	5.05	6.56	3.53
$C_{13}H_{21}Cl_7$	1.27	2.85	5.35
$C_{13}H_{20}Cl_8$	0.11	0.51	5.47
$C_{13}H_{19}Cl_9$	<0.02	0.05	2.91
$C_{13}H_{18}Cl_{10}$	<0.02	<0.02	0.23
calcd Cl content (%)	51.8	54.2	60.5
specified Cl content (%)	51.0	55.5	63.0

[a] CH_4/CH_2Cl_2−NICI was applied.

Characterization of Technical PCA Mixtures by CH_4/CH_2Cl_2-NICI. The composition of three technical PCA mixtures was determined. The dominating ion adduct formation and the more uniform response factors for different congeners should allow a more precise analysis of the congener and homologue fractions. The relative concentrations of the observed congener and homologue groups are summarized in Table 2 and Figure 3. In contrast to CH_4-ECNI, CH_4/CH_2Cl_2-NICI enabled also the detection of tri- and tetrachloro congeners. The reproducibility of the composition was good (standard deviation below 6% for the major components and below 15% for the less abundant components, $n = 5$). The chlorine content calculated on the basis of the relative quantities of congeners and homologues deviated only about 1−2.5% from the values specified by the manufacturer (see Table 2). Data obtained with CH_4-ECNI were 2−7% off.

The linearity of CH_4/CH_2Cl_2-NICI was investigated for four major components ($C_{11}H_{19}Cl_5$, $C_{11}H_{18}Cl_6$, $C_{12}H_{21}Cl_5$, $C_{12}H_{20}Cl_6$) in two technical PCA mixtures with 51.0 and 55.5% chlorine content. A linear range of 2−100 ng of technical PCAs was found ($r^2 > 0.998$, six measuring points). This is sufficient for quantitative analysis. CH_4-ECNI gave similar results for the Cl_6 components.

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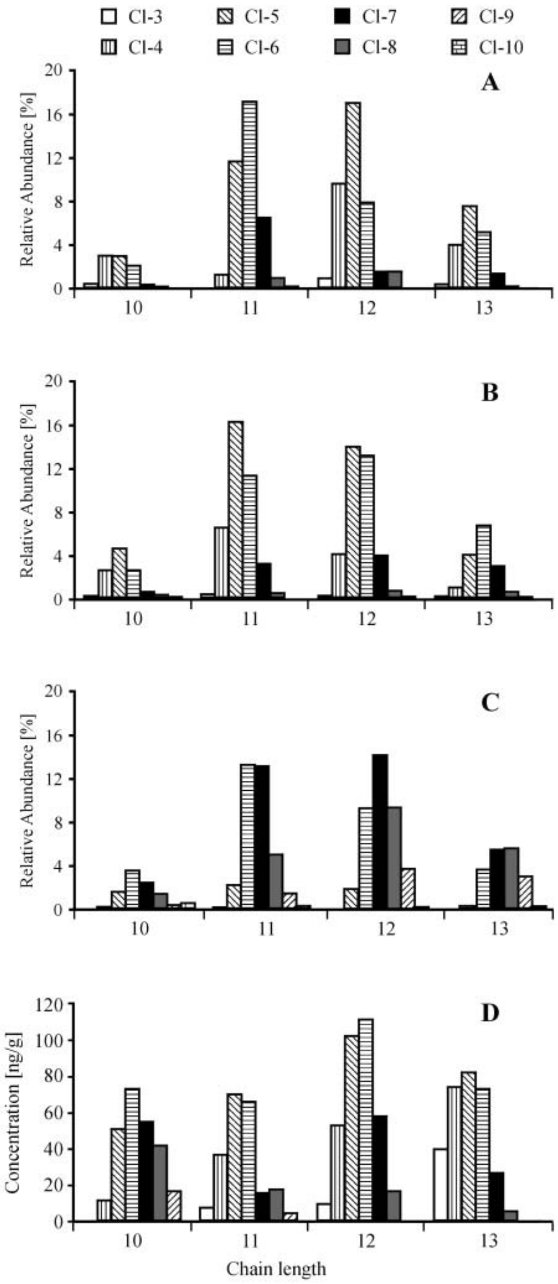

Figure 3. Congener and homologue patterns of three technical PCA mixtures (A−C) and of an extract of liver from North Sea dab (D) obtained with CH_4/CH_2Cl_2-NICI. The declared chlorine contents were 51.0 (A), 55.5 (B), and 63.0% (C).

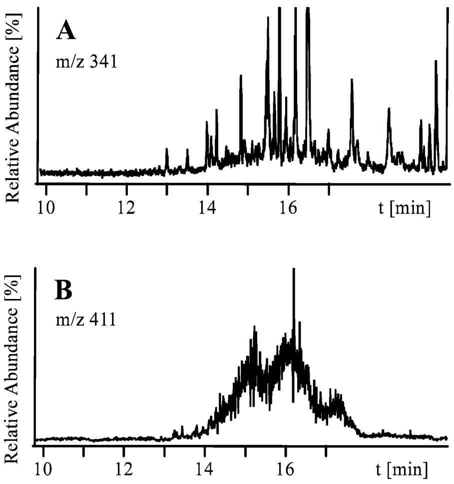

Figure 4. Mass chromatograms of a liver extract from North Sea dab. The mass traces for the $C_{12}H_{20}Cl_6$ isomers are shown. (A) CH_4-ECNI, $[M - Cl]^-$ (*m/z* 341, most intense signal of the isotope cluster). (B) CH_4/CH_2Cl_2-NICI, $[M + Cl]^-$ (*m/z* 411, most intense signal of the isotope cluster).

Table 3. Congener- and Homologue-Specific PCA Concentrations (ng/g Wet Weight) in a Pooled Sample of North Sea Dab (*L. limanda*)

PCA isomer	concn (ng/g)	PCA congener	concn (ng/g)
$C_{10}H_{18}Cl_4$	2.0	$C_{12}H_{23}Cl_3$	1.8
$C_{10}H_{17}Cl_5$	9.7	$C_{12}H_{22}Cl_4$	10.1
$C_{10}H_{16}Cl_6$	13.9	$C_{12}H_{21}Cl_5$	19.6
$C_{10}H_{15}Cl_7$	10.4	$C_{12}H_{20}Cl_6$	21.3
$C_{10}H_{14}Cl_8$	8.0	$C_{12}H_{19}Cl_7$	11.1
$C_{10}H_{13}Cl_9$	3.1	$C_{12}H_{18}Cl_8$	3.1
$C_{11}H_{21}Cl_3$	1.4	$C_{13}H_{25}Cl_3$	7.5
$C_{11}H_{20}Cl_4$	7.0	$C_{13}H_{24}Cl_4$	14.1
$C_{11}H_{19}Cl_5$	13.4	$C_{13}H_{23}Cl_5$	15.7
$C_{11}H_{18}Cl_6$	12.6	$C_{13}H_{22}Cl_6$	13.9
$C_{11}H_{17}Cl_7$	2.8	$C_{13}H_{21}Cl_7$	5.1
$C_{11}H_{16}Cl_8$	3.3	$C_{13}H_{20}Cl_8$	1.0
$C_{11}H_{15}Cl_9$	0.7		
mean Cl content:	54.5%		

Application to a Biota Sample. A cleaned-up liver extract of North Sea dab (*L. limanda*) was analyzed with CH_4-ECNI and CH_4/CH_2Cl_2-NICI. As can be seen from Figure 4, the sample cleanup on Florisil did not remove other polychlorinated compounds completely, which then interfered in the quantification of PCAs by CH_4-ECNI. Only the mass trace for the congeners with the elemental composition $C_{12}H_{20}Cl_6$ is shown. The CH_4/CH_2Cl_2 reagent gas mixture suppressed the ionization of these compounds completely and also improved the signal-to-noise ratio. The “noise” within the hump is caused by quick changes in composition since it is much higher than that of the baseline on both sides. This opens the possibility to determine PCA levels without interferences even by low-resolution mass spectrometry. Quantitative isomer-specific results are given in Table 3, and the congener and homologue pattern is presented in Figure 3D. The PCA sum concentration was 213 ng/g (wet weight, w/w), which is comparable to the range of 100−1700 ng/g w/w reported in the literature for fish and determined mainly by high-resolution mass spectrometry.[15] Sensitivity and selectivity of CH_4-ECNI were not sufficient to quantify PCAs in this sample (see Figure 4). The mean chlorine content was calculated to 54.5%, which is comparable to technical mixtures. However, the observed congener and homologue profile in the liver sample deviated substantially from the analyzed technical PCA mixtures (Figure 3). Therefore, expressing PCAs as a certain amount of a technical mixture might introduce systematic deviations as also found for other complex classes of polychlorinated pollutants such as PCBs. Consequently, PCA levels should be given on a congener- and homologue-specific basis.

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CONCLUSIONS

The addition of dichloromethane to methane allowed us to enhance both sensitivity and selectivity for PCAs compared to conventional CH_4-ECNI. The introduction of dichloromethane required only a very simple instrument modification resulting in a stable and reproducible reagent gas pressure and composition. Quite similar response factors were obtained for PCA congeners of different degrees of chlorination compared to CH_4-ECNI. This allowed use of technical mixtures as reference standards for a congener- and homologue-specific analysis. Furthermore, CH_4/CH_2Cl_2-NICI suppressed sufficiently interferences from other polychlorinated pollutants, enabling the determination of short-chain PCAs (C_{10-13}) by low-resolution mass spectrometry even in marine biota.

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Paper III:

DETERMINATION OF TOTAL POLYCHLORINATED *n*-ALKANE CONCENTRATION IN BIOTA BY ELECTRON IONIZATION-MS/MS

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Determination of Total Polychlorinated *n*-Alkane Concentration in Biota by Electron Ionization-MS/MS

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Electron ionization (EI) tandem mass spectrometry (MS/MS) allowed the fast determination of the total concentration of short- and medium-chained polychlorinated n-alkanes (PCAs) in biota. EI fragment ions common to all PCAs could be identified. Collision-induced dissociations (CIDs) were carried out by ion trap and triple quadrupole EI-MS/MS. CIDs of m/z 91 → 53 (limit of detection (LOD) 0.15 ng/μL), 102 → 65 (LOD = 0.2 ng/μL), and 102 → 67 (LOD = 0.1 ng/μL) were applied for the determination of the total short- and medium-chain PCA concentration in pooled fish liver samples (North Sea dab, cod, flounder) from the North Sea and from the Baltic Sea using both MS technologies. Total PCA concentrations were in the range of 88−607 ng/g. Accuracy was controlled with spiked samples and deviated not more than 15% from expected values.

Polychlorinated n-alkanes (PCAs) are complex technical mixtures. They are sold commercially as “chlorinated paraffins” with a chlorination degree between 30 and 70% and a linear chain length of C_{10}−C_{13} (short-chain PCAs or sPCAs), C_{14}−C_{17} (medium-chain PCAs or mPCAs) or $C_{>17}$ (long-chain PCAs). The number of theoretically possible congeners, homologues, diastereomers, and enantiomers is huge (far beyond 10 000 compounds[1]), which explains the complexity of the resulting technical products. Due to their physical properties, PCAs have a wide range of application such as fire retardants, plasticizers in PVC, additives in adhesives, and mainly as extreme pressure additives in lubricants and cutting oils.[2]

The vapor pressures of some PCA congeners and mixtures of homologue groups are between 5×10^{-1} and 1×10^{-4} Pa.[3] Other persistent organic chemicals such as polychlorinated biphenyls show similar vapor pressures and are known to undergo atmospheric long-range transport.[4] In addition, PCAs are of concern due to their toxicological properties and their capacity to bioaccumulate. Widespread and unrestricted PCA use, combined with the properties mentioned above, has resulted in their presence in aquatic and terrestrial food webs in rural and remote areas.[2,5,6] However, the information about environmental levels of PCAs is limited.[7,8] PCA analyses are usually performed by high-resolution gas chromatography (HRGC) coupled to electron capture negative ionization (ECNI) mass spectrometry (MS) and is based on the detection of the $[M - Cl]^-$ ions.[8,9] An alternative chemical ionization method has been published recently using dichloromethane as reagent gas, which leads to the nearly exclusive formation of $[M + Cl]^-$ ions and a better sensitivity for lower chlorinated congeners.[10]

Normally, high-resolution mass spectrometry is employed,[9,11] but determination of PCAs in biota with low-resolution mass spectrometry is also possible.[8] All methods have to detect each possible homologue and congener mass in the selected ion monitoring mode. Due to the many PCA congeners and isomers present eluting as broad signals within a few minutes, this requires several injections of the same sample (which makes PCA analysis expensive and time-consuming) or the use of complex set of retention time windows. This is also one of the main reasons for the currently limited information about environmental levels of PCAs.

