CLEAN-UP AND QUANTIFICATION OF SHORT AND MEDIUM CHAIN POLYCHLORINATED *n*-ALKANES IN FISH, FISH OIL AND FISH FEED

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Introduction

Polychloro-n-alkanes (PCAs), synonymous with chloroparaffins (CPs), have been produced for industrial use since the early thirties by radical-induced chlorination of *n*-alkanes (C_{10}-C_{38})[1,2]. Actually, PCAs represent the largest group of chlorinated hydrocarbons produced in the European Community and in North America and are among the most intensively utilized chlorinated hydrocarbons[3]. Their production increased by 0-1 % per year between 1989 and 1998, and it has been estimated that this figure will amount to 1-2 % per year through 2003[4]. Commercially available products present a very complex mixture of PCA compounds containing 10 % to 72 % chlorine by weight and standard analytical methods do not permit separation and identification of these. They are classified into short chain C_{10}-C_{13}, medium chain C_{14}-C_{17}, and long chain $C_{>17}$ PCAs. Of particular interest are the short chain PCAs, which have the greatest potential for environmental release and the highest toxicity of all PCA products[5].

Determination of PCAs in environmental matrices and foodstuffs is very difficult. The greatest problem arises from the complex composition of PCAs and the substantial differences in GC peak patterns in environmental samples as compared to the technical products. Another problem is that a number of chlorinated organic substances, especially toxaphene compounds, partially co-elute with PCAs when using recommended clean-up procedures, and thus interfere with an accurate identification and quantification of them in environmental samples[6].

Here, we describe a new three step clean-up method for the selective separation of short and medium chain PCAs from other interfering chlorinated organic compounds. Additionally, the results of their quantification in fish, fish oil, cod liver oil and fish feed samples by short column GC/ECNI-MS with low resolution[6,7] (R=1000) are presented and discussed.

Materials and Methods

Materials. The solvents and materials used were quality grade "for residue analysis". H_2SO_4, 95-97 %, as well as (a) SiO_2 60, 0.2-0.5 mm and (b) SiO_2 60, 0.063-0.200 mm (Merck) were used. All standards of defined concentrations were obtained from Ehrenstorfer, Augsburg, Germany. The fish samples included species consumed in Europe. Fish oil and cod liver oil were obtained from different countries in Europe and America. The fish feed was of German origin.

Methods. The clean-up procedure comprises three separation steps. The first one includes simultaneous column extraction of the fatty (lipid) material from the homogenised sample matrix

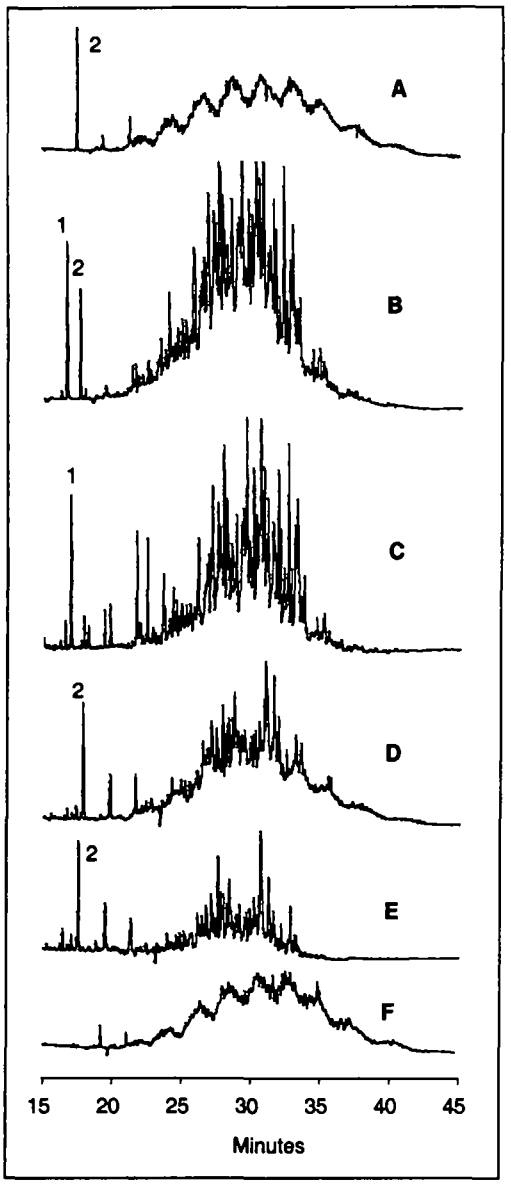

Fig. 1: Gas chromatograms of **A**: a commercial C_{10}-C_{13} PCA, 63 % chlor ; **B**: commercial C_{10}-C_{13} PCA, 63 % chlor, technical toxaphene and other interfering compounds in the extract after the first clean-up step; **C** and **D**: extract 1 and 2, respectively after the second step; **E** and **F**: fraction 27-32 min and 23-27 min, respectively, after the third step. **1**: hexachlorobenzene, **2**: γ-hexachlorocyclohexane

