Congener Specific Determination and Levels of Polychlorinated Naphthalenes In Cod Liver Samples from Norway

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1. Introduction

Polychlorinated naphthtalenes (PCN) show, like polychlorinated biphenyls (PCB), strong chemical and thermal stability and are highly persistent in the environment. PCN are a class of 75 compounds. Mixtures with different degrees of chlorination (f. ex. Halowax 1000, 1013, 1014) were manufactured for use as dielectric fluids, flame retardants and fungicids. PCN are also formed by combustion processes in the presence of chlorine[1,2]. Recent results of enzyme induction assays (EROD and AHH) indicate dioxin-like toxicity. Polychlorinated naphthalenes seem to have the same enzyme inducting activity as some non-ortho PCB. 2,3,7,8-TCDD toxicity equivalent factors are proposed for some congeners[3,4]. Recently, some congeners have been synthesized and separated which allows the congener specific determination of PCN[5].
In this paper we describe a highly specific method for clean-up and quantification of polychlorinated naphthalenes in biological samples. It is close to our standard dioxin method and allows a parallel determination of PCDD/F, non-*ortho* PCB, and PCN. Results are given for PCN concentration in cod liver from different sites of southern Norway.

2. Experimental

PCN standard compounds were obtained as separate solutions with a concentration of 10 ng/µl in iso-octane (Promochem, Wesel, Germany). Since no ^{13}C-labeled PCN compounds are commercially available we used ^{13}C-2,3,7,8-TCDD and ^{13}C-1,2,3,7,8-PeCDD as internal standards and ^{13}C-1,2,3,4-TCDD as recovery standard. The ^{13}C-labeled compounds were obtained from CIL (Woburn, MA 0181, USA).

All solvents were of pesticide grade quality (Merck). The following adsorbents were used: basic aluminium oxide, silica and activated charcoal. Rinsing and activation have been described in detail earlier[6].
For sample extraction and clean-up a multi column chromatography system[6] have been used. Column 1, a glass column with 45 mm i.d. and 100 cm length, was filled with 2 cm of sodium sulfate, 30 g of silica, and 30 g of KOH-coated silica (bottom to top). Column 2, a glass column with 21 mm i.d and 25 cm length, was filled with 12,5 cm KOH-coated silica and 12,5 cm silica (bottom to top). Column 3, a glass column with 9 mm i.d. and 14 cm length, was filled with activated charcoal (AX21) suspended on glass fibres. All three

columns and a solvent reservoir were connected together with three four-way valves (Hamilton) and teflon tubes. Nitrogen purified with an activated charcoal filter was used as a pressure source.

A 5 g sample was ground with dried sodium sulfate in a household mixer and placed on top of the KOH-treated silica layer of column 1. The remaining volume was filled with 650 ml cyclohexane/dichloromethane 1+1. The sample was passed through column 1 and 2 and eluted into column 3. Pesticides, mono- and di-*ortho* PCB and other undesired sample compounds were removed from column 3 with 75 ml cyclohexane/dichloromethane 1+1 and 75 ml dichloromethane. PCN, PCDD/PCDF and non-*ortho* PCB were eluted with 40 ml toluene in a reversed flow direction. Column 3 can be reused after flushing with 100 ml portions of toluene, methanol, and toluene in a reverse-flow direction. The sample extract was transferred to a 50 ml round-bottom flask with a centrifuge tube fused to the bottom. Some droplets of tetradecane were added as a keeper and the solvent was evaporated to dryness using a slight vacuum and a gentle flow of nitrogen. Alternatively a TurboVap 500 solvent evaporator (Zymark Co., Hopkinton, MA01748, USA) was used.
The residue was dissolved in 1 ml of hexane and transferred to a 15 ml centrifuge tube. 8 - 10 ml of concentrated sulfuric acid was added and the mixture was allowed to stay over night. Afterwards the hexane was transferred with a Pasteur pipette to a new centrifuge tube. The sulfuric acid was washed three times with 0,5 ml hexane. All hexane fractions were combined and washed once with ultra purified water to remove acid traces. The underlaying water phase was removed with a pipette and the hexane extract was dried with sodium sulfate. A final clean-up was done on a glass column with 15 mm i.d. and 20 cm length filled with 4 g of silica and a top layer of 1 g sodium sulfate. The filled column was washed with 30 ml of hexane/diethylether 9+1. Afterwards the sample extract was transferred to the column and eluted with 30 ml of hexane/diethylether 9+1. After adding some droplets of nonane as a keeper the solvent was evaporated to approx. 50 µl and transferred to a autosampler vial with a 100 µl insert. The extract was blown down to near dryness and spiked with 10 µl of the recovery standard.

Separation of the PCN congeners were carried out on a HP 5890 II gas chromatograph with the following conditions: DB5ms fused silica capillary (30m x 0,22mm x 0,12µm); He as carrier gas (80kPa); injector temperature 250°C; interface line 280°C; slitless injection of 2 µl sample volume at 100°C, 2 min at 100°C; 100-170°C at, 170-270°C at 3°C/min for 10 min. Quantifcation was done on a VG AutoSpec at a resolution of 10000 (5% valley) and 