INTERPRETATION OF CONGENER GROUP PATTERNS OF SHORT CHAIN CHLORINATED PARAFFINS BY PRINCIPAL COMPONENTS ANALYSIS

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Introduction

Chlorinated paraffins (CPs) are commercial mixtures used for example as plasticisers and flame retardants in plastics, paints and sealants. However, CPs have also been identified as environmental contaminants of particular concern due to their persistence in the environment, their tendency to bioaccumulate in food chains, and their occurrence in remote regions of the globe far from any potential sources.¹ CPs are mixtures of thousands of distinct compounds. They are divided according to their carbon chain length into short chain CPs (SCCPs, C_{10} - C_{13}), medium chain CPs (MCCPs, C_{14} - C_{17}) and long chain CPs (LCCPs, $C_{>17}$). The degree of chlorination can vary between 30 and 70%. The complexity of CP mixtures poses unique challenges to analytical chemists. Consequently, there is limited information about environmental behaviour and levels of CPs. This is most particularly true for the environmental transformation of CPs; there is currently no comprehensive information about the changes in CP compositions in the environment.

During the last 10 years many of the problems in CP analysis have been overcome.² It has been shown that high resolution gas chromatography combined with electron ionization tandem mass spectrometry (HRGC-EI-MS/MS) is powerful, fast and cost efficient screening technique to measure the total CP amount in different matrices.³ HRGC combined with electron capture negative ionization (ECNI) mass spectrometry is currently the method of choice for the determination of SCCPs and MCCPs.^{1,4} Furthermore, the evaluation of the CP congener group patterns is possible by HRGC-ECNI-MS.⁵

Principal components analysis (PCA) is a useful tool to identify similarities and differences in a complex dataset. This technique has been often applied in PCB studies to determine characteristic congeners and to elucidate possible PCB sources.^{6,7} Hüttig and Oehme applied PCA to show similarities in the CP patterns present in sediments.⁸ In this work PCA was used to identify characteristic congener groups in CP mixtures and to evaluate the differences between technical SCCP mixtures and CPs in fish from Northern Europe and from the Baltic Sea. SCCP congener group patterns were determined HRGC-ECNI-low resolution MS.

Methods and Materials

CP mixtures: The following CP mixtures were used to compare the CP congener patterns in biota to that of technical CP mixtures and CP standard mixtures: Three SCCP standard mixtures (C_{10-13} , 51.5%, 55.5% and 63% chlorine, 100 ng/µl, solutions in cyclohexane) were obtained from Ehrenstorfer (Augsburg, Germany). The technical mixtures, Hordalub 17 (49% Cl), Hordalub 80 (56% Cl), and Hordalub 500 (69% Cl) were obtained from Hoechst (Frankfurt, Germany) and diluted to 100 ng/µl in cyclohexane. Additionally, Cereclor 60 L (59% Cl) and Cerechlor 70 L (69% Cl), both from Imperial Chemical Industries (ICI, London, England), were diluted to 107 ng/µl in cyclohexane.

Cod samples. Six cod liver samples (*Gadus morhua*) were obtained from the Norwegian Institute for Air Research in Tromsø. Two cods were caught close to the Northern Norwegian coast (Lofoten, A1, A4), two south of Iceland (Vestmannaeyjar, A3, A6) and two north of Iceland (Akureyri, A2, A5; see Figure 1). Eight cod samples (*Gadus morhua*) were provided by the German Federal Research Centre for Fisheries (Hamburg, Germany). Fish were captured in August 2002 and in August 2003 at two locations in the southern Baltic Sea. Fish of the same size was selected for analysis. The fishes were dissected immediately after their capture and the lipid rich liver was used for analysis.



Figure 1: Map of the capture locations of the cod samples.

Experimental details. Information about standards, chemicals, clean-up, quality control, instrumentation, and quantification, are published in detail elsewhere and therefore only briefly described.^{4,5,9,10} Samples were homogenized with a tenfold excess of anhydrous sodium sulphate. 10 ng of ${}^{13}C_{10}$ -*trans*-chlordane (internal standard, purity 99%, Cambridge Isotope Laboratories, USA) in 10 µl of cyclohexane were added and the sample extracted with 250 ml of *n*-hexane/CH₂Cl₂ (1+1, v/v) in a glass column. After concentration, lipids were removed by column chromatography on 40 g of silica gel impregnated with 44% (weight) of conc. H₂SO₄. The lipid-free sample was eluted with 120 ml of *n*-hexane/CH₂Cl₂ (1+1, v/v). A further fractionation was carried out on 16 g of Florisil[®] (1.5% water content) with 85 ml of *n*-hexane (fraction 1), 5 ml of CH₂Cl₂ (fraction 2) and 65 ml of CH₂Cl₂ (fraction 3). The last fraction contained all CPs. 10 ng of ε -hexachlorocyclohexane (Ehrenstorfer, Germany) in 10 µl of cyclohexane were added as recovery standard to the concentrated CP fraction before analysis.