(fish [g] : Na_2SO_4 [g] : sea sand [g] = 1:4:2), decomposition of lipids with **(a)** $SiO_2/H_2SO_{4(c)}$ (44 % $H_2SO_{4(c)}$, w/w), and isolation of the lipid-soluble contaminants, (Fig.1, **B**). All this is performed using a glass column of 75.0 cm length and 3.8 cm i.d. filled successively with glass wool → sea sand → 10 g **(a)** SiO_2 → **(a)** $SiO_2/H_2SO_{4(c)}$ (44 % $H_2SO_{4(c)}$, w/w)/sea sand → 10g **(a)** SiO_2 → 20 g Na_2SO_4 → 50 g fish /200 g Na_2SO_4/100 g sea sand → 5 g Na_2SO_4. The amounts of **(a)** $SiO_2/H_2SO_{4\,(c)}$ (44 % $H_2SO_{4(c)}$, w/w) and sea sand were calculated in the following way: **(a)** SiO_2/H_2SO_4 (44 % $H_2SO_{4(c)}$, w/w) [g] = 0.12 × fish [g] × fat [%] and sea sand [g] = 0.08 × fish [g] × fat [%]. Eluent: *n*-hexane/dichloromethane (DCM) 1:1 (eluent [ml]: fish sample [g] = 10 : 1).

The second step involves the column decomposition of the remaining lipids (0.01 %) in the 300 µl concentrated *n*-hexane (free of DCM) extract, as well as the separation of the PCAs from the most part of the interfering compounds. It is carried out using a small EPA glass column (22.0 cm length × 0.7 cm i.d.), filled successively with glass wool → sea sand → 0.3 g Na_2SO_4 →1 g **(b)** $SiO_2/H_2O_{(d)}$ (30 % $H_2O_{(d)}$, w/w) → 1 g **(a)** SiO_2/H_2SO_4 (44 % $H_2SO_{4(c)}$, w/w) → 0.1 g **(b)** $SiO_2/H_2O_{(d)}$ (3 % $H_2O_{(d)}$, w/w) and eluted sequentially with 8 ml *n*-hexane (interfering compounds fraction, Fig. 1, **C**) and 7 ml *n*-hexane:DCM 1:1 (PCAs fraction, Fig. 1, **D**). The *n*-hexane/DCM extract was dried, resolved in 200 µl THF, and PCAs were separated quantitatively from the rest of interfering compounds by a final Gel Permeation Chromatography (GPC) step on a Phenogel 5µ 50 Å using tetrahydrofurane (THF) as eluting solvent (Fig. 1, **E** and **F**).

For the development of this procedure and recovery efficiency, several simulated environmental samples were prepared as follows: 42 g SiO_2 and 8 g pure sun-flower oil were spiked with different doses of C_{10}-C_{13} PCAs of varied chlorine content, C_{14}-C_{17} PCA,

52 % chlorine (w/w), as well as toxaphene and other selected chlorinated pollutants (separate or together). The samples were cleaned as described above and analysed by HRGC/ECD (Fig. 1). Concerning the fish and cod liver oils, the simulated samples were prepared without SiO_2 in the same way, diluted in *n*-hexane/DCM 1:1 (solvent [ml]: fish oil [g] = 10:1) and then cleaned as described. In this connection, the amounts of (a) SiO_2/H_2SO_4 (c) (44 % $H_2SO_{4(c)}$, w/w) and sea sand were: (a) SiO_2/H_2SO_4 (44 % $H_2SO_{4(c)}$, w/w) [g] : sea sand [g] : fish oil [g] = 10:7:1. Eluent: *n*-hexane/DCM 1:1 (eluent [ml]: fish oil [g] = 15:1).

GPC, GC and GC/MS parameters. The GPC system consisted of a L-6000A HPLC pump (Merck) with metal column (300 mm × 7.80 mm, Phenomenex) filled with Phenogel 5μ, 5Å, flow rate 0.25 ml/min THF, injection volume 200 μl, temperature 25° C, retention indicator mix of dioctyl-, dibutyl- and diethylphthalate and a UV-Detektor, λ=280 nm (Merck). The GC analyses were performed on a Dani 86.10 gas chromatograph, column DB5, 30 m x 0.25 mm i.d., film thickness 0.25 μm (J&W). Injector 230° C, carrier gas N_2, splitless 45 s, ECD 290° C, temp. program: 90° C (2 min) to 160 °C at 10° C/min (1 min), to 280° C at 5° C/min (20 min). For GC/MS measurements an HP 5890 Series II gas chromatograph with a short quartz capillary column 1.3 m x 0.15 mm i.d. without stationary phase, coupled to a Finnigan Mat 8200 MS was used. Carrier gas He (0.69 ml/min), injector 280° C, split 1:4 (3 ml/min), temp. program: isotherm 250° C, ECNI mode with CH_4 as reactand gas, low resolution (R=1000), interface 250° C, ion source temp. 240-250° C, electron beam energy 120 eV, ion accelerating voltage 3 kV.

