Organohalogen Compounds, Volume 60, Pages 444-447 (2003)

ANALYSIS OF SHORT CHAIN POLYCHLORINATED N-ALKANES IN FISH SAMPLES BY HRGC-NICI-LRMS

Margot Reth, Zdenek Zencak and Michael Oehme

Organic Analytical Chemistry, University of Basel, Neuhausstr. 31, 4057 Basel, Switzerland

Introduction

Chlorinated paraffins consist of a complex mixture of polychlorinated n-alkanes (PCAs) with a chlorination degree between 30 and 70% by weight and are subdivided into short chain (C_{10}-C_{13}), medium chain (C_{14}-C_{17}) and long chain (>C_{17}) PCAs[1]. They are used mainly as additives in metal working fluids and find minor uses in rubbers, paints and coatings, sealant/adhesives, leather processing and textiles[1,2].

Short chain PCAs are classified as persistent and their high octanol-water partition coefficient (log K_{ow} 4.4 - 8, depending on the degree of chlorination) implies a high potential for bioaccumulation[2]. Short chain PCAs have already been found in biota, sediments, air and water. PCAs are classified as very toxic to aquatic organisms and may cause long term adverse effects in the aquatic environment[2]. Although the use of PCAs has been voluntarily reduced from 13 000 tons in 1994 to 4 000 tons by the European industry in 1998, the amount of imported PCAs is still huge[2]. Despite their properties and their wide application very limited knowledge is available about metabolic pathways and toxicokinetics of PCA congeners compared to other organochlorine compounds such as PCBs or toxaphenes[1].

Main reasons are the complex composition of the technical mixtures (thousands of congeners) and the lack of simple quantification methods. Currently, high resolution gas chromatography (HRGC) coupled to high resolution mass spectrometry (HRMS) in the negative ion chemical ionization (NICI) mode is the standard method for PCA determination[1]. HRMS shows low detection limits and high selectivity. However, this instrumentation is not suitable for routine analysis since it is not affordable for many laboratories.

In this work a simple clean-up method combined with HRGC-NICI low resolution (LR) MS is presented which has sufficient selectivity and sensitivity for a study of the fate of PCAs in the environment. To show the applicability of the method to real samples, the total amount of PCAs and their isomeric pattern were determined in different fish liver samples from the North Sea and Baltic Sea.

Materials and Methods

Chemicals and solvents. Solvents for pesticide residue analysis were obtained from Scharlau (Barcelona, Spain). Solid PCA technical mixture ($C_{10\text{-}13}$, purity 100%, 55.5% chlorine) as well as ε-hexachlorohexane (ε-HCH, solution in cyclohexane, 10 ng/µl) were purchased from Ehrenstorfer

GmbH (Augsburg, Germany). [$^{13}C_{10}$]-*trans*-chlordane was obtained from Cambridge Isotope Laboratories (Andover, USA). Florsil®PR (60-100 mesh) and sodium sulfate (Pestanal®) was purchased from Fluka (Buchs, Switzerland), silica gel (200-400 mesh, 0.035-0.070 mm) from CU Chemie Uetikon AG (Uetikon, Switzerland). All three chemicals were dried over night at 600 °C and afterwards cooled for 6 h at 130 °C before usage.

Fish samples. Fish samples were collected in the North Sea and Baltic Sea (see Table 1 for a selection) by the Federal Research Centre for Fisheries (Hamburg, Germany) in August 2002. Pool liver samples of North Sea dab (*Limanda limanda*) and cod (G*adus morhua*) were composed out of 5 individuals.

Table 1. Fish species used for PCA analysis, their capture location, sex and total liver weight.

