DETERMINATION OF CHLORINATED PARAFFINS IN HUMAN BREAST MILK BY HRGC-ECNI-LRMS

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Introduction

Chlorinated paraffins (CPs) also known as polychlorinated n-alkanes (PCAs) are complex mixtures consisting of thousands of isomers which can even not be completely separated by HR-GC[1,2]. The technical mixtures are gained by chlorination of n-alkane feedstock under forcing conditions. By their chain lengths CPs are classified as short (C_{10}-C_{13}), middle (C_{14}-C_{17}) and long (C_{18}-C_{30}) chain CPs (SCCP/MCCP/LCCP). In regard to their intended use the chlorine content varies between 30 and 70%[1,2]. Numerous commercial products with different compositions are available[1,2]. For these reasons the analysis of CPs is extremely difficult and only limited information on CP concentrations in environment are available[1,2].

The application range of CPs is widely spread e.g. as fire retardants, plasticizers or as additives in paints, sealants or rubber[1,2].

The acute toxicity of CPs is low, but in dependence of chain length and chlorine contents, CPs feature a more or less great bioaccumulation potential[1,2]. Because of their physico-chemical properties SCCPs possess a higher bioaccumulation potential and a greater risk of release[1,2]. Therefore at time it is reviewed if SCCPs are persistent organic pollutants (POP) according to the Stockholm convention[1].

So far only few data on CPs in human breast milk are available[3,4]. As part of the Bavarian Monitoring of Breast Milk (BAMBI) Study, sixty human breast milk samples were examined for SCCPs and MCCPs.

Material and methods

Chemicals and standards: CP mixtures with defined chain lengths und varying chlorine contents synthesized as described elsewhere[5] were used for mixing the correlation-standards. Technical SCCP- (63% chlorine), MCCP mixtures (42%, 52%, and 57% chlorine) and ε-hexachlorocyclohexane (HCH) were obtained from Ehrenstorfer (Augsburg, Germany). Solvents were purchased from LGC (Wesel, Germany). Silica gel, sulfuric acid, sodium hydroxide as well as sodium sulfate came from Merck (Darmstadt, Germany).

Extraction and clean-up: About 50 - 100 ml of breast milk was centrifuged at 3000 rpm, temperature set to 5 °C, for 10 minutes to obtain the milk fat, which was homogenized with sodium sulfate. This mixture was filled in a chromatographic column (l= 30 cm, ID= 3 cm), fat was extracted using a solvent mixture (n-hexane/acetone, 2:1, v:v). Solvent was evaporated to dryness, using a rotary evaporator and gentle stream of nitrogen. About 1 g of milk fat was weighted in a screw cap tube, solved in 9 ml n-hexane and internal-standard (IS)-solution consisting of 1,1,1,3,10,11-hexachloroundecane and 1,1,1,3,6,7,10,12,12,12-decachlorododecane added. After that sulfuric acid treatment for degradation of fatty acids and some interfering compounds followed. Next clean-up step was a mini acid-basic-silica-gel-column. Solvent was removed until dryness and finally residue was taken up by a solution of ε-HCH (injection standard) in cyclohexane.

Instrumentation: A Shimadzu QP 2010 Plus GC-LRMS system operating in ECNI mode, with methane as reactant gas, was used. Chromatographic separation was carried out on a 15 m x 0.25 mm x 0.1 µm DB 5 ms column with helium as carrier gas (1.2 ml/min). PTV injection (3 µl) was done per autosampler, temperature program started at 110 °C (1 min) and was fast heated up to 275 °C. Ion source temperature was kept at 150 °C; interface temperature was set 250 °C. GC oven temperature program started at 100 °C (1 min) rising by 20 °C/min up to 310 °C held for 3 minutes.

SIM mode was used for detection of selected fragment ions listed in table 1, m/z 255 was applied for ε-HCH.

Quantification: Correlation functions between detector response and chlorine content for SCCPs as well as for MCCPs followed a polynomial trend of second order. Samples' chlorine content was estimated by ratios of

fragment ion areas, and used to determine proper response factor, which was applied to correct signal areas. Details of quantification procedure will be published elsewhere.

Table 1: Selected fragment ions of SCCPs and MCCPs:

313	327	347	361	368	375	381	382	389	395	396	403
409	417	423	431	437	445	451	459	465	479	493	

Quality control: Milk fat gained from mixed human milk samples was used for quality control experiments. Eight samples, each 1 g of fat, were dissolved in n-hexane, and IS-solution added. Six samples additionally were spiked with 5 µg of a technical SCCP mixture, 63% chlorine and 25 µg of a technical MCCP mixture with 52% chlorine content. Quality control samples were worked up as mentioned before. The intraday reproducibility (n=6) provided relative standard deviations of 14.2% for SCCPs and 13.6% for MCCPs. The recovery rates for SCCPs laid between 87% and 124%, respectively 79% and 119% for MCCPs.