Our research group already demonstrated that electron ionization (EI) combined with ion trap tandem mass spectrometry (MS/MS) is a selective and sensitive alternative to ECNI-MS for the analysis of toxaphenes.[12] However, the strong fragmentation of PCAs under EI conditions gives mass spectra without compound-specific information such as chain length or number of chlorine atoms. This work shows that this disadvantage can be beneficial for a “total” PCA analysis, since some fragments are present in all PCA mass spectra below m/z 110. An increased risk of interferences, an insufficient sensitivity, and a rather high chemical

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background from the remaining sample matrix in this low-mass range does not allow a detection by low-resolution EI-MS. However, a further fragmentation by tandem mass spectrometry might open the possibility to achieve a satisfactory selectivity and sensitivity. In this work, the potential of ion trap EI-MS/MS combined with fast gas chromatography was investigated for routine screening of PCAs in biota. In addition, EI-MS/MS by triple quadrupole mass spectrometry was also evaluated. The benefits and limitations of both techniques are presented including a full method validation. Both techniques were applied to the determination of total concentrations of short- and medium-chained PCAs in fish liver.

EXPERIMENTAL SECTION

Chemicals and Solvents. Technical sPCA (chlorine contents of 51.5, 55.5, and 63.0%) and mPCA mixtures (chlorine contents of 42.0, 52.0, and 57.0%) with concentrations of 100 ng/μL in cyclohexane and a mixture of single sPCA congeners (2,5,6,9-tetrachlorodecane, 1,2,9,10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane, and 1,2,5,6,9,10-hexachlorodecane, 10 ng/μL each in cyclohexane) were purchased from Ehrenstorfer (Augsburg, Germany). $^{13}C_{10}$-trans-Chlordane (99%) at a concentration of 100 ± 10 ng/μL in nonane was obtained from Cambridge Isotope Laboratories (Andover, MA). Cyclohexane, dichloromethane, and n-hexane for pesticide residue analysis were purchased from Scharlau (Barcelona, Spain). Florisil PR (60−100 mesh) and sodium sulfate (Pestanal) were obtained from Fluka (Buchs, Switzerland), and silica gel (200−400 mesh, 0.035−0.070 mm) was from CU Chemie Uetikon AG (Uetikon, Switzerland). Before use, all three chemicals were treated at 600 °C overnight and afterward kept for 6 h at 130 °C.

Environmental Samples. Fish was caught in the North Sea and Baltic Sea by the Federal Research Center for Fisheries (Hamburg, Germany) in August 2002 (for species and location see Table 3). Pooled liver samples were composed of five individuals. The cleanup method is described briefly, since more details have been published elsewhere.[8] Samples were homogenized with a 10-fold excess of Na_2SO_4. A 10-ng sample of $^{13}C_{10}$-trans-chlordane (internal standard) in 10 μL of cyclohexane was added and the sample column extracted with 250 mL of n-hexane/CH_2Cl_2 (1+1, v/v). The cleanup of the extracts removed fat by column chromatography on 40 g of silica gel impregnated with 44% H_2SO_4. The lipid-free sample was eluted with 120 mL of n-hexane/CH_2Cl_2 (1+1, v/v). Then, adsorption chromatography on 16 g of Florisil (1.5% H_2O water content) was carried out with 60 mL of n-hexane (fraction 1), with 6 mL of CH_2Cl_2 (fraction 2), and with 60 mL of CH_2Cl_2 (fraction 3). The last one contained all PCAs. Quantification was carried out with a technical sPCA mixture of 55.5% chlorine content as reference. The obtained chromatographical signals were integrated “from baseline to baseline” including the whole PCA signal. The analysis of spiked fish or fish oil was performed in the same way. A 1500-ng sample of a technical sPCA mixture or 1500 ng of a sPCA and of a technical mPCA mixture was spiked to 4−5 g of fish oil or to 8−10 g of homogenized mackerel muscle.

Instrumentation. Gas chromatographic separations combined with ion trap MS were performed on a Star 3400 CX (Varian, Walnut Creek, CA) gas chromatograph. A fused-silica capillary (9-cm length, 0.05-mm i.d.) was connected in front of a fused-silica capillary column (5-m length, 0.25-mm i.d.) coated with 0.25 μm of DB35-MS (35% cross-linked phenylmethylpolysiloxane, J&W Scientific, Folsom, CA). The injector was equipped with a glass liner filled with glass wool and set to 250 °C. Helium (99.999%, Carbagas) was used as carrier gas at an inlet pressure of 68.9 kPa (10 psi). The temperature program was as follows: 100 °C isothermal for 1 min, then 50 °C/min to 300 °C, and isothermal for 4 min. Splitless injections (splitless time 0.75 min) of 2.5-μL volume were carried out with a Varian 8200 autosampler. A Saturn 2000 (Varian) ion trap MS detector was employed. The transfer line temperature was set to 280 °C, the ion trap temperature to 250 °C, and the manifold temperature to 40 °C. The instrument was tuned regularly with perfluorotributylamine using the fragment masses m/z 69, 131, 264, 414, 464, and 502. EI-MS spectra were recorded at 70 eV and a scan range of m/z 50−500 (emission current 10 μA, scan time 0.8 s, trap target 25 000 counts). EI-MS/MS conditions were as follows: 70 eV electron energy, emission current 70 μA, scan range m/z 50−300, scan time 0.3 s, trap target 20 000 counts, maximum ionization time 25 ms, and collision-induced dissociation (CID) at an isolation time of 5 ms and excitation time of 20 ms working in the resonant waveform. Selected masses and mass-related ion trap MS/MS parameters are listed in Table 1. The precursor ion m/z 383 $[M - Cl]^+$ and the product ion m/z 276 $[M - 4Cl]^+$ were chosen for $^{13}C_{10}$-trans-chlordane (excitation amplitude 0.85 V and excitation storage level m/z 169.0).

In addition, a 1200 triple quadrupole MS (Varian) was employed. Gas chromatographic separations were performed on a CP-3800 (Varian) gas chromatograph equipped with a fused-silica capillary column (30-m length, 0.25-mm i.d.) coated with 0.25 μm of VF-5MS (5% cross-linked phenylmethylpolysiloxane, Factor Four, Varian). The injector temperature was set to 275 °C. Helium was used as carrier gas at a constant flow of 2 mL/min. The temperature program was as follows: 100 °C isothermal for 1 min, then 50 °C/min to 300 °C, and isothermal for 4 min. Splitless injections (splitless time 1.5 min) of 2.5-μL volume were carried out with a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland). The mass spectrometer was tuned regularly with perfluorotributylamine using the fragment masses m/z 69, 131, 218, 414, 464, and 502. The transfer line temperature was set to 250 °C, the ion source temperature to 200 °C, and the manifold temperature to 40 °C. EI-MS spectra were recorded at 70 eV and a scan range of m/z 50−500 (emission current 300 μA, scan time 0.5 s). Conditions for EI-MS/MS were as follows: 70 eV electron energy, emission current 300 μA, dwell time 0.2 s, resolution of Q1 at 0.8 and of Q3 at 1.2, and argon as CID gas at 0.12−0.15 Pa (0.9−1.1 mTorr). Selected masses and collision energies are listed in Table 1. The precursor ion m/z 383 $[M - Cl]^+$ and the product ion m/z 276 $[M - 4Cl]^+$ were chosen for $^{13}C_{10}$-trans-chlordane (collision energy −21 V).

RESULTS AND DISCUSSION

Selection of Precursor Ions. EI mass spectra of PCAs show a strong fragmentation and no molecular ions. Main fragments are formed by consecutive loss of chlorine radicals, by elimination of HCl, and by fragmentation of the carbon chain (see Figure 1). Due to the high degree of fragmentation and the nearly complete absence of ions above m/z 300, the mass spectra do not contain any structure-specific information like chain length or degree of

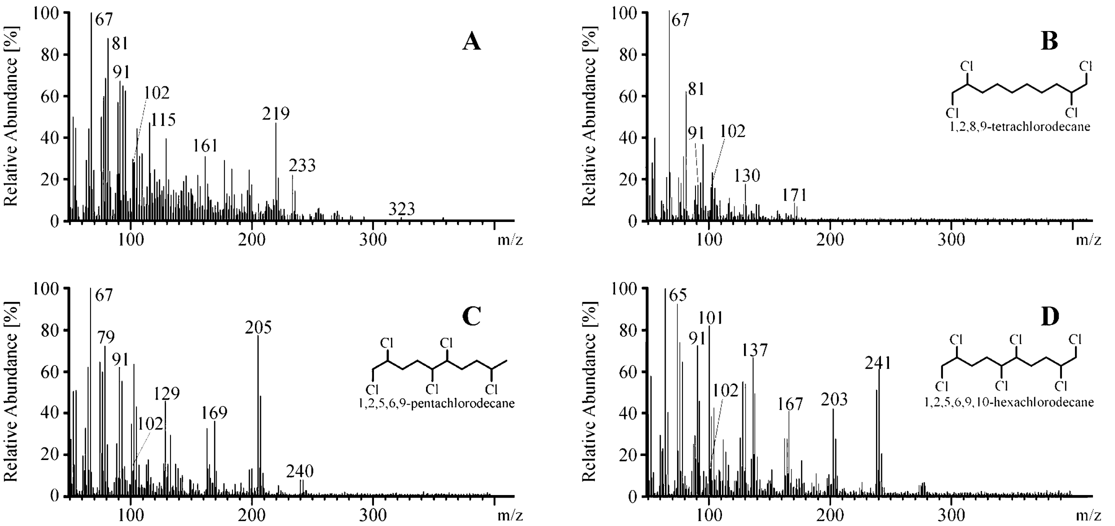

Figure 1. Ion trap EI mass spectra (scan m/z 50−500) of a technical sPCA mixture with a chlorine content of 55.5% at a retention time of 4.4 min (A) and of three single sPCA congeners (B) 1,2,9,10-tetrachlorodecane, (C) 1,2,5,6,9-pentachlorodecane, and (D) 1,2,5,6,9,10-hexachlorodecane).

Table 1. Ion Trap and Triple Quadrupole MS/MS Parameters for Selected Fragment Ions Common to All PCA Congeners and for the Internal Standard $^{13}C_{10}$-*trans*-Chlordane

		ion trap		triple quadrupole
precursor ion (m/z)	product ion (m/z)	excitation amplitude (V)	excitation storage level (m/z)	collision energy (V)
77 $[C_6H_5]^+$	51 $[C_4H_3]^+$	0.2	35.0	−15.0
79 $[C_6H_7]^+$	51 $[C_4H_3]^+$	0.2	35.0	−21.0
81 $[C_6H_9]^+$	79 $[C_6H_7]^+$	0.2	35.4	−8.0
91 $[C_7H_7]^+$	65 $[C_5H_5]^+$	0.2	39.9	−9.5
91 $[C_7H_7]^+$	53 $[C_4H_5]^+$	2.0	39.9	−10.0
102 $[C_5H_7Cl]^+$	65 $[C_5H_5]^+$	0.6	44.7	−10.0
102 $[C_5H_7Cl]^+$	67 $[C_5H_7]^+$	a	a	−18.0
104 $[C_8H_8]^+$	77 $[C_6H_5]^+$	0.7	45.6	−14.0
383 $[M - Cl]^+$	276 $[M - 4Cl]^+$	0.85	169.0	−21.0

[a] Hardly detectable with the ion trap mass spectrometer.

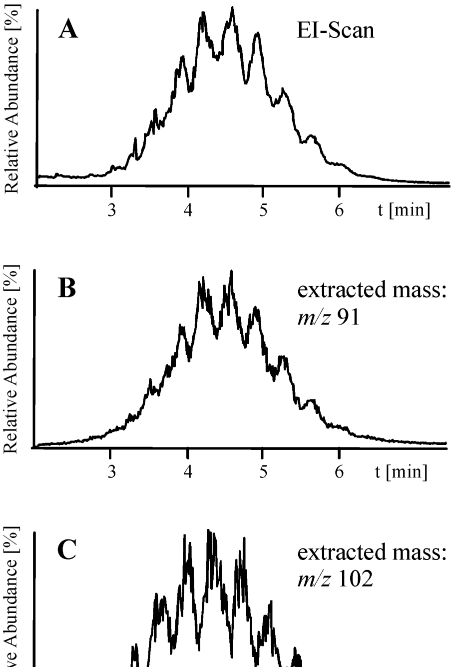

Figure 2. Ion trap EI-MS chromatograms of a technical sPCA mixture with 55.5% chlorine content: (A) total ion range m/z 50−500, (B) extracted mass m/z 91, and (C) extracted mass m/z 102.

chlorination. However, some of the fragments in the mass range of <110 u were present in any mass spectrum. The signal shape of the extracted mass chromatograms for m/z 77, 79, 81, 91, 102, and 104 for technical PCAs was very similar to the full-scan mode (see Figure 2). This confirms that these fragments are present in (almost) all PCA congeners at comparable relative abundances. Therefore, they should be suited for a determination of the total PCA concentration in samples.