Identification and Quantification. The PCA compounds in selected environmental samples were identified and quantified by comparison of mass spectra with those of polychlorinated C_{10}, C_{11}, C_{12}, and C_{13}, with alkanes 45, 50, 55, 60, 65, and 70 % chlorine content (w/w) synthetized in our laboratory, as well as with those of a C_{14}-C_{17} PCA standard with 52 % chlor (w/w). The quantification was performed by integration of the ions 313/349/379/413/447 (C_{10}), 327/361/397/431/463 (C_{11}), 341/375/411/445/481 (C_{12}), 353/389/423/459/493 (C_{13}), 369/402/438/473 (C_{14}) and 416/453/487 (C_{15}). Total PCA concentrations have been calculated as the sum of concentrations of the single PCAs.

Results and Discussion

The fat removal after the first step of this procedure was 99.9 % and the extract contains short and medium chain PCAs, as well as other H_2SO_4 resistant chlorinated organic compounds, including toxaphene. After the second step, the recovery of toxaphene, PCB 209, OCS, mirex, p,p´-DDE and α-cis-chlordane in the *n*-hexane fraction was > 90 %. The *n*-hexane:DCM fraction contain besides PCAs (≥ 90 %) the rest of toxaphene (about 9-10 %), β, δ, γ-HCH (> 90 %) and p,p´-DDD and p,p´-DDT (≈ 30 %). The recoveries of C_{10}-C_{13} PCA with 45 %, 56 % and 63 % chlorine (w/w) after the third step amounted to 90 %, 92 % and 94 % (Fig. 1 **A** and **F**), respectively. The recovery of C_{14}-C_{17} PCA, 52 % averaged to 93 %.

The levels of short and medium chain PCAs in the fish, fish oil and fish feed analyzed (on a fat weight basis) are presented in Tab. 1. The total amounts of C_{10}-C_{17} PCAs measured was 135 to 294 μg/kg fat for fish samples, 1719 for fish feed sample, 130 to 362 for fish oil samples, and 238 to 500 μg/kg for cod liver oil samples. The highest amount of total PCAs were found in the fish feed sample (1719 μg/kg fat), whereby the medium chain PCAs were dominated (90.2 %).

Table 1: Concentrations of short and medium chain PCAs in fish, fish products, and fish feed

Sample, year and origin	Fat [a] (%)	Mean values of C_{10}-C_{17} PCAs (μg/kg fat)							
		ΣC_{10}	ΣC_{11}	ΣC_{12}	ΣC_{13}	ΣC_{10}-C_{13}	ΣC_{14}	ΣC_{15}	ΣC_{14}-C_{17} [b]
Sprat, 1993, England	5.8	23	41	27	91	182	49	33	82
Redfish, 1994, Norway	5.4	13	34	35	33	115	26	13	39
Salmon, 1994, Chile	15.4	26	30	24	8	88	49	36	85
Herring, 1994, Norway	14.5	18	51	22	44	135	41	27	68
Mackerel, 1996, North Sea	13.2	16	33	50	7	106	19	10	29
Halibut, 1994, Norway	12.7	32	135	39	31	237	41	16	57
Sardine, 1999, Greece	14.4	15	73	30	47	165	51	14	65
Trout, 1999, Germany	11.1	17	81	6	10	114	60	39	99
Fish feed, 1999 Germany	20.8	47	40	34	48	169	1017	533	1550
Fish oil, Germany		11	17	20	21	69	121	70	191
Fish oil, Germany		7	14	12	11	44	57	29	86
Fish oil, England		28	87	10	8	133	50	13	63
Fish oil, Iceland		27	110	57	90	284	48	30	78
CL [c] oil, France		9	8	16	24	57	147	101	248
CL oil, Iceland		54	92	175	39	360	85	26	111
CL oil, USA		11	60	41	21	133	64	41	105
CL oil, England		23	206	100	56	385	82	33	115

[a]: the fat content was determined gravimetrically; [b]: C_{16} and C_{17} PCAs were not detected; [c]: Cod liver

It is evident from the results summarised in Tab. 1, that in samples coming from North Atlantic (England, Norway, North Sea, and Iceland) the C_{10}-C_{13} PCAs are present in higher concentrations than those of C_{14}-C_{17} PCAs. The relative ratio between these two groups of substances lie between 2 to 3. In contrast to that, in fish oil from Germany and cod liver oil from France the C_{14}-C_{17} PCAs could be found significantly in higher concentrations than the C_{10}-C_{13} PCAs. Very unusual is the C_{14}-C_{17} PCAs level determined in fish feed originated from Germany. At the present time it is not possible to give an satisfactory explanation for this behaviour.

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