Chlorinated paraffins in human milk from Germany analyzed by HRGC-EI-MS/MS

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Introduction

Chlorinated paraffins (CPs) are very complex mixtures of polychlorinated *n*-alkanes containing thousands of different isomers. 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%. CPs are produced as bulk chemicals in large quantities (300,000 t/year) and are mainly utilized as additives in metal working fluids, as flame retardants and as plasticizers[1].

CPs have a low acute toxicity to mammals, however, information about carcinogenic properties in mice and aquatic toxicity is available. Furthermore, CPs are classified as persistent and their physical properties imply a high potential for bioaccumulation as well as for global long-range atmospheric transport. SCCPs and MCCPs have already been found in biota, sediments, air and water[1]. But almost no data about current CP concentrations in humans are available, although CPs have already been detected in human adipose tissue in 1985[2].

The analysis of CPs is very demanding and time-consuming. A new method for the fast determination of the total CP concentration by electron ionization (EI) tandem mass spectrometry (MS/MS) was published in 2004[3]. Three fragmentations were observed, which are common to all CPs. The method proved to be enough sensitive and selective for the analysis of CPs in environmental samples. In this study CPs were determined for the first time in human milk by HRGC-EI-MS/MS. A comparison to polychlorinated biphenyl (PCB) concentrations is also included in this work.

Methods and Materials

Human milk samples. Six milk samples were collected in the summer of 2004 and in January 2005 from volunteering nursing mothers living in Baden-Württemberg (South Germany). Samples were randomly selected and do not represent a specific group of mothers. The age of the donors was 36 years in average (range of 29-38 years).

Clean-up. Information about the clean-up, purity and pretreatment of chemicals, standards and solvents is published in detail elsewhere and, therefore, only briefly described [4,5]. Ca. 50 g of milk was centrifuged (3000 rpm, 4°C) for ten minutes. The cream was separated and melted in a water bath of 50°C. Anhydrous sodium sulphate was added until the cream was powdery. Lipids were extracted with *n*-hexane and filtrated with a glass funnel filled with a pre-cleaned piece of cotton wool and sodium sulphate. Lipid extraction was repeated 3-4 times. Solvents were evaporated with a speed vac. Residues of solvent were evaporated in a preheated sand bath under nitrogen for 5-10 minutes[5]. 10 ng of $^{13}C_{10}$-*trans*-chlordane (internal standard, purity 99%, Cambridge Isotope Laboratories, USA) in 10 µl of cyclohexane were added to the lipid extract. Lipids were removed by column chromatography on 40 g of silica gel impregnated with 44% (weight) of conc. H_2SO_4. The lipid-free sample was eluted with 100 ml of *n*-hexane/CH_2Cl_2 (1+1, v/v). A further fractionation was carried out on 16 g of Florisil (1.5% water content) with 60 ml of *n*-hexane (fraction 1), 5 ml of CH_2Cl_2 (fraction 2) and 60 ml of CH_2Cl_2 (fraction 3). The last fraction contained all CPs. 10 ng of ε-hexachlorocyclohexane (ε-HCH, 10 ng/µl, Ehrenstorfer, Germany) in 10 µl of cyclohexane were added as recovery standard to the concentrated CP fraction before analysis. Method blanks consisted of 20 g of sodium sulfate (preheated at 600 °C for 6 h). Reference solutions for quantification contained 1500 ng of SCCPs (55% Cl, 100 ng/µl, Ehrenstorfer, Germany), 10 ng of ε-HCH and 10 ng of $^{13}C_{10}$-*trans*-chlordane in 150 µl of cyclohexane. The

determination of PCBs followed the principles of German Official Methods[5].

Instrumentation. Details about parameters for the HRGC-EI-MS/MS analysis are published in detail elsewhere and therefore only briefly described[3]. Gas chromatographic separations were performed on a CP-3800 (Varian, USA) gas chromatograph equipped with a fused silica capillary column (15 m length, 0.25 mm id) coated with 0.25 mm of DB5-MS (5% phenyl-methylpolysiloxane, J&W Scientific, USA). The temperature program was: 100 °C isothermal for 1 min, then 50 °C/min to 300 °C and isothermal for 4 min. Splitless injections (splitless time 3 min) of 2.0 ml volume were carried out with a Combi Pal autosampler (CTC Analytics, Switzerland). A 1200 triple quadrupole MS (Varian, USA) was employed. Conditions for EI-MS/MS were as follows: 70 eV electron energy, emission current 300 mA, 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). For the determination of the total CP concentration the following fragmentations and collision energies were used: *m/z* 91 $[C_7H_7]^+$ to *m/z* 53 $[C_4H_5]^+$ (-10 V), *m/z* 102 $[C_5H_7Cl]^+$ to *m/z* 65 $[C_5H_5]^+$ (-10 V) and *m/z* 102 $[C_5H_7Cl]^+$ to *m/z* 67 $[C_5H_7]^+$ (-18 V). Limits of quantification were 0.5, 0.5 and 0.3 ng/µl, respectively. Results are given as the average of all three fragmentations. The precursor ion *m/z* 383 $[M\text{-}Cl]^+$ and the product ion *m/z* 276 $[M\text{-}4Cl]^+$ were chosen for the internal standard $^{13}C_{10}$-*trans*-chlordane (-21 V).