Selectivity Increase by Ion Trap MS/MS. The fragments common in all PCA mass spectra have rather low mass-to-charge ratios and are not very specific for PCAs. Interferences from other sample components eluting within the retention time range of PCAs are therefore possible for detection by EI-MS. To increase selectivity further, fragmentation by ion trap MS/MS was carried out. The parameters for the studied CIDs are listed in Table 1.

Since PCA mixtures cannot be resolved by any HRGC column, only a short chromatographic column was used (5-m length, 0.25-mm i.d.) to lower the detection limits further. Since the He flow into the ion trap has to be <2 mL/min for good precision of the CID process, an uncoated restriction capillary of 9 cm and 0.05-mm i.d. was mounted in front of the short separation capillary limiting the flow to 1.8 mL/min at 100 °C (68.9 kPa column head pressure). The applied fast temperature program allowed a total separation time of 9 min and resulted in an increase in sensitivity by a factor of 2.

Calibration functions were linear for all CIDs listed in Table 1 within 1−100 ng/μL technical sPCA mixture with 55.5% Cl content (R^2 >0.99, 6−7 measuring points). Limits of detection (LODs) at a signal-to-noise ratio (S/N) of 3:1 were in the range of 0.7−5

Table 2. LODs at a S/N of 3:1 and LOQs at a S/N of 10:1 for the CID Fragmentations Given in Table 1 as Well as Errors in the Analysis of Spiked Samples[a] for the Ion Trap and the Triple Quadrupole Mass Spectrometer

	ion trap			triple quadrupole		
CID (m/z)	LOD (ng/μL)	LOQ (ng/μL)	error in the quantification of spiked samples (%)	LOD (ng/μL)	LOQ (ng/μL)	error in the quantification of spiked samples (%)
77 → 51	5	15	367	4	13	351
79 → 51	3	9	119	6	10	118
81 → 79	3	9	684	2	5	292
91 → 53	0.7	2	14	0.15	0.5	17
91 → 65	2	5	215	2	5	212
102 → 65	0.7	2	24	0.2	0.5	15
102 → 67[b]				0.1	0.3	5
104 → 51	3	9	535	1	4	420

[a] n = 5, 1500 ng of a technical sPCA mixture with 55.5% chlorine content added to fish oil or fish filet. Lowest errors are marked in boldface type. [b] Hardly detectable with the ion trap mass spectrometer.

ng/μL and limits of quantification (LOQs, S/N 10:1) between 2 and 15 ng/μL, depending on the selected fragmentation reaction (see Table 2).

The selectivity of the different CIDs was tested with spiked fish oil and filet from mackerel. Only the accuracy of the CID of m/z 91 → 53 and m/z 102 → 65 was acceptable for the screening purpose of the method. These CIDs showed maximum errors of 15−20% (repeated for five spiked samples) and had also the lowest LODs (Table 2) and best linearity ($R^2 > 0.996$, range 1−100 ng/μL, 7 calibration points). They were therefore selected for screening of the liver samples. All the other fragmentations showed a positive systematic error of >150% (Table 2).

The ion m/z 102 is also present in the mass spectra of chlorinated benzenes. However, these are either removed by the cleanup process or are so volatile that they elute within the solvent signal at the employed temperature program. The fragment ion m/z 91 and its product ion m/z 53 are present in the mass spectra of, for example, alkylated aromates, but again such compounds elute much earlier than the PCAs. Therefore, quantifications were carried out with the CID m/z 102 → 65 and confirmed with m/z 91 → 53.

Simultaneous Detection of sPCAs and mPCAs. mPCAs cannot be separated from sPCAs by any standard cleanup procedure and therefore are also present in the sample extracts. Therefore, the applicability of the developed EI-MS/MS method for the determination of mPCAs was evaluated as well. mPCAs formed fragments similar to sPCAs. The relative response factors of three technical sPCA and three technical mPCA mixtures with different degrees of chlorination showed a standard deviation of 14% for m/z 102 → 65 and of 21% for m/z 91 → 53. This demonstrates clearly that the response is influenced neither by the degree of chlorination nor by the chain length. This is a further advantage, since PCA composition differs considerably between technical mixtures and also compared to biota.[8,10] Due to strongly varying response factors,[10] the analysis of PCAs by ECNI-MS requires a careful selection of the quantification standard in order to match the PCA distribution in the sample.[9] The small variations of the response factors under EI-MS/MS conditions make this procedure unnecessary and reduce the risk of systematic errors.

Accuracy tested with samples spiked with 1500 ng of each sPCA and mPCA gave results similar to those for sPCAs only (±22% for m/z 102 → 65 and ±8% for m/z 91 → 53, two parallels). Therefore, the developed method is also suitable for the simultaneous determination of the sum of sPCAs and mPCAs.

Detection by Triple Quadrupole MS/MS. To detect artifacts caused by the long residence time in the ion trap and to eventually increase sensitivity, the developed method was transferred to a triple quadrupole mass spectrometer. The same CIDs as in the ion trap were observed in the collision cell of the triple quadrupole instrument and the instrument parameters optimized correspondingly (Table 1). However, a few more fragments of low abundance were observed in the mass range of <50 u.

Moreover, the precursor ion m/z 102 fragmented both to m/z 65 (collision energy of −18 V) as observed for the ion trap and to m/z 67 at a lower collision energy of −10 V. The latter fragment was also observed in the trap but not selected, since it was only abundant, when low excitation amplitudes (<0.25 V) were applied. The CID m/z 102 → 67 was included in the evaluation of the triple quadrupole method, since it was reproducible.

All CIDs showed a good linearity for the concentration range 0.5−100 ng/μL ($R^2 > 0.99$, 7−9 measuring points) with LODs (S/N 3:1) in the range of 0.1−6 ng/μL and LOQs (S/N 10:1) of 0.3−13 ng/μL, depending on the selected fragmentation (see Table 2).

Again, the accuracy tested with five spiked samples was satisfactory for m/z 91 → 53 (±17%) and m/z 102 → 65 (±15%). The additionally selected m/z 102 → 67 gave the best results (±5%) and was therefore also included in the analysis of fish liver. As for the ion trap, the fragmentations with the best accuracy had also the lowest LODs (Table 2) and best linearity ($R^2 > 0.999$, range 0.5−100 ng/μL, 9 calibration points).

Detection of mPCAs by Triple Quadrupole MS/MS. mPCAs showed the same CID as sPCAs and could therefore be determined by EI-MS/MS as well. Relative response factors of three sPCA and three mPCA technical mixtures with different degrees of chlorination showed a standard deviation of 14% for m/z 102 → 67, of 11% for m/z 102 → 65, and of 13% for m/z 91 → 53. Mass chromatograms of a technical sPCA and mPCA mixture as well as of a 1+1 mixture are given in Figure 3 as example.

Analysis of Fish Liver. The ion trap and triple quadrupole EI-MS/MS methods were applied to the quantification of s+mPCAs in fish liver from the North Sea and the Baltic Sea. Quantification

Table 3. Total Concentrations of Short- and Medium-Chain (s+m) PCAs in Fish Liver Obtained by Different CID Fragmentations and Ion Trap or Triple Quadrupole MS and by ECNI-MS

						total s+mPCAs (ng/g)		
no.	fish species	capture location	lipid (%)	sex[a]	CID (m/z)	ion trap	triple quadrupole	ECNI-MS[b]
1	North Sea dab (Limanda limanda)	North Sea 54°17′N/7°30′E	50	f	91 → 53	405	342	316
					102 → 65	338	265	
					102 → 67	c	292	
2	cod (Gadus morhua)	North Sea 54°43′N/2°08′E	44	nd	91 → 53	99	95	121
					102 → 65	105	88	
					102 → 67	c	114	
3	North Sea dab (Limanda limanda)	North Sea 54°30′N/2°16′E	52	f	91 → 53	607	491	470
					102 → 65	521	503	
					102 → 67	c	581	
4	North Sea dab (Limanda limanda)	North Sea 56°17′N/2°05′W	54	f	91 → 53	357	172	24
					102 → 65	203	219	
					102 → 67	c	192	
5	North Sea dab (Limanda limanda)	North Sea 55°31′N/4°41′E	31	f	91 → 53	449	304	229
					102 → 65	258	324	
					102 → 67	c	267	
6	cod (Gadus morhua)	Baltic Sea 54°47′N/13°07′E	49	nd	91 → 53	535	440	332
					102 → 65	351	359	
					102 → 67	c	449	
7	flounder (Platichtys flesus)	Baltic Sea 54°46′N/13°18′E	33	m/f	91 → 53	138	197	282
					102 → 65	188	175	
					102 → 67	c	231	
8	flounder (Platichtys flesus)	Baltic Sea 54°46′N/13°18′E	33	m/f	91 → 53	na	99	342
					102 → 65		83	
					102 → 67		108	
9	North Sea dab (Limanda limanda)	Baltic Sea 54°32′N/10°39′E	42	f	91 → 53	na	146	218
					102 → 65		90	
					102 → 67		115	

[a] nd, not determined; na, not analyzed; f, female; m, male. [b] Values taken from Reth et al.[13] [c] The fragmentation m/z 102 → 67 was not used for the analysis by ion trap MS.

was performed with a technical sPCA mixture containing 55.5% Cl. The determined concentrations are compared in Table 3. Ion trap and triple quadrupole EI-MS/MS chromatograms from fish sample 3 are shown in Figure 4 as an example. s+mPCA concentrations varied between 99 and 607 ng/g (wet weight) for the ion trap and between 88 and 581 ng/g (wet weight) for the triple quadrupole.

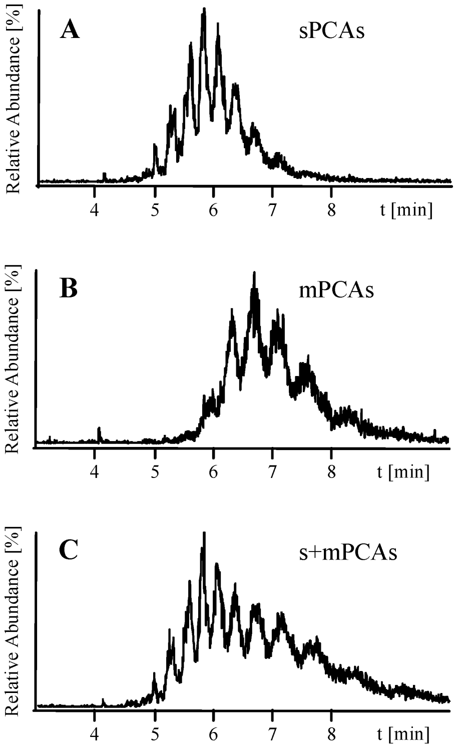

Figure 3. Triple quadrupole EI-MS/MS chromatograms recorded with the CID *m/z* 102 → 65 of a technical sPCA mixture with 55.5% Cl (A), of a technical mPCA mixture with 52.0% Cl (B), and of a 1+1 mixture (C).

Concentrations determined with different ion trap CIDs differed in maximum by 28% and in average by 15%. The triple quadrupole showed much more consistent results for the employed CIDs. Differences were <15% and often within 10% except for sample 9 (24%). The results of both MS techniques were comparable taking the deviations between the applied CIDs into account.

Moreover, the principal difference of robustness between ion trap and triple quadrupole mass spectrometers was confirmed. The latter achieved a far better precision and lower LODs than the ion trap. Deviations of parallel CID analysis of the same fish liver extract were <9% for the triple quadrupole and <21% for the ion trap.

The analysis of these samples by HRGC-ECNI-MS showed similar results for samples 1−3 and 5−7 (difference lower than 40%, Table 3) whereas considerable differences were observed for samples 4, 8, and 9.[13] However, ECNI-MS cannot be taken as the absolute reference for accuracy, since this method does not detect lower chlorinated PCAs, and as already shown by Tomy et al.[14] and later by Coelhan et al.,[15] the result of ECNI analysis is strongly dependent on the choice of the reference material giving errors of up to 100% or more.