No.	Fish species	Location	Sex	Total liver weight
		North Sea		
1	North Sea dab (*Limanda limanda*)	54°15´N/7°29´E	f	4.4 g
2	North Sea dab (*Limanda limanda*)	54°39´N/2°12´E	f	4.9 g
3	Cod (*Gadus morhua*)	54°39´N/2°12´E	nd	10.5 g
		Baltic Sea		
4	Cod (*Gadus morhua*)	54°45´N/13°20´E	nd	9.9 g

nd: not determined

Sample clean-up. Up to 10 g of liver were homogenized with a 10-fold excess of water free sodium sulfate in a Rondo 500 household mixer (Tefal, France). For lipid extraction the homogenized liver sample was dry-packed into a glass column (30 cm long, 2.0 cm i.d.) and a 1 cm layer of water free sodium sulfate was placed on top. 10 ng of [$^{13}C_{10}$]-*trans*-chlordane (internal standard) were added onto the water free sodium sulfate prior to column extraction of lipids with 250 ml of n-hexane/dichloromethane (1+1, v/v). The extract was concentrated with a Turbo Vap 500 (Zymark, Hutchinson, USA). For lipid elimination a glass column (30 cm long, 2.0 cm i.d.) filled with 1 g of water free sodium sulfate, 40 g of a silica gel/sulfuric acid (44%) mixture and 1 g of water free sodium sulfate was conditioned with a n-hexane/dichloromethane (1+1, v/v) mixture. The concentrated fat extract was transferred to the column and eluted with 100 ml of n-hexane/dichloromethane (1+1, v/v) under an air pressure of 96.5 hPa generated by a diaphragm pump. The eluate was evaporated to 0.5 ml and transferred to a Florisil column (20 cm long, 1.5 cm i.d.) packed with 1 g of water free sodium sulfate, 16 g of Florisil (deactivated with 1.5% of water) and 1 g of water free sodium sulfate. The column was conditioned with n-hexane and the sample eluted with 60 ml of n-hexane and 5 ml of dichloromethane (prefraction, containing PCBs and toxaphenes) and 60 ml of dichloromethane (PCA-fraction). The PCA-fraction was concentrated, taken up in cyclohexane and finally the volume was reduced to 100 µl. 10 ng of ε-HCH were added as recovery standard to the sample extract before further analysis by HRGC-NICI-LRMS.

Instrumentation. The chromatographic separations were carried out on a HP 5890II (Hewlett Packard, Palo Alto, USA) gas chromatograph equipped with a split/splittless injector and a fused silica capillary column (15 m, 0.25 mm i.d.) coated with a 0.25 µm thick film of DB35-MS (35% crosslinked phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). Sample volumes of 1.5 µl

were injected in the splitless mode at an injector temperature of 275 °C. Helium (99.999%, Carbagas, Basel, Switzerland) was employed as carrier gas at a column 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, isothermal for 10 min.

A MS Engine HP 5989B (Hewlett Packard, Palo Alto, USA) was used in the NICI mode using methane (99.995%, Carbagas, Basel, Switzerland) as reagent gas at a pressure of 0.9 Torr. The mass spectrometer was tuned to optimal performance using perfluorotributylamine at *m/z* 283, 414 and 452. The electron energy was 100 eV. The transfer line temperature was 280 °C, the ion source temperature 200 °C and the quadrupole temperature 100 °C. Compounds were detected in the selected ion monitoring (SIM) mode at a dwell time of 100 ms per ion using the $[M\text{-}Cl]^-$ fragments of each PCA isomer at the mass of the two most intense isotopes and of the recovery standard ε-HCH (*m/z* 254.88). For the internal standard the $[M]^-$ ion (*m/z* 419.81) was recorded.

Results and Discussion

Method validation. PCA recoveries in spiked samples (1.5-25 µg technical PCA mixture) were about 90%. The recovery of the internal standard was about 70% in real samples. The linearity was good (R^2>0.997, 7 measuring points for the range between 1 and 100 ng technical PCA mixture for $C_{11}H_{18}Cl_6$ and $C_{12}H_{20}Cl_6$). The detection limit for the two major components $C_{11}H_{18}Cl_6$ and $C_{12}H_{20}Cl_6$ was 1 ng/µl of technical PCA mixture at a signal to noise ration of 3:1, the quantification limit was 2 ng/µl of technical PCA mixture at a signal to noise ratio of 10:1. A total PCA amount of 75 ng/g fish was still quantifiable including minor components of the PCA mixture.

Fish samples. The total PCA concentrations analysed in the liver samples (see Table 2) ranged between 90 and 287 ng/g of wet weight. These concentrations are comparable to other published concentrations for fish (100-1700 ng/g of wet weight) determined mainly by HRMS[3].