LOD/Q: Bovine milk fat was isolated as described above for human milk. For determination of detection (LOD) and quantification (LOQ) limits bovine milk fat was fortified with four differing levels of self-mixed $C_{10\text{-}13}$:60% and $C_{14\text{-}16}$:48% standards. LOD level was defined as S/N 3:1 and LOQ as S/N 10:1. The LOD and LOQ values varied dependent from the chain length. Results are summarized in table 2.

Table 2: LOD/Q levels of $C_{10\text{-}13}$:60% and $C_{14\text{-}16}$:48% standard

$C_{10\text{-}13}$:60%						$C_{14\text{-}16}$:48%				
[ng/g lw]	C_{10}	C_{11}	C_{12}	C_{13}	$C_{10\text{-}13}$	[ng/g lw]	C_{14}	C_{15}	C_{16}	$C_{14\text{-}16}$
LOD	0.9	1.9	2.3	5.6	10.7	LOD	11.2	18.3	31.1	60.6
LOQ	2.8	6.5	9.4	18.7	37.4	LOQ	37.4	55.4	93.6	186.4

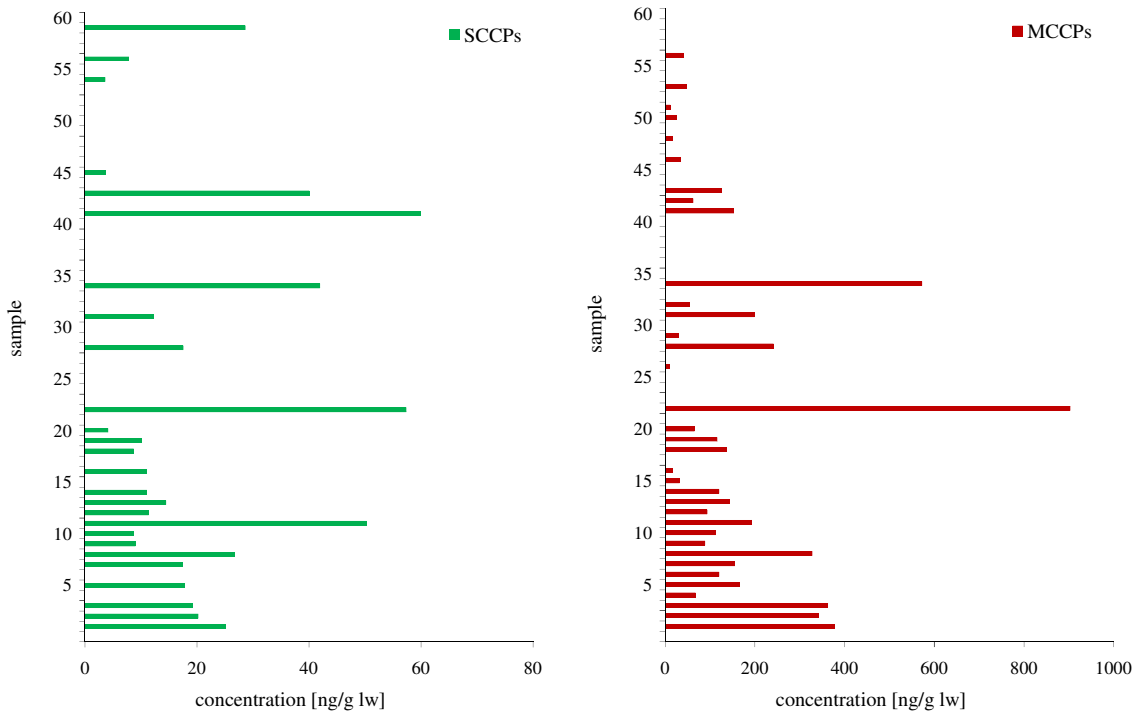

Figure 1: SCCP and MCCP concentrations in the human breast milk samples

Results and discussion:
In figure 1 the CP concentrations of the 60 human breast milk samples are displayed.
MCCPs could be detected in 58% of the samples, the concentrations varied from 9.6 ng/g lipid weight (lw) up to 903.0 ng/g lw with a median value of 115.4 ng/g lw. About one third of the determined MCCP concentrations were in the range between LOD and LOQ level, mainly the tetradecane chain was present in the samples. In 43% of the samples SCCPs were observed. The highest SCCP concentration was 59.9 ng/g lw, a median value of 15.9 ng/g lw was calculated for samples with detectable SCCP amounts. The majority of the samples featured SCCP concentrations lower than LOQ level, excepting three samples. As can be taken out of figure 1 the amounts of MCCPs predominated obviously the SCCP concentrations.

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