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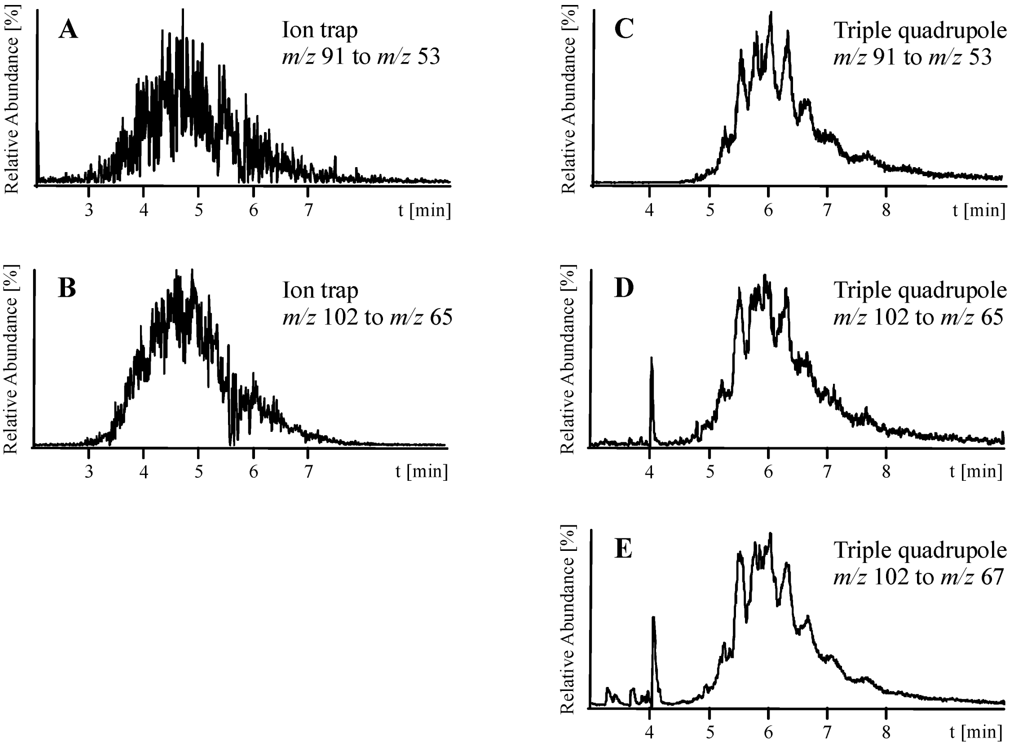

Figure 4. EI-MS/MS chromatograms of a North Sea dab liver extract (sample 3, Table 3) recorded by ion trap (A, B) and triple quadrupole (C, E). CIDs of m/z 91 → 53 (A, C), 102 → 65 (B, D), and 102 → 67 (E) were applied.

CONCLUSIONS

Both ion trap and triple quadrupole MS have proved to be suited for fast determination of PCAs in biota. However, PCA concentrations in biota showed to be close to the LOQ of the ion trap mass spectrometer. The triple quadrupole provided lower LODs and better reproducibility, which justifies the higher price of this instrument. In the future, our research group will apply the triple quadrupole for PCA screening in biota and sediments.

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Paper IV:

EVALUATION OF FOUR MASS SPECTROMETRIC METHODS FOR THE ANALYSIS OF POLYCHLORINATED *n*-ALKANES

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Evaluation of four mass spectrometric methods for the analysis of polychlorinated *n*-alkanes

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Abstract

The suitability of four mass spectrometric methods for the analysis of polychlorinated *n*-alkanes (PCAs, also called chlorinated paraffins) was evaluated and compared using spiked and fish liver samples. Electron ionization tandem mass spectrometry (EI–MS/MS) as well as electron capture negative ionization (ECNI) combined with low and high resolution mass spectrometry and CH_4/CH_2Cl_2-negative ion chemical ionization (NICI) low resolution mass spectrometry were investigated. All methods showed an accuracy of <21% for the analysis of spiked fish samples. However, the analysis of real samples showed deviations of up to 46% between the four mass spectrometric methods. The influence of the selected reference standard on quantification was also evaluated. The use of a quantification standard with a degree of chlorination deviating from that of the sample can result in differences of >100% for the ECNI methods. EI–MS/MS and CH_4/CH_2Cl_2-NICI led to errors of maximum 17% and 33%, respectively, independent from the degree of chlorination of the used reference standard.

Keywords: Polychlorinated *n*-alkanes; PCAs; Chlorinated paraffins; Intercomparison; Mass spectrometry

1. Introduction

Polychlorinated *n*-alkanes (PCAs) are highly complex technical mixtures, which are also known as chlorinated paraffins. These mixtures have a chlorination degree between 30% and 70% and a linear chain length of C_{10}–C_{13} (short chain PCAs or sPCAs, also called short chain chlorinated paraffins (SCCPs)), C_{14}–C_{17} (medium chain PCAs or mPCAs, also called medium chain chlorinated paraffins (MCCPs)) or $C_{>17}$ (long chain PCAs) [1]. PCAs are of concern due to their toxicological properties and their capability to bioaccumulate. As consequence of their widespread and unrestricted use and the properties mentioned above, PCAs are present in aquatic and terrestrial food webs in rural and remote areas [2–5].

Little attention has been paid to the analysis of these compounds in the past. Therefore, analytical methods and information about environmental levels are limited. However, recently sPCAs, have been included in the list of substances for priority action of the Convention for the Protection of the Marine Environment of the North-East Atlantic (The "OSPAR Convention") [6], in the list of priority dangerous substances of the European water framework directive [7,8] and in that of selected substances for immediate priority action of the Helsinki Commission (HELCOM) [9]. Consequently, environmental levels of PCAs should be monitored more extensively in the near future, which will require reliable analytical methods. Nowadays PCA analysis is mainly performed by electron capture negative ionization (ECNI) combined with high resolution (HRMS) [10] or low resolution mass spectrometry (LRMS) [11,12]. However, ECNI has some disadvantages: The use of different technical mixtures as quantification standards can lead to considerable deviations in the results [13,14]. Additionally, lower chlorinated PCAs ($Cl_{<5}$) are hardly detectable, and the data processing is quite time consuming.

Recently, alternative approaches for the mass spectrometric analysis of these compounds have been reported. A CH_4/CH_2Cl_2 gas mixture has been used as reagent for

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negative ion chemical ionization (NICI) in combination with LRMS [15]. The addition of dichloromethane to the reagent gas favored the formation of chlorine adduct ions and reduced the dependence of the response factors from the degree of chlorination. Similar results could also be achieved by Moore et al. using metastable atom bombardment as ionization technique [16]. Electron ionization tandem mass spectrometry (EI–MS/MS) was proposed for the simultaneous detection of all short and medium chain PCA congeners [17]. This approach is based on fragment ions with low mass-to-charge ratios, which are common to all (or most) PCAs and has the advantage, that the response factors of different PCA mixtures are not dependent on their degree of chlorination. Additionally, the use of ECNI–LRMS monitoring the $[HCl_2]^-$ and $[Cl_2]^-$ ions has been reported [18]. Similarly to EI–MS/MS this technique allows the determination of the total PCA concentration, but congener and homologue specific analysis is not possible. Moreover, liquid chromatography combined with chloride enhanced atmospheric pressure chemical ionization mass spectrometry was used for the analysis of PCAs [19]. However, the suitability of this technique for environmental samples was not proven yet.

The aim of this work was to investigate the comparability of results obtained by high resolution gas chromatography coupled to ECNI–HRMS, ECNI–LRMS, CH_4/CH_2Cl_2-NICI–LRMS and EI–MS/MS and to point out advantages and limitations of these techniques. The comparison was carried out with both spiked fish tissue and fish liver samples from the North Sea and Baltic Sea.

2. Experimental

2.1. Chemicals and solvents

Technical sPCA (chlorine contents of 51.5%, 55.5% and 63.0%) and mPCA mixtures (chlorine contents of 42.0%, 52.0% and 57.0%) at concentrations of 100 ng/μl in cyclohexane were purchased from Ehrenstorfer (Augsburg, Germany). $^{13}C_{10}$-*trans*-chlordane (99%) at a concentration of 100 ± 10 ng/μl in nonane was obtained from Cambridge Isotope Laboratories (Andover, USA). Cyclohexane, dichloromethane and *n*-hexane for pesticide residue analysis were purchased from Scharlau (Barcelona, Spain). Florisil®PR (60–100 mesh) and sodium sulfate (Pestanal®) were obtained from Fluka (Buchs, Switzerland) and silica gel (200–400 mesh, 0.035–0.070 mm) from CU Chemie Uetikon AG (Uetikon, Switzerland). Before use, all three chemicals were treated at 600 °C overnight and afterwards kept for 6 h at 130 °C.

2.2. Environmental samples

Fish samples were collected in August and September 2002 by the German Federal Research Center for Fisheries (liver samples 1–3: cod (*Gadus morhua*) captured at 54°51′N and 14°01′E; liver samples 4 and 5: North Sea dab (*Limanda limanda*) captured at 54°31′N and 10°39′E and 55°30′N and 4°40′E, respectively). Spiked samples were prepared adding 1500 ng of a technical sPCA mixture (55.5% Cl content), or 1500 ng of each of sPCAs (55.5% Cl content) and mPCAs (52% Cl content) to 8–10 g of homogenized mackerel muscle. The clean-up method is described in brief, since more details have been published elsewhere [12,20]. Samples were homogenized with a tenfold excess of Na_2SO_4. $^{13}C_{10}$-*trans*-chlordane (10 ng, internal standard) in 10 μl of cyclohexane were added and the sample column-extracted with 250 ml of *n*-hexane/CH_2Cl_2 (1 + 1, v/v). The clean-up of the extracts included removal of lipids by column chromatography on 40 g of silica gel impregnated with 44% H_2SO_4. The lipid-free sample was eluted with 120 ml of *n*-hexane/CH_2Cl_2 (1 + 1, v/v). Then, adsorption chromatography on 16 g of Florisil® (1.5% H_2O water content) was carried out with 60 ml of *n*-hexane (fraction 1), with 7 ml of CH_2Cl_2 (fraction 2) and with 60 ml of CH_2Cl_2 (fraction 3). The last one contained all PCAs.

2.3. Instrumentation

ECNI–HRMS analysis was performed on a VG AutoSpec (Micromass, Manchester, UK) coupled to a HP 5890II (Hewlett-Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (25 m length, 0.20 mm i.d.) coated with a 0.33 μm thick film of HP-1 (dimethylpolysiloxane, J&W Scientific, Folsom, USA) and 1 μl was injected in the splitless mode. The injector temperature was 260 °C. Helium (99.999%) was used as carrier gas at an inlet pressure of 137.8 kPa (20 psi). The temperature program was as follows: 150 °C isothermal for 2 min, then 7 °C/min to 260 °C, isothermal for 8 min, increased to 280 °C at 10 °C/min and then isothermal for 13 min. The mass spectrometer was operated in the ECNI mode using argon as reagent gas at a source housing pressure of 2×10^{-5} mbar. A resolution of 12,000 at an acceleration voltage of 6 kV was employed. The temperature of the ion source was set to 170 °C, the filament emission current was set to 0.3–1 mA and the electron energy was in the range 25–40 eV. Perfluorokerosene was used as calibration compound. The most abundant isotopes of the $[M - Cl]^-$ ions of PCAs and of the $[M]^-$ and $[M - 4Cl - 2H]^-$ ions of $^{13}C_{10}$-*trans*-chlordane were detected in the selected ion monitoring (SIM) mode with dwell times of 50 ms each [10].

For the ECNI–LRMS and the CH_4/CH_2Cl_2-NICI–LRMS analysis, gas chromatographic separations were performed on a HP 5890II (Hewlett-Packard, Palo Alto, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m length, 0.25 mm i.d.) coated with a 0.25 μm thick film of DB5-MS (5% crosslinked phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA) and 1.5 μl were injected in the splitless mode. The injector temperature was 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was used as carrier gas at an inlet pressure of 68.9 kPa (10 psi). The temperature program was as follows: 100 °C isothermal for 2 min, then 10 °C/min to 260 °C and isothermal for 10 min. A MS Engine HP 5989B (Hewlett-Packard, Palo Alto, USA) was employed in the negative ion mode. The transfer line temperature was kept at 275 °C, the ion source temperature at 200 °C and the quadrupole temperature at 100 °C. Methane (99.995%, Carbagas, Basel, Switzerland) was used at a reagent gas pressure of 1.0–1.6 mbar (0.8–1.2 Torr) in ECNI. The most abundant isotopes of the $[M-\mathrm{Cl}]^-$ ions of PCAs and of the $[M]^-$ ion of $^{13}C_{10}$-*trans*-chlordane were detected in the SIM mode with dwell times of 75 ms each [11]. For the CH_4/CH_2Cl_2-NICI–LRMS analysis, dichloromethane was introduced through a modified transfer line. The reagent gas pressure was 2.0 mbar (1.5 Torr) and the CH_4/CH_2Cl_2 ratio was 80 + 20. The $[M+\mathrm{Cl}]^-$ ions of PCAs and of the $[M]^-$ ion of $^{13}C_{10}$-*trans*-chlordane were detected in the SIM mode with dwell times of 75 ms each using the most abundant isotope signals[15].

For EI–MS/MS analysis, a 1200 triple quadrupole MS (Varian, Wallnut Creek, USA) was employed. Gas chromatographic separations were performed on a CP-3800 (Varian, Wallnut Creek, USA) gas chromatograph equipped with a fused silica capillary column (15 m length, 0.25 mm i.d.) coated with 0.25 μm of DB-5MS (5% crosslinked phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). The injector temperature was set to 275 °C. Helium was used as carrier gas at a constant flow of 2 ml/min (linear flow velocity of 74 cm/s). The temperature program was as follows: 100 °C isothermal for 1 min, then 50 °C/min to 300 °C and isothermal for 4 min. Splitless injections (splitless time, 1.5 min) of 2.5 μl volume were carried out with a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland). The transfer line temperature was set to 275 °C, the ion source temperature to 200 °C and the manifold temperature to 40 °C. Conditions for EI–MS/MS were as follows: 70 eV electron energy; emission current, 300 μA; dwell time, 50 ms; resolution of Q1 at 0.8 and of Q3 at 1.2; and argon as CID gas at 0.12–0.15 Pa (0.9–1.1 mTorr). PCAs were detected using the *m*/*z* $102 \rightarrow 67$ (collision energy, −10 V), $102 \rightarrow 65$ (collision energy, −18 V) and $91 \rightarrow 53$ (collision energy, −10 V) fragmentation reactions. The precursor ion *m*/*z* 383 $[M-\mathrm{Cl}]^+$ and the product ion *m*/*z* 276 $[M-4\mathrm{Cl}]^+$ were chosen for $^{13}C_{10}$-*trans*-chlordane (collision energy, −21 V).