Table 2. PCA levels determined in liver of North Sea dab and cod (ng/g of wet weight) from the North Sea and Baltic Sea.

No.	Sample	PCAs [ng/g of wet weight]				Total PCAs [ng/g of wet weight]
		Σ C_{10}	Σ C_{11}	Σ C_{12}	Σ C_{13}	
1	North Sea dab	14	32	58	66	170
2	North Sea dab	59	97	86	45	287
3	Cod	16	37	27	10	90
4	Cod	22	53	49	19	142

The obtained congener and homologue patterns for cod and North Sea dab liver samples and for a technical PCA mixture with 55.5% chlorine content are shown in Figure 1. Differences in the PCA patterns were found depending on the species. This is presumably caused by bioaccumulation and metabolization.

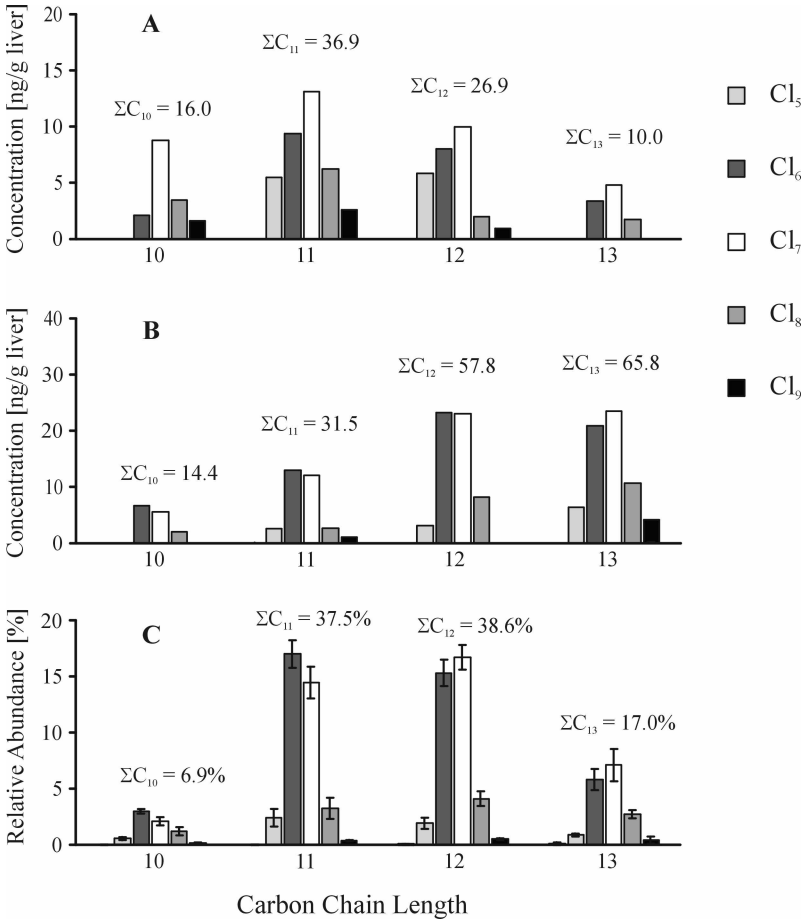

Figure 1. Relative distribution of PCA congeners found in cod liver (A), North Sea dab liver (B) and in a technical PCA mixture (C_{10}-C_{13}, 55.5% chlorine, n = 4).

Acknowledgements

This work was supported by the German Federal Environmental Agency and the Swiss National Science Foundation (no.20-64817.01).

References

1. Muir D., Stern G. and Tomy G. (2000) in: The Handbook of Environmental Chemistry (Springer), Vol. 3 Series, Anthropogenic Compounds, Chapter Chlorinated Paraffins, ISBN 3-540-65338-6.
2. OSPAR Commission (2001) OSPAR Background Document on Short Chain Chlorinated Paraffins, No.141, ISBN 0-946956-77-4.
3. Borgen A. R., Schlabach M., Kallenborn R. and Fjeld E. (2001) Organohalogen Compd. 52, 75.