DICHLOROMETHANE AS NICI REAGENT GAS FOR THE SELECTIVE DETECTION OF POLYCHLORINATED n-ALKANES

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Introduction

Polychlorinated n-alkanes (PCAs) 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), C_{14}-C_{17} (medium chain PCAs) or >C_{17} (long chain PCAs)[1]. 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[1]. They are also replacements for other persistent chemicals such as polychlorinated biphenyls[1,2].

Due to a slow dispersion into the environment, high lipophilicy and persistence[2], PCAs can be found in aquatic and terrestrial food webs in rural and remote areas[1]. To date there is limited information about environmental levels of PCAs[3-5]. 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) combined with electron capture negative ionization (ECNI) mass spectrometry (MS) employing CH_4 as reagent gas. However, the application of high resolution mass spectrometry (HRMS) is desirable to achieve a better selectivity and sensitivity[5], since it is difficult to separate completely PCAs from other polychlorinated pollutants such as chlordanes or toxaphenes during sample clean-up.

In this work a CH_4/CH_2Cl_2 reagent gas mixture was used for the selective ionization of PCAs. This technique allowed to overcome some drawbacks of conventional CH_4-ECNI such as the simultaneous formation of [M-HCl]⁻, [M-Cl]⁻ and [M+Cl]⁻, variations of response factors by about one order of magnitude due to the strong influence of the number and position of chlorine atoms as well as reduced sensitivity for lower chlorinated PCAs.

Methods and Materials

Chemicals and sample clean-up. 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 PCA C_{10-13} technical mixtures with chlorine contents of 51.0%, 55.5% and 63.0% were purchased from Ehrenstorfer (Augsburg, Germany). Dichloromethane (99.8%, Scharlau, Barcelona, Spain) was used as reagent gas for the NICI experiments. ¹³C_{10}-trans-chlordane (Cambridge Isotope Laboratories, Mandover MA) was used as internal standard for quantification and octachloro-naphthalene (Ehrenstorfer, Augsburg, Germany) as recovery standard.

Two pooled samples from North Sea dab (*Limanda limanda*) livers (5.151 g and 4.391 g) caught in the North Sea (54° 15.64 N, 7° 29.79' E) were each homogenized with 40 g of Na_2SO_4

after the addition of 10 ng of $^{13}C_{10}$-trans-chlordane (internal standard) and extracted with 250 ml of n-hexane/dichloromethane (1+1). The extracts were subjected to a sequential clean-up consisting of removal of fat by chromatography on 40 g H_2SO_4(44%)/silica gel (elution with 80 ml n-hexane/dichloromethane 1+1) and adsorption chromatography (16 g Florisil deactivated with 1.5% H_2O, Fraction 1: 40 ml n-hexane, Fraction 2: 10 ml n-hexane/dichloromethane 85+15, Fraction 3: 30 ml n-hexane/dichloromethane 85+15 and 25 ml n-hexane/dichloromethane 1+1). Fraction 3 contained the PCAs. 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. 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 id) coated with a 0.25 μm thick film of DB35-MS (35% crosslinked phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). The injector temperature was 250 °C. Helium 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, Palo Alto, USA) was employed in the NICI mode. 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 ECNI was carried out at a reagent gas pressure of 1.0-1.6 mbar (0.8-1.2 Torr). CH_2Cl_2 was introduced through a modified transfer line by adding a T-piece and a small glass bulb of 6 ml volume filled with 5 ml of dichloromethane. A needle valve was used to meter the dichloromethane pressure. CH_4/CH_2Cl_2-NICI was performed at a total reagent gas pressure of 2.0 mbar (1.5 Torr) measured at the transfer line inlet and a CH_4/CH_2Cl_2 ratio of 80+20.

Results and Discussion

CH_2Cl_2 as reagent gas. In contrast to conventional ECNI, which leads to the formation of [M+Cl]⁻, [M-Cl]⁻ and [M-HCl]⁻ the use of CH_2Cl_2 as reagent formed almost exclusively [M+Cl]⁻ adduct ions. This resulted in an increased selectivity and strongly reduced interferences between different PCA congeners. Additionally, 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 maximum a factor of 2.4 (factor of 22 for conventional ECNI conditions).

A better uniformity of the response factors and the almost exclusive formation of [M+Cl]⁻ ions allowed a more precise characterization of technical PCA mixtures and their application as reference standards. A comparison of the characterization of a technical PCA mixture with 51.0% chlorine content by the two ionization techniques is shown in Figure 1. The chlorine content calculated on the basis of the relative quantities of congeners and homologues deviated only by 1-2.5% from the value specified by the manufacturer when employing CH_4/CH_2Cl_2-NICI. Compositions obtained with CH_4-ECNI were 2-7 % off. In contrast to conventional CH_4-ECNI, the use of CH_2Cl_2 also allowed the detection of tri- and tetrachloro congeners. The linearity of the new ionization method was good (R^2>0.998, 6 concentrations between 2 and 100 ng technical