Chromatographic separations were performed on an HP 5890II (Hewlett Packard, USA) gas chromatograph equipped with a split/splitless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 μ m thick film of DB5-MS (5% phenyl-methylpolysiloxane, J&W Scientific, USA). The temperature programme was as follows: 100 °C, isothermal for 2 min, then 15 °C/min to 280 °C and isothermal for 8 min. An HP 5989B (Hewlett Packard, USA) mass spectrometer was employed in the ECNI mode using methane (99.995%, Carbagas, Switzerland) as reagent gas. The most abundant isotopes of the [M-Cl]⁻ ions of CPs (quantification and confirmation ion) were detected in the selected ion monitoring (SIM) mode.⁹

Principal components analysis (PCA). PCA was conducted using the software Statistica (Version 5.5; 1999 Edition, StatSoft, Inc., Tulsa, USA) for multivariate data analysis. The N variables were the relative abundance of each CP congener group (C_{10-13} with five to eleven chlorine atoms, N = 28 for SCCPs). Data were normalized so that the sum of all congener groups was 100%. The loading plot and score plot were obtained after normal-varimax rotation. Loading plots indicate relationships among variables. Score plots give the positions of the samples in the coordinates of the principal components. Similar samples are represented by points located close to each other.¹¹

Results and Discussion

PCA of SCCP mixtures: Similarities and differences in the congener group patterns of the technical and standard CP mixtures were compared using PCA. The first and second principal components (PC1 and PC2) accounted for 62.6% and 21.8% of the total variance, respectively. Results for the CP mixtures revealed that SCCPs congeners clustered into two main groups according to their number of chlorine atoms. PC1 was positively correlated with high-chlorinated SCCPs ($>Cl_8$) and negatively with low-chlorinated SCCPs ($<Cl_7$), indicating that SCCP mixtures were mainly differentiated according to their chlorine content. CP mixtures with similar chlorine content were located closely in the score plot, showing similarities between the Hordalub



mixtures and the standard mixtures of similar chlorine content. The carbon chain length has no or only little influence.

Figure 2: Principal components analysis score plot (A) and loading plot (B) for five technical SCCP formulations, three SCCP standards, 14 SCCP patterns determined in cod from northwest Europe (A1-A6) and from the Baltic Sea (OS1, OS8-OS13 and OS15). Chlorine contents were calculated from the ECNI-MS data.

PCA of SCCPs in cod: PCA was conducted to compare the congener group patterns of the cod liver samples from the Baltic Sea and from northwest Europe with those of technical CP mixtures. The CP concentrations measured in these samples are discussed elsewhere.⁵ The plots of PCA scores and loading factors for the first two principal components are shown in Figure 2. Congener groups mainly clustered into low- and high-chlorinated SCCPs (see loading plot, Figure 2, B). Additionally, PC2 was positively correlated with $C_{12}H_{19}Cl_7$, $C_{10}H_{15}Cl_7$ and $C_{10}H_{14}Cl_8$.

Cod samples could be distinguished due to their geographic origin in the score plot (Figure 2, A). Two groups could be identified additionally to the technical CP mixtures. Cod samples from northwest Europe (A1 to A6) were characterised by C_{10} and C_{12} congeners and were located differently to cod from the Baltic Sea. Three cod samples from the Baltic Sea were positioned closely to technical mixtures (OS1, OS8 and OS9). Additionally, five cod samples from the Baltic Sea showed differences to the technical CP mixtures and to the other samples (OS10-OS13, OS15). They were characterized by C_{13} congeners. Their proportion of C_{13} congeners was high (between 19 - 28%) compared to that of the technical CP mixtures.

PCA of SCCP congener group patterns obtained by HRGC-ECNI-MS allows a precise comparison of different SCCP congener group patterns. The SCCP composition in fish captured far away from industrialised areas and possible CP sources was different from technical SCCP mixtures. However, the chlorine contents (59.5 - 62.8%) in the fish were similar to the chlorine content of most technical SCCP mixtures.

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