2.4. Quantification

PCA quantification was performed with a technical sPCA mixture (55.5% Cl content) and a technical mPCA mixture (52% Cl content) for all four methods. The detailed procedure for ECNI–MS has been previously described by Tomy et al. [10]. This quantification method was also applied for CH_4/CH_2Cl_2-NICI–LRMS. Additionally, the problems due to mass overlap were reduced for LRMS as described by Reth and Oehme [11]. The technical sPCA mixture with 55.5% chlorine content was used as reference for the determination of the total s + mPCA concentration by EI–MS/MS.

3. Results and discussion

3.1. Limits of detection

The technical details of all detection methods applied in this work are described in detail elsewhere [10,11,15,17]. Therefore, only a description of their performance is given here. PCA quantification by ECNI–HRMS has been extensively described by Tomy et al. [10]. The authors reported limits of detection (LODs) of 60 pg/μl of technical sPCA mixture (70% Cl content) for their instrumentation. This sensitivity could not be achieved on the high resolution mass spectrometer used in this work, which showed an LOD of 1 ng/μl of sPCA mixture with a degree of chlorination of 55.5%. The explanation for this deviation is the lower degree of chlorination of the sPCA standard used in this work. It is well known, that the congener response increases with the degree of chlorination under ECNI conditions. Consequently, a higher chlorinated PCA mixture leads to a higher response for the main congeners. The LODs for ECNI–LRMS and for CH_4/CH_2Cl_2-NICI–LRMS were 1 ng/μl of sPCA mixture with a chlorination degree of 55.5% (detection of the most abundant congeners). EI–MS/MS showed lower LODs in the range of 0.15–0.3 ng/μl of sPCA mixture (55.5% Cl content) depending on the selected fragmentation.

3.2. Analysis of standards and spiked samples

Two standards were used to evaluate the quantification of PCAs and a possible interference by mPCAs. Standard one contained 1500 ng of a sPCA mixture (55.5% Cl content) and standard two 1500 ng of the sPCA and 1500 ng of the mPCA mixture (52% Cl content). The results are summarized in Table 1. Interday reproducibility (five measurements over several months) was 8% or less for all techniques. For standard 1, all four techniques led to an acceptable result (bias < 10%). For standard 2, ECNI–LRMS did not allow to eliminate completely the interferences from mPCAs with similar mass-to-charge ratios to sPCAs. Therefore, the deviation from the expected amount was higher (14%). However, also the bias of ECNI–HRMS increased (24%) despite the higher resolution. The reason of this deviation is not clear. In contrast, the determined mPCA amount was lower than expected for both ECNI techniques, leading to a good accuracy for the overall content. CH_4/CH_2Cl_2-NICI–LRMS allowed to determine both sPCAs and mPCAs with good accuracy in the two standards. Also EI–MS/MS showed errors below 10%.

To evaluate the interference of matrix, three mackerel muscle samples (s + mPCAs < 1 ng/g) were spiked with PCAs (samples 1 and 2 with 1500 ng sPCAs and sample 3 with 1500 ng of sPCAs and mPCAs each). The results

Table 1
Quantification of s- and mPCAs in standard solutions using the four mass spectrometric methods (single measurement)

Compound	Expected (ng)	ECNI–HRMS (ng, %)	ECNI–LRMS (ng, %)	CH_4/CH_2Cl_2-NICI–LRMS (ng, %)	EI–MS/MS (ng, %)
Standard 1					
sPCAs	1500	1350 (10)	1590 (6)	1590 (6)	1420 (5)
Standard 2					
sPCAs	1500	1870 (24)	1710 (14)	1590 (6)	n.p.
mPCAs	1500	1140 (24)	1370 (9)	1510 (1)	n.p.
s + mPCAs	3000	3010 (1)	3080 (3)	3100 (3)	2760 (8)

n.p., not practicable. Standard 1 contained 1500 ng of sPCAs with 55.5% Cl content and standard 2 contained 1500 ng of sPCAs and mPCAs each with Cl contents of 55.5% and 52%, respectively. The relative deviations (%) from the expected values are given in parenthesis.

Table 2
Quantification of s- and mPCAs in spiked fish samples using the four mass spectrometric methods (single measurement)

Compound	Expected (ng)	ECNI–HRMS (ng, %)	ECNI–LRMS (ng, %)	CH_4/CH_2Cl_2-NICI–LRMS (ng, %)	EI–MS/MS (ng, %)
Sample 1					
sPCAs	1500	1720 (14)	1560 (5.7)	1810 (20)	1610 (7)
Sample 2					
sPCAs	1500	1190 (21)	1610 (7.2)	1300 (13)	1580 (5)
Sample 3					
sPCAs	1500	1730 (15)	1750 (17)	1290 (14)	n.p.
mPCAs	1500	1360 (9)	1510 (1)	1450 (3)	n.p.
s + mPCAs	3000	3090 (3)	3260 (9)	2740 (9)	2950 (2)

n.p., not practicable. Samples 1 and 2 contained 1500 ng of sPCA with 55.5% Cl content and sample 3 contained 1500 ng of sPCAs and mPCAs each with Cl contents of 55.5% and 52%, respectively. The relative deviations (%) from the expected values are given in parenthesis.

obtained with the different techniques are summarized in Table 2. Interday reproducibility (five measurements over several months) of ECNI–LRMS for these samples was <6% for sPCAs and <9% for mPCAs. CH_4/CH_2Cl_2-NICI–LRMS had an interday reproducibility of <13% for sPCAs and <12% for mPCAs whereas EI–MS/MS had an interday reproducibility <10%. Most results showed errors of 15% or less. Only for CH_4/CH_2Cl_2-NICI–LRMS and sample 1 as well as ECNI–HRMS and sample 2, the deviation was ca. 20%. The analysis of sample 3 containing both sPCAs and mPCAs showed similar results for all three s/m-PCA specific techniques (errors of ca. 15% for the determination of sPCAs and of ca 10% for mPCAs). The total PCA concentration determined by EI–MS/MS was in good agreement with the results obtained with the other techniques.

3.3. Analysis of fish samples

Since the results obtained for spiked samples showed good accuracy and repeatability with all detection methods, five fish liver samples were cleaned-up and analyzed. The results are summarized in Table 3. Usually, total PCA concentration in fish liver from the North and Baltic Sea is between 20 and 600 ng/g wet weight [20].

The total PCA concentration obtained by the different techniques showed a relative standard deviation of 30% or less for all fish liver samples. This error is acceptable taking into account that the interday reproducibility (five measurements) for these samples was <12% for sPCAs and <18% for mPCAs (ECNI–LRMS), <28% for sPCAs and <10% for mPCAs (CH_4/CH_2Cl_2-NICI–LRMS) and <20% for s + mPCAs (EI–MS/MS).

The differences between the results of ECNI–LRMS and ECNI–HRMS were in the range of the interday reproducibility. The determination of mPCAs showed good agreement between low and high resolution ECNI–MS for samples 2 and 3. For sample 1, a slightly higher mPCA concentration was obtained by ECNI–LRMS. This might be explained by possible interferences by other compounds not resolved by LRMS. The analysis of sPCAs by ECNI–LRMS was not possible for sample 3, since it contained too much lipid (>6 g), which overloaded the clean-up method. Therefore, the extract contained a considerable amount of polychlorinated compounds like PCBs, toxaphenes and chlordanes interfering the ECNI–LRMS analysis. The detection of mPCAs in sample 3 was possible even by ECNI–LRMS, since the medium chained PCA congeners form ions with mass-to-charge ratios different from those of the interfering compounds. Samples 4 and 5 were not analyzed by ECNI–HRMS, which was not available in-house.

Most PCA concentrations determined by CH_4/CH_2Cl_2-NICI–LRMS were lower than those determined by the ECNI methods. Only in sample 5, the sPCA concentration obtained by CH_4/CH_2Cl_2-NICI–LRMS was higher. This technique considerably suppresses the ionization of other compounds, so that sPCA analysis was possible for sample 3. Also EI–MS/MS was not disturbed by interfering compounds in the extract. Fig. 1 shows the mass chromatograms for the sPCA congener $C_{12}H_{14}Cl_7$ in sample 3 obtained by the different detection methods.

Table 3
Quantification of s- and mPCAs in fish samples using the four mass spectrometric methods

Compound	ECNI–HRMS (ng/g)	ECNI–LRMS (ng/g)	CH_4/CH_2Cl_2-NICI–LRMS (ng/g)	EI–MS/MS (ng/g)	Mean (ng/g)	Relative standard deviation (%)
Sample 1						
sPCAs	57	43	21	n.p.	40	
mPCAs	52	75	40	n.p.	56	
s + mPCAs	109	118	61	84	93	28
Sample 2						
sPCAs	30	19	23	n.p.	21	
mPCAs	21	25	25	n.p.	24	
s + mPCAs	51	44	48	59	48	18
Sample 3						
sPCAs	13	[a]	20	n.p.	24	
mPCAs	77	76	37	n.p.	82	
s + mPCAs	90	n.p.	57	66	94	27
Sample 4						
sPCAs	n.a.	48	42	n.p.	45	
mPCAs	n.a.	130	61	n.p.	96	
s + mPCAs	n.a.	178	103	117	133	30
Sample 5						
sPCAs	n.a.	37	141	n.p.	89	
mPCAs	n.a.	221	112	n.p.	167	
s + mPCAs	n.a.	258	253	298	270	9

n.a., not analysed; n.p., not practicable.

[a] ECNI–LRMS determination of sPCAs in this sample was not possible due to an overloaded clean-up.

3.4. Problems of quantification

The PCA concentrations in fish liver obtained with the different methods varied more than for the spiked samples (ca. 30% compared to 15%). One possible reason could be, that the same PCA mixture was added to the spiked samples and used for quantification. It has already been shown that the congener composition strongly varies between different technical products and, in addition, in the environment. Tomy et al. [13] and later Coelhan et al. [14] showed that the use of PCA mixtures with a different degree of chlorination as quantification standard for ECNI may give deviations of up to 100%. Table 4 summarizes the data of the analysis of three standards of sPCAs with a different degree of chlorination against different PCAs mixtures used as reference. As can be seen, ECNI causes huge errors (>100%), due to the difference in the degree of chlorination between sample and standard material. CH_4/CH_2Cl_2-NICI–LRMS showed a lower dependency reducing the errors to <32%. This is a considerable advantage, since a perfect match between the chlorine content of standard and sample is less important. Finally, the best results were obtained by EI–MS/MS. This technique is not influenced by the degree of chlorination of the quantification standard, and errors were <17% even when extremely different PCA mixtures were selected as standard. This makes EI–MS/MS, originally developed for screening, very attractive for the quantitative determination of total PCA concentrations, although it cannot be applied for the study of congener patterns and is unable to differentiate between short and medium chained PCAs.

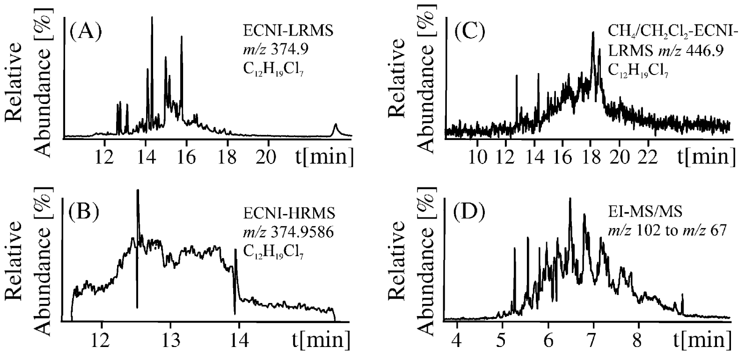

Fig. 1. Mass chromatograms for the congener $C_{12}H_{19}Cl_7$ in sample 3. Quantification of sPCAs was not possible by ECNI–LRMS due to an overloaded clean-up (A). ECNI–HRMS (B); CH_4/CH_2Cl_2-NICI–LRMS (C) and EI–MS/MS for the fragmentation *m/z* 102–67 (D) had a sufficient selectivity for quantification. The chromatograms were obtained with different chromatographic conditions.

Table 4
Quantification of three sPCA solutions using different sPCAs as reference standard

Standard	51[a]		55[a]		63[a]	
Sample	55[b]	63[b]	51[b]	63[b]	51[b]	55[b]
ECNI–LRMS	4300 (190)	15600 (940)	522 (65)	5440 (260)	143 (90)	413 (72)
CH_4/CH_2Cl_2-NICI–LRMS	1770 (18)	1210 (19)	1270 (15)	1030 (31)	1860 (24)	1980 (32)
EI–MS/MS	1590 (6)	1450 (3)	1430 (5)	1323 (12)	1700 (14)	1750 (17)

The spiked amount was 1500 ng for all solutions. The relative errors (%) from the expected result are given in parenthesis.

[a] Sample: sPCA, %Cl.

[b] Standard: sPCA, %Cl.

3.5. Congener patterns

One important step of the quantification of PCAs as described by Tomy et al. is the determination of the congener and homologue composition, which also gives important information about differences in environmental samples [10]. EI–MS/MS does not provide this kind of information, but all other techniques do. Fig. 2 shows the congener pattern of a sPCA mixture (55.5% Cl content) obtained with ECNI–HRMS, ECNI–LRMS and CH_4/CH_2Cl_2-NICI–LRMS. The correction factors described by Tomy et al. were applied for the ECNI methods. They were not used the CH_4/CH_2Cl_2-NICI–LRMS, since the response of different congeners is not dependent on the number of chlorine atoms.

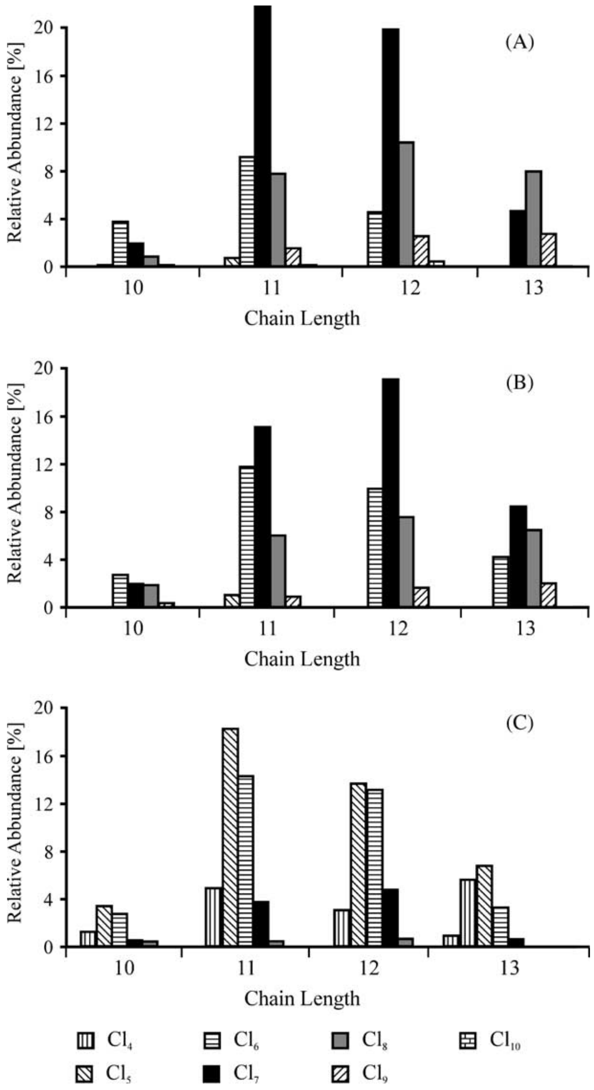

Fig. 2. sPCA congener and homologue patterns of a technical sPCA mixture with a degree of chlorination of 55.5% determined by ECNI–HRMS (A); ECNI–LRMS (B) and CH_4/CH_2Cl_2-NICI–LRMS (C).

As can be seen from Fig. 2, C_{12} was the main homologue group for ECNI–LRMS and C_{11} for ECNI–HRMS. Additionally, some differences were observed in the pattern of the same homologue group. It is not possible to say which technique gave the true composition. However, both methods allow to chose a quantification standard with a pattern close to the sample, which is important to reduce the error of the quantification. The use of the CH_4/CH_2Cl_2 reagent gas mixture allowed also the detection of lower chlorinated compounds ($Cl_{4–5}$). Therefore, and since there is no increase in the response factors for higher chlorinated congeners, the congener distribution is shifted to lower chlorinated congeners. Since the real composition of PCA mixtures is unknown, it is not possible to say which technique gives the true composition. Moreover, a direct comparison of the congener patterns obtained by conventional ECNI and by CH_4/CH_2Cl_2-NICI may lead to erroneous conclusions.

4. Conclusions

All mass spectrometric methods were comparable for the analysis of standards and spiked samples giving results with satisfying accuracy. Variability of the results was reduced to the instrumental methods, since the same solutions were analyzed and used for quantification. However, for the analysis of fish liver some differences in the results were observed. Main reasons are probably a “not perfect” matching of the PCA composition of the sample and the reference material as well as the variable influence of the degree of chlorination on the response factors. This problem can have a strong influence on the results obtained by ECNI–MS. Consequently, a careful selection of the quantification standard is necessary when ECNI is applied. EI–MS/MS and CH_4/CH_2Cl_2-NICI–MS showed little dependency on the degree of chlorination of the standard used for quantification, which is a considerable advantage for these methods. Moreover, comparable results were obtained by HRMS and LRMS. This confirms that interferences between PCAs forming ions with the same *m*/*z* can be reduced even if LRMS is applied. Finally, ECNI–LRMS requires more thorough sample clean up than the other methods.

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Paper V:

CHLORIDE-ENHANCED ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY OF POLYCHLORINATED *n*-ALKANES

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Chloride-enhanced atmospheric pressure chemical ionization mass spectrometry of polychlorinated *n*-alkanes

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The use of high-performance liquid chromatography combined with chloride-enhanced atmospheric pressure chemical ionization for the determination of polychlorinated *n*-alkanes (PCAs, also called chlorinated paraffins or CPs) is described as an alternative to gas chromatographic methods. Atmospheric pressure chemical ionization in the negative ion mode formed exclusively $[M+Cl]^-$ adduct ions and suppressed fragmentation when a chlorinated solvent was added. Limits of detection were 1–2 ng/µL for technical PCA mixtures. Response factors for single short-chain PCA homologues with different degrees of chlorination varied by not more than a factor of 6.5. The developed method was applied for the determination of the composition of technical PCA mixtures as well as for the analysis of PCAs in household commodities. Medium-chain PCAs were found in paint samples at concentrations of 8.2–11.5% (w/w), compared with 7.4–11.5% obtained by gas chromatography combined with electron ionization tandem mass spectrometry. Copyright © 2004 John Wiley & Sons, Ltd.

The introduction of atmospheric pressure ionization techniques has led to an amazing development of liquid chromatography combined with mass spectrometry (LC/MS).[1] However, routine application of electrospray ionization (ESI) and of atmospheric pressure chemical ionization (APCI) has been mainly focused on polar compounds. The analysis of nonpolar and semipolar compounds still poses challenges.[2]

During recent years, different strategies have been developed to ionize nonpolar compounds by ESI or APCI.[3] These are coordination ion spray ionization,[4] on-line electrochemical conversion,[5] and atmospheric pressure electron capture negative ionization.[6] Moreover, a careful choice of the composition of the mobile phase is essential.[7] In this way, nonpolar compounds such as polycyclic aromatic compounds[7,8] or hexabromocyclododecane have been detected.[9]

Polychlorinated *n*-alkanes (PCAs), also called chlorinated paraffins, are complex technical mixtures with a chlorination degree between 30 and 70% and a linear chain length of C_{10}–C_{13} (short-chain PCAs or sPCAs), C_{14}–C_{17} (medium-chain PCAs or mPCAs) or $>C_{17}$ (long-chain PCAs of lPCAs). These mixtures contain a huge number of structural isomers, diastereomers and enantiomers (theoretically, more than 10 000 compounds[10]), which explains the complexity of the resulting technical products. Due to their chemical and physical properties, PCAs find application as fire retardants, plasticizers in PVC, adhesives, and as extreme pressure additives in lubricants and cutting oils.[11] Due to continuous release into the environment during the past decades, PCAs can be found in aquatic and terrestrial food webs in rural and remote areas. Most PCA analyses have been performed by high-resolution gas chromatography (HRGC) coupled to electron capture negative ionization (ECNI) mass spectrometry (MS),[12,13] although alternatives based on the use of electron ionization tandem mass spectrometry (EI-MS/MS)[14] and of metastable atom bombardment[15] have been reported. The routinely applied HRGC/ECNI-MS methods are time-consuming, and quantification is strongly dependent on the degree of chlorination of the standard.[16]

To our knowledge, PCAs have never been analyzed by LC/MS before. Due to the low polarity of these compounds, conventional ESI or APCI is not suited for their ionization. Recently, our group showed that the use of a CH_4/CH_2Cl_2 reagent gas mixture for negative ion chemical ionization (NICI) enhanced the formation of chloride adduct ions of PCAs.[17] Major benefits were suppression of the ionization of other interfering chlorinated compounds, and response factors less dependent on the degree of chlorination. Generally, the ionization processes of APCI are quite similar to those of classical chemical ionization.[18] Therefore, the chloride adduct formation observed in NICI should also take place under APCI conditions.

This work had the aim to investigate the formation of $[M+Cl]^-$ adducts of PCAs under chloride-enhanced APCI (Cl^--APCI) conditions and to overcome some of the limitations of the HRGC/MS methods. The applicability of

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Cl^--APCI for routine analysis of PCAs was evaluated. Details are given about method optimization, detection limits as well as linear range. Aspects complementary to conventional CH_4-ECNI and CH_4/CH_2Cl_2-NICI are also discussed. Finally, the composition of technical mixtures and PCA concentrations in household commodities were determined to demonstrate the applicability of Cl^--APCI.

EXPERIMENTAL

Chemicals and solvents

Single PCA congeners (2,5,6,9-tetrachlorodecane, 1,2,9,10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane and 1,2,5,6,9,10-hexachlorodecane) as well as several technical sPCA mixtures (51.5, 55.5 and 63.0% Cl content), mPCA mixtures (42.0, 52.0% and 57.0% Cl content) and lPCA mixtures (36.0, and 49.0% Cl content) with concentrations of 100 ng/μL in cyclohexane were purchased from Ehrenstorfer (Augsburg, Germany). Standard solutions of PCAs with a defined chain length (C_{10} PCAs of 50, 60, and 69.8% Cl content; C_{11} PCAs of 50, 60, and 70.4% Cl content; C_{12} PCAs of 50 and 70% Cl content; and C_{13} PCAs of 50, 60, and 70% Cl content) were kindly provided by Dr. Coelhan from the Technical University of Munich. A solution of ε-hexachlorocyclohexane at a concentration of 10 ng/μL in cyclohexane was purchased from Ehrenstorfer and used as internal standard for HRGC/EI-MS/MS analysis. A solution containing diethylhexylphthalate (40 ng/μL), dibutylphthalate (20 ng/μL), diisononylphthalate (1000 ng/μL), diisodecylphthalate (1000 ng/μL), benzylbutylphthalate (45 ng/μL) and di-*n*-octylphthalate (45 ng/μL) in cyclohexane was prepared from pure substances purchased from Fluka (Buchs, Switzerland) and Sigma-Aldrich (St. Louis, MI, USA). Cyclohexane, dichloromethane, ethyl acetate and acetone, all of pesticide residue analysis grade (>99.8%), were purchased from Scharlau (Barcelona, Spain) and chloroform (>99.8%) from J. T. Becker (Deventer, The Netherlands).

Household commodities samples

Paint for swimming pools, paint for garage floors, window putty, a cable conduit, a cable duct, children's inflatable balloons and ink for computer printers were purchased from a local supermarket. Except for the ink, all samples gave a positive flame test for halogens in the presence of copper (green color of the flame). The soluble samples (ink, paints and putty; 5–25 mg) were dissolved in 5 mL of dichloromethane, and filtered through a 0.45 μm PTFE filter (Sri Scientific, New Brunswick, USA). The other samples were cut into fine pieces, and 0.2–0.6 g were extracted with 60 mL of acetone/dichloromethane/ethyl acetate (1:1:1, v/v) overnight. The extract was filtered through a 0.45 μm PTFE filter. The filtrates were washed with 60 mL of dichloromethane, and the resulting solution was evaporated to dryness. Finally, the residues were taken up in 5 mL of dichloromethane. These solutions were injected directly for HPLC/MS analysis, whereas 10 ng of ε-hexachlorocyclohexane (internal standard) in 10 μL of cyclohexane were added to 200 μL of this extract prior to HRGC/EI-MS/MS. PCA recoveries for soluble samples were determined by adding 37.5 μg sPCAs (55.5% Cl content) to 5 mL dichloromethane and treating this solution as described above. PCA recoveries of nonsoluble samples were determined by spiking 37.5 μg sPCAs (55.5% Cl content) into 300 mg of a low-density polyethylene plastic bag containing no PCAs. Sample preparation was carried out as described above.

Instrumentation

HPLC separations were performed on a column of 250 mm length and 4.6 mm i.d. packed with Nucleosil silica gel (5 μm particles, 100 Å pore size; Macherey-Nagel, Germany). Samples were injected with a PAL autosampler (CTC Analytics, Switzerland) overfilling twice an external loop of 20 μL and using dichloromethane as rinsing solution. Several eluents were evaluated for HPLC (e.g. *n*-heptane, tetrahydrofuran, dichloromethane, *n*-heptane/chloroform 7:3 v/v, cyclohexane/chloroform 7:3 v/v at flow rates in the range 0.5–1.0 mL/min). If not present in the eluent, chloroform (or dichloromethane) was added post-column with a HP 1050 (Hewlett Packard, Palo Alto, USA) pump at a flow rate of 0.3 mL/min using a T-connection. The application of chloroform as mobile phase at a flow rate of 0.8 mL/min was the simplest and gave results comparable to the other eluents for the ionization of PCAs. A Rheos 2000 pump (Flux Instruments, Switzerland) was used for all experiments.

An ion trap mass spectrometer (LCQ; Thermo Finnigan, Palo Alto, USA) was employed in the APCI negative ion mode. The instrument was tuned for maximum yield of the most abundant isotope signals of the chloride adduct ion of $C_{13}H_{22}Cl_6$ (*m/z* 424.9) using an sPCA mixture with 51.5% Cl content. The following source parameters were applied: nitrogen sheath gas flow, 50 arbitrary units (ca. 12.5 L/min); auxiliary gas flow, 10 arbitrary units (ca. 3.5 L/min); ionization current of the corona discharge, −5 μA; heater temperature, 200°C; and heated capillary at 150°C and −5 V. The scan range was set to *m/z* 200–800.

To control enhancement or suppression of the ionization of PCAs by other compounds present in the samples, the extract of a polyethylene bag and 43 ng of a mixture of diethylhexylphthalate, dibutylphthalate, diisononylphthalate, diisodecylphthalate, benzylbutylphthalate and di-*n*-octylphthalate were injected on the HPLC column. A solution of 100 ng/μL lPCA was added post-column at a flow rate of 25 μL/min with a syringe pump during the HPLC separation.

Data obtained by GC combined by EI-MS/MS[14,16] or ECNI-MS,[13,16] and CH_4/CH_2Cl_2-NICI-MS,[16,17] were compared with the newly developed method. Detailed descriptions of these analytical techniques are given in the references.

RESULTS AND DISCUSSION

Selection of the Cl^- source

Previous studies using CH_2Cl_2 as reagent gas for NICI demonstrated the ability of CPs to form $[M+Cl]^-$ ions. They can be used for quantification if an excess of chloride anions is present.[17] This could also be confirmed for APCI-MS using different concentrations (30–100%) of CH_2Cl_2 or $CHCl_3$ in the mobile phase or adding it post-column via a T-connection. The ionization yield obtained with the different eluents was comparable. Finally, $CHCl_3$ was preferred and used as

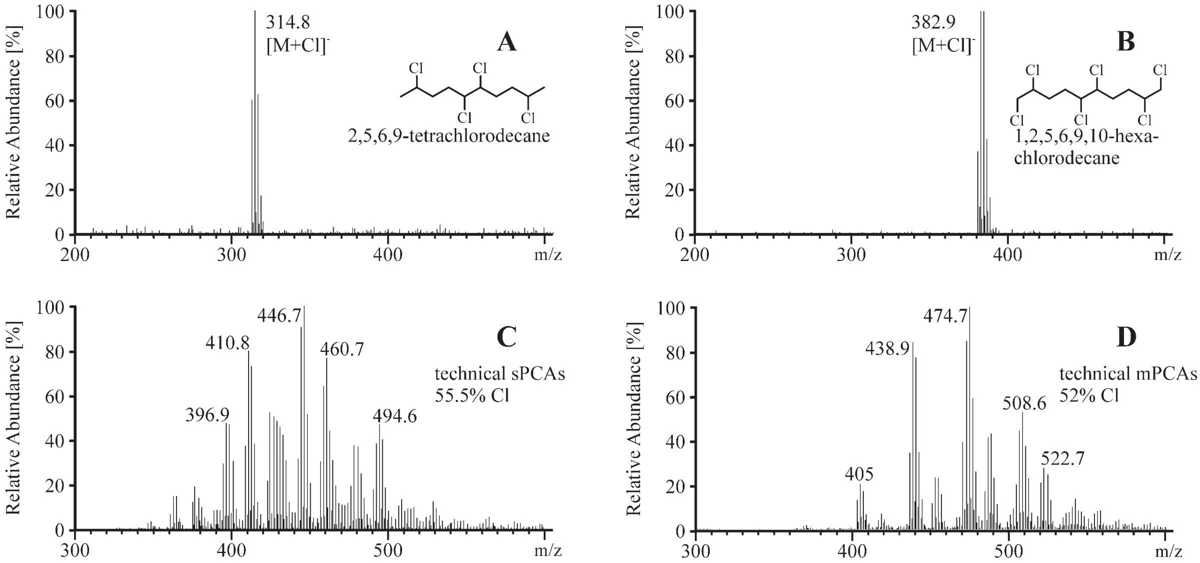

Figure 1. Cl^--APCI(−) mass spectra of two sPCA congeners (A: 2,5,6,9-tetrachlorodecane, B: 1,2,5,6,9,10-hexachlorodecane), an sPCA mixture with 55.5% Cl content (C) and a mPCA mixture with 52% CP content (D).

eluent for normal-phase chromatography, since it provided good chromatography and the simplest instrumental setup.

Ionization of single congeners

In contrast to conventional ECNI forming $[M{+}Cl]^-$, $[M{-}Cl]^-$ and $[M{-}HCl]^-$, Cl^--APCI gave exclusively $[M{+}Cl]^-$ adducts (see Fig. 1 for examples). This resulted in an increased selectivity and reduced mass interferences between PCA congeners (e.g. $[M{+}Cl]^-$ of $C_{11}H_{20}Cl_4$ and $[M{-}Cl]^-$ of $C_{11}H_{18}Cl_6$ have both *m*/*z* 327). The response factors of different single congeners such as 2,5,6,9-tetrachlorodecane, 1,2,9,10-tetrachlorodecane, 1,2,5,6,9-pentachlorodecane and 1,2,5,6,9,10-hexachlorodecane varied by a factor of 5 compared with >20 for ECNI and 2.4 for CH_2Cl_2-NICI (response factors calculated on mass basis using the signal of the most intensive isotope).

Analysis of technical mixtures

The response factors of technical mixtures (calculated on mass basis using the sum of the signals of all congeners) were less dependent on the degree of chlorination than observed for conventional ECNI. They varied by a factor <3 between sPCA mixtures of 51, 55.5 and 63% chlorine content compared with 9 for conventional ECNI and 1.4 for CH_4/CH_2Cl_2-NICI. Figure 2 shows the homologue and congener distribution obtained for sPCA, mPCA and lPCA mixtures (average of five measurements). A relative distribution of the homologue groups (C_{14}, C_{15}, etc.) similar to ECNI was observed for mPCA mixtures. However, compared with CH_4/CH_2Cl_2-NICI and ECNI, a higher abundance of longer chained homologues was found for sPCA mixtures. In contrast to CH_4/CH_2Cl_2-NICI, no lower chlorinated congeners (Cl_{3-4}) could be detected in the technical PCA mixtures.

Solutions of single sPCA homologues (C_{10}, C_{11}, C_{12} and C_{13}) with different degrees of chlorination (50, 60 and 70% Cl content) were analyzed to investigate the dependence of the response factor on the length of the carbon chain and on the degree of chlorination. Figure 3 shows the response factors obtained for different sPCA homologues with a degree of chlorination of 50 and 60%. The response factor of the homologues with a degree of chlorination of 50% varied by a

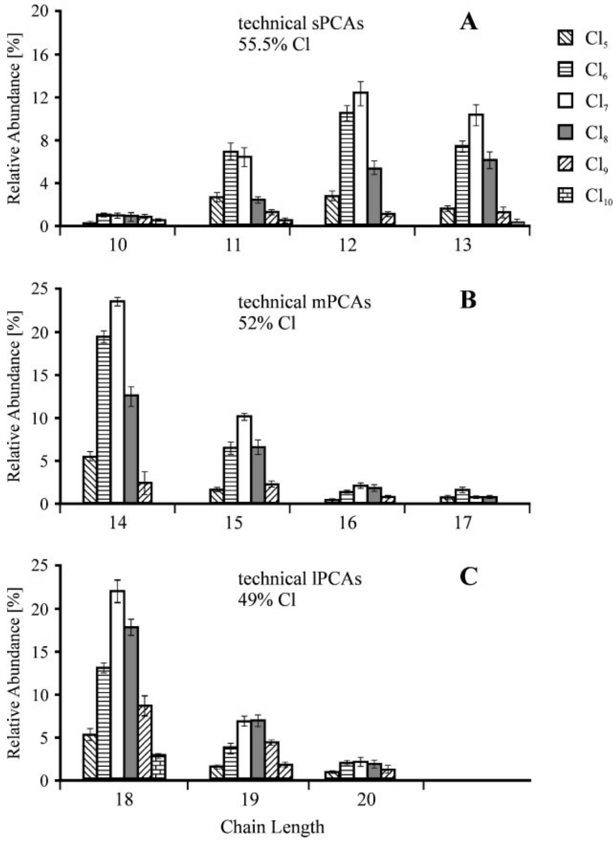

Figure 2. Congener and homologue distribution obtained by chloride-enhanced APCI-MS for an sPCA (A, 55.5% Cl), an mPCA (B, 52% Cl) and a lPCA mixture (C, 49% Cl). Error bars of the average of five measurements are given.

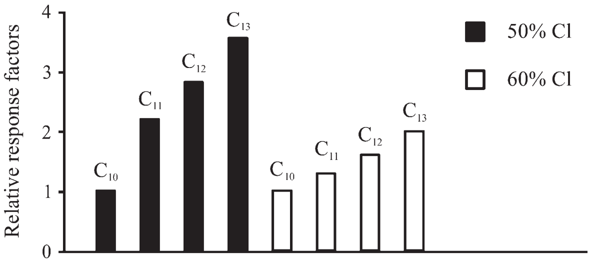

Figure 3. Cl^--APCI(−) response factors of C_{10}, C_{11}, C_{12} and C_{13} PCA mixtures with a degree of chlorination of 50 and 60%. Response factors were calculated on mass basis using the sum of the signals of all congeners. The results are normalized to the C_{10} homologues and the average of three measurements is given.

factor of 3.5 between the C_{10} and the C_{13} PCAs (factor of 2 for homologues with 60% Cl content). The higher the degree of chlorination the lower was the influence of the length of the carbon chain on the response. For comparison, response factors for ECNI-MS analysis of these compounds varied by a factor of 2 (homologues with 50% Cl content) and 1.5 (60% Cl content). CH_4/CH_2Cl_2-NICI-MS showed variations of 1.5 for homologues with 50% and 60% Cl content.

A major drawback of the normally applied ECNI technique is the strong dependence of the response factor on the degree of chlorination of the PCA mixture. To a minor degree, this was also observed for Cl^--APCI. For example, the response factors of C_{10} PCAs with Cl contents of 50 and 70% varied by a factor of 6.5 (3.4 for the C_{11} PCA mixtures). The degree of chlorination had a lower influence on the response factors of homologues with longer carbon chains. For comparison, response factors for ECNI-MS varied by a factor of 37 (C_{10} homologues) and 12 (C_{11}-homologues). CH_4/CH_2Cl_2-NICI-MS showed variations of factor 1.4 (C_{10} homologues) and 1.8 (C_{11} homologues).

Compared with ECNI, Cl^--APCI-MS allowed a reduction in the dependence of the response factors on the degree of chlorination in a similar way as for CH_4/CH_2Cl_2-NICI-MS. This is a considerable advantage, since it reduces the error caused by differences in the degree of chlorination of PCAs between standard and sample, which can be >100% for ECNI-MS.[19] In comparison with CH_4/CH_2Cl_2-NICI, the use of Cl^--APCI-MS allowed a shorter analysis time and did not require any modifications of the instrumentation.[17]

Linearity and limits of detection

For the sPCA mixture with 55.5% Cl content the limit of detection (LOD) at a signal-to-noise (S/N) ratio of 3:1 was 1.5 ng/μL technical mixture (30 ng injected) extracting the mass of the main congener $C_{12}H_{19}Cl_7$ only. For the mPCA and the lPCA mixtures, with 52 and 49% Cl content, the LODs (S/N 3:1) were 1 ng/μL technical mixture (20 ng injected) detecting $C_{14}H_{23}Cl_7$ and $C_{18}H_{31}Cl_7$. In full scan mode the LOD (S/N 3:1) was 0.8 ng/μL for the sPCA mixture and 0.8 ng/μL for the mPCA and lPCA mixtures. For comparison, the LODs (S/N 3:1) of ECNI-LRMS were 1 ng/μL for the sPCA mixture and 0.5 ng/μL for the mPCA mixture. The linearity was good between 3 and 100 ng/μL of sPCA, mPCA and lPCA mixtures (55.5, 52 and 49% Cl content, respectively) in the full scan mode as well as extracting the masses of the main congeners of each homologue group ($R^2 > 0.99$, 7 concentrations).