PCA mixture for $C_{11}H_{19}Cl_5$, $C_{11}H_{18}Cl_6$, $C_{12}H_{21}Cl_5$ and $C_{12}H_{20}Cl_6$ using a technical PCA mixture with 55.5% chlorine content).
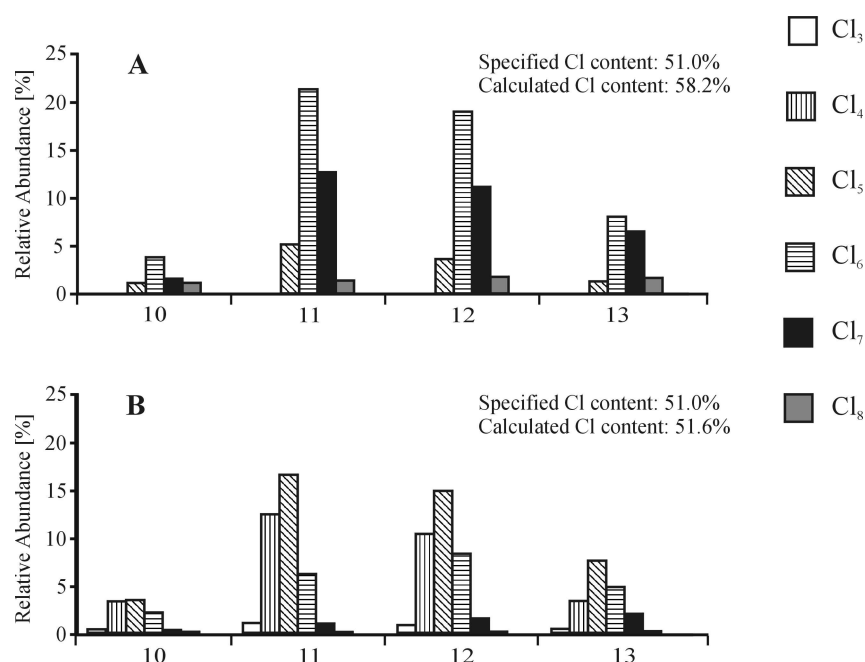
Figure 1. Congener and homologue patterns of a technical PCA mixture with 51.0% Cl content obtained with CH_4-ECNI (A) and CH_4/CH_2Cl_2-NICI (B).

The application of CH_4/CH_2Cl_2 as reagent gas resulted in an increased selectivity due to the selective formation of $[M+Cl]^-$ adducts reducing the interferences between different PCA congeners and homologues. Ionization by CH_4-ECNI and CH_4/CH_2Cl_2-NICI was also compared for α-hexachlorocyclohexane, trans-chlordane, PCB 153, p,p'-dichlorodiphenyl-trichloroethane and toxaphene #62 (Parlar nomenclature). All compounds except PCB 153 formed chloride adducts. However, their response was suppressed resulting in a gain of selectivity for PCAs of at least one order of magnitude when applying CH_4/CH_2Cl_2-NICI.

Application of CH_4/CH_2Cl_2-NICI to biota. Two cleaned-up extracts of North Sea dab livers were analyzed with both CH_4-ECNI and CH_4/CH_2Cl_2-NICI. The applied sample clean-up did not remove other polychlorinated compounds completely (e.g. toxaphenes and chlordanes) leading to interferences in the quantification of PCAs in CH_4-ECNI mode. The co-elution of such compounds with the broad PCA signals is shown in Figure 1. Use of CH_4/CH_2Cl_2 as reagent gas mixture allowed to suppress the ionization of these compounds almost completely and also improved the signal-to-noise ratio of PCAs. This opens the possibility to determine PCA levels in biota without interferences even by low resolution mass spectrometry.
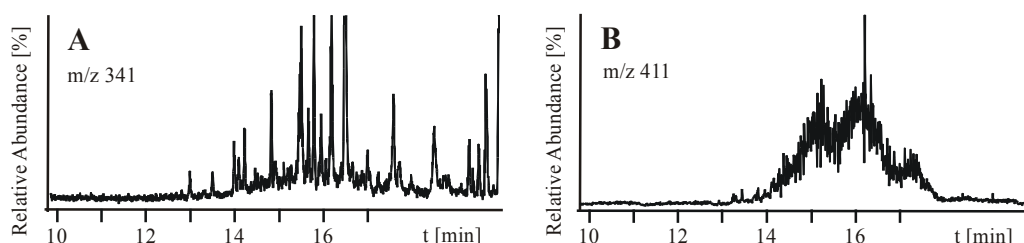
Figure 2. Mass chromatograms of a liver extract from North Sea dab. The mass traces for the $C_{12}H_{20}Cl_6$ isomers are shown. A: CH_4-ECNI, $[M-Cl]^-$ (*m/z* 341, most intense signal of the isotope cluster), PCAs not detectable. B: CH_4/CH_2Cl_2-NICI, $[M+Cl]^-$ (*m/z* 411, most intense signal of the isotope cluster), PCA signal above the limit of detection.

The total PCA concentrations in the biota samples were 213 ng/g and 619 ng/g (wet weight) which is comparable to values reported in the literature for fish (100-1700 ng/g wet weight) determined mainly by HRMS[6]. The amounts are slightly higher than those determined by conventional ECNI due to the additional detection of lower chlorinated PCAs (tri- and tetrachloroalkanes). The mean chlorine content (54.5% and 54.4% respectively) was comparable to the technical mixtures. However, the observed congener and homologue profile in the fish samples deviated substantially from the analyzed technical PCA mixtures.

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