Results and Discussion

Analysis by EI-MS/MS. Figure 1 shows the EI-MS/MS mass chromatograms of a human milk sample and a standard mixture containing both SCCPs (55% Cl) and MCCPs (52% Cl). All three fragmentations showed the typical CP signal (a hump of thousands of unresolved CP isomers).

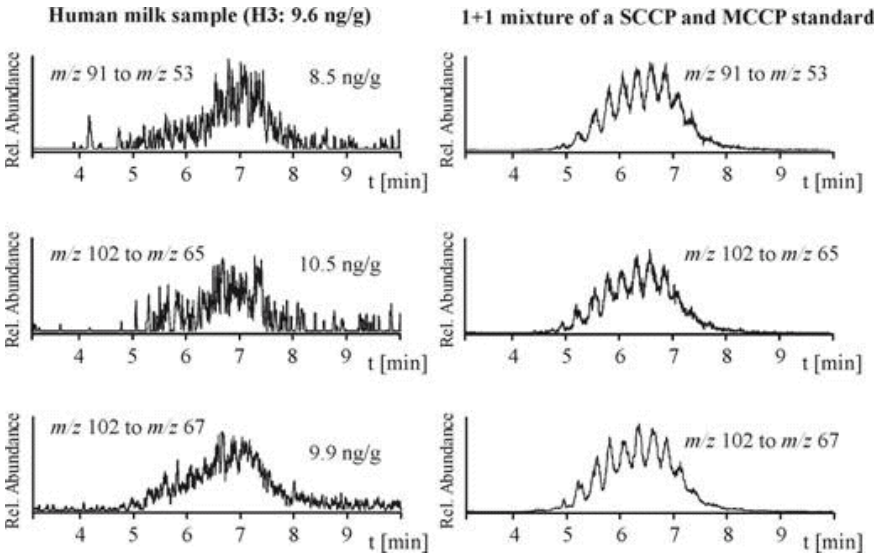

Figure 1: Triple quadrupole EI-MS/MS chromatograms of a human milk sample (sample no. H3, Table 1) and a 1+1 mixture of a SCCP (55% Cl) and a MCCP (52% Cl) standard. CIDs of m/z 91 $\rightarrow$ 53, m/z 102 $\rightarrow$ 65 and m/z 102 $\rightarrow$ 67 were applied.

CP concentrations. Recoveries ranged between 69 and 102%. Total CP concentrations of the human milk samples are listed in Table 1. CP concentrations were between 2.6 and 9.6 ng/g wet weight (ww) and were in a similar range as the total PCB concentration (sum of PCBs 26, 52, 101, 153, 138, 180 and 118).

Table 1: Total CP concentrations (ng/g wet weight and lipid weight) and PCB concentrations in human milk samples from South Germany. Lipid content (%) is also given. Results in brackets did not meet the quality control criterion of being 10 times higher than the average method blank (0.2 ng/g).

Sample No.	Total CP concentration [ng/g ww]	Lipid content [%]	Total CP concentration [ng/g lw]	PCB concentration[a] [ng/g lw]
H1	3.3	6.3	52	153
H2	(1.9)	n.d.	(55)[b]	320
H3	9.6	n.d.	275[b]	251
H4	2.6	n.d.	76[b]	75
H5	5.2	2.4	216	212
H6	(1.7)	3.5	(49)	126

a: Sum of PCBs 28, 52, 101, 153, 138, 180 and 118; b: Calculated with an assumed lipid content of 3.5%; n.d. not determined; ww: wet weight; lw: lipid weight.

Conclusions. As for the first time shown in 1985 CPs are still detectable in human milk. Currently, CP concentrations are in a similar range as PCB concentrations taking into account that the sum of the three most characteristic congeners (PCB 153, 138, 180) represents 55 - 70% of the total PCB content in human milk[6]. EI-MS/MS proved to be a suitable method with a sufficient selectivity and sensitivity for the determination of CPs in human milk.

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