Quantification

Since no suitable internal standard was found, quantification by external standard was used. The reproducibility of the full scan signal area of PCA standards, expressed as relative standard deviation, was <8% (five injections over 12 h). Influence of the matrix on the ionization was controlled by adding post-column a constant amount of a lPCA solution and injecting on the HPLC column the extract of a plastic bag (containing no halogenated compounds) or a standard solution of phthalates. These compounds are also used as additives at concentrations up to 5–40% of the total weight. Therefore, a mixture of 43 μg of six different phthalates was selected. In both cases no fluctuations of the baseline could be observed, which means that these compounds neither enhanced nor suppressed the ionization of PCAs. Figure 4 shows the chromatograms obtained.

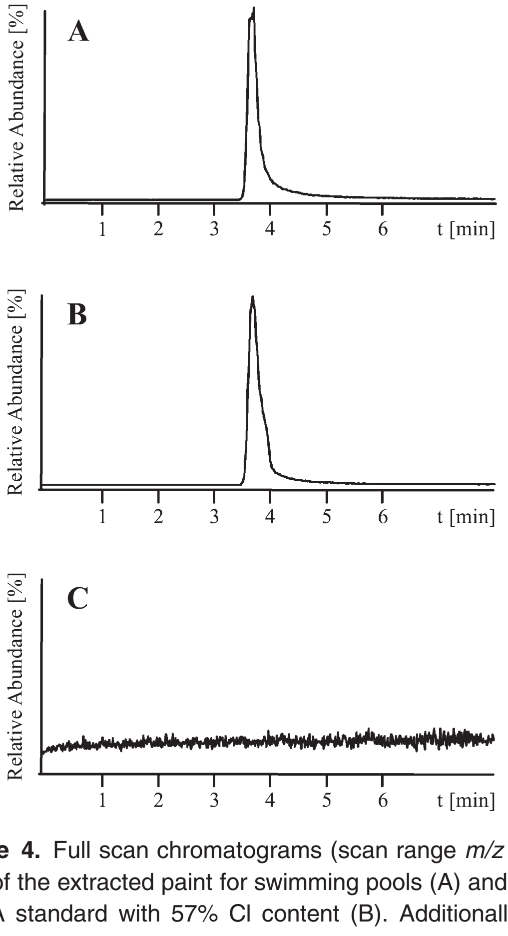

Figure 4. Full scan chromatograms (scan range *m/z* 300–700) of the extracted paint for swimming pools (A) and of an mPCA standard with 57% Cl content (B). Additionally, the control of ionization interferences by phthalates is shown (C, extracted ion chromatogram of *m/z* 530.9, $[C_{18}H_{31}Cl_7+Cl]^-$). 43 μg of six different phthalates were separated and a solution of 100 ng/μL lPCA (49.0%) was added post-column at a flow rate of 25 μL/min. For (C) the y-axis was enlarged ca. 100 times.

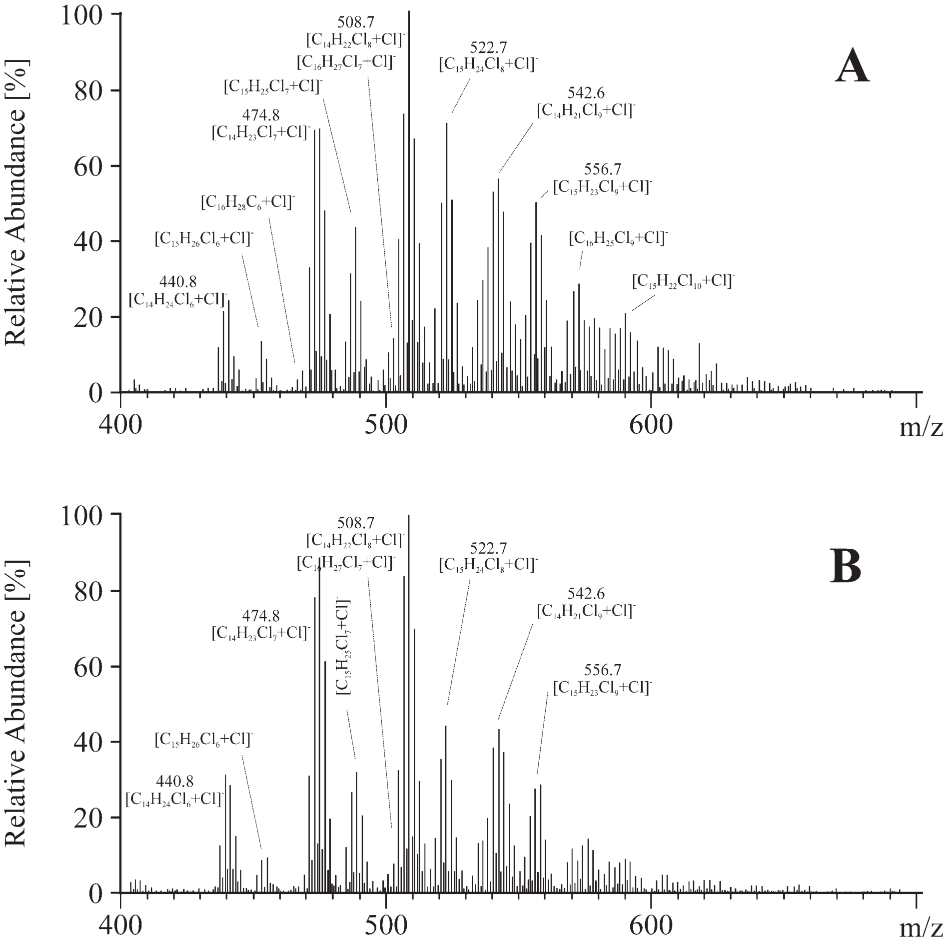

Figure 5. Cl^--APCI(−) mass spectra of the paint for garage floors (A) and of the mPCA standard with 57% Cl content, which was used for quantification (B). The main congeners are indicated.

Although the variations in the response factors of different PCA mixtures were lower than for ECNI-MS, quantification was carried out with a PCA standard of a composition most similar to the sample. The PCA mass spectra of the samples allowed the estimation of the PCA composition (chain length and degree of chlorination). This was necessary to identify which kind of PCAs is present in the sample (e.g. sPCAs or mPCAs), since some PCA congeners have isotope signals with the same nominal mass-to-charge ratio (e.g. $C_{11}H_{16}^{37}Cl_2^{35}Cl_6$ and $C_{16}H_{28}^{37}Cl^{35}Cl_5$), which cannot be distinguished by low-resolution MS.[13] The ability of ion traps to acquire complete mass spectra at low analyte concentrations (100 ng of PCAs are sufficient) was an advantage. These full scan mass spectra allowed us to distinguish between different congeners with the same mass-to-charge ratios by evaluating their chlorine isotope signals.

Analysis of household commodities

PCA recovery was 96–102% for nonsoluble samples and 99.5–100.5% for soluble samples. Paint for swimming pools, paint for garage floors, window putty, two kinds of cable conduit, inflatable balloons and ink for computer printers were extracted and the extracts analyzed for PCAs. Only the paints for swimming pools and garage floors contained PCAs. The chromatogram of the paint for swimming pools sample is shown in Fig. 4(A).

Interpretation of the recorded mass spectra allowed identification of chain length and degree of chlorination of the PCAs present in the sample. The mass spectrum of the sample of paint for garage floors is shown in Fig. 5. As can be seen, the main PCA congeners were $C_{14}H_{23}Cl_7$, $C_{14}H_{22}Cl_8$, $C_{14}H_{21}Cl_9$, $C_{15}H_{25}Cl_7$, $C_{15}H_{24}Cl_8$ and $C_{15}H_{23}Cl_9$. Minor congeners are indicated in the mass spectrum. Only mPCAs could be identified in this sample, and the congeners present indicated a composition similar to the mPCA standard with 57% Cl content. Therefore, this PCA mixture was used as standard for quantification. Table 1 shows the relative abundances of the PCA congeners and homologues present in the paint samples and in the quantification standard.

The two paints had mPCA contents of 8.2 ± 0.1% (paint for swimming pools, n = 3) and 11.5 ± 0.4% (paint for garage floors, n = 3). Analysis by HRGC/EI-MS/MS gave concentrations of 7.4 and 11.5%, respectively. The results of the two methods were in very good agreement considering the difficulties of quantification using technical mixtures as standard.

Table 1. Relative congener and homologue abundances (expressed in %) of the mPCAs detected in the paint samples and of the technical mPCA mixture used for quantification (specified Cl content 57%). The precision of three replicates is given

PCA isomer	mPCA standard	Paint for garage floors	Paint for swimming pools
$C_{14}H_{25}Cl_5$	0.5 ± 0.2	<0.3	0.3 ± 0.1
$C_{14}H_{24}Cl_6$	5.8 ± 0.8	3.6 ± 0.12	2.0 ± 0.2
$C_{14}H_{23}Cl_7$	18.7 ± 0.7	11.7 ± 0.5	7.6 ± 0.6
$C_{14}H_{22}Cl_8$	21.4 ± 0.4	16.5 ± 1.6	9.3 ± 0.3
$C_{14}H_{21}Cl_9$	9.9 ± 1.0	9.6 ± 0.9	5.4 ± 0.3
$C_{14}H_{20}Cl_{10}$	2.0 ± 0.1	2.7 ± 0.5	2.1 ± 0.7
$C_{15}H_{27}Cl_5$	0.2 ± 0.1	<0.3	<0.3
$C_{15}H_{26}Cl_6$	1.7 ± 0.2	1.2 ± 0.3	2.6 ± 0.2
$C_{15}H_{25}Cl_7$	6.8 ± 0.3	7.2 ± 0.4	7.9 ± 0.7
$C_{15}H_{24}Cl_8$	10.2 ± 0.4	12.5 ± 1.0	11.7 ± 1.14
$C_{15}H_{23}Cl_9$	6.6 ± 1.0	8.6 ± 0.3	7.3 ± 0.8
$C_{15}H_{22}Cl_{10}$	1.7 ± 0.2	3.1 ± 0.3	2.6 ± 0.3
$C_{15}H_{21}Cl_{11}$	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
$C_{16}H_{28}Cl_6$	0.3 ± 0.1	0.5 ± 0.1	1.7 ± 0.1
$C_{16}H_{27}Cl_7$	1.8 ± 0.1	2.5 ± 0.1	5.6 ± 0.8
$C_{16}H_{26}Cl_8$	2.9 ± 0.1	4.8 ± 0.2	9.2 ± 1.8
$C_{16}H_{25}Cl_9$	2.5 ± 01	4.2 ± 0.5	6.6 ± 0.5
$C_{16}H_{24}Cl_{10}$	0.8 ± 0.2	1.7 ± 0.2	2.1 ± 0.4
$C_{16}H_{23}Cl_{11}$	<0.3	<0.3	<0.3
$C_{17}H_{31}Cl_5$	0.3 ± 0.1	<0.3	0.3 ± 0.1
$C_{17}H_{30}Cl_6$	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.4
$C_{17}H_{29}Cl_7$	1.2 ± 0.2	1.3 ± 0.2	2.6 ± 0.3
$C_{17}H_{28}Cl_8$	1.5 ± 0.4	2.3 ± 0.3	5.0 ± 0.7
$C_{17}H_{27}Cl_9$	1.2 ± 0.3	2.2 ± 0.2	3.9 ± 1.2
$C_{17}H_{26}Cl_{10}$	0.5 ± 0.1	<0.3	1.6 ± 0.3
$C_{17}H_{25}Cl_{11}$	<0.3	<0.3	<0.3
Calculated Cl content (%)	57.8	58.1	57.2

CONCLUSIONS

Chloroform in the HPLC eluent allowed ionization of PCAs by APCI. This technique was applied for the quantitative detection of PCAs in plastic materials. The new method proved to be a good alternative to the normally applied gas chromatographic methods, especially due to the rapidity of the analysis. Only HRGC/EI-MS/MS has a comparably short analysis time, but does not provide any information about the composition of PCAs in the sample. Applicability of the new method to other kinds of samples should therefore be investigated.

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Eidesstattliche Erklärung

Ich erkläre, dass ich die Dissertation „Specific Alternative Mass Spectrometric Methods for the Trace Analysis of Chlordanes and Polychlorinated *n*-Alkanes" selbständig nur mit der darin angegebenen Hilfe verfasst und bei keiner anderen Fakultät der Universität Basel eingereicht habe.

Basel, 23. August, 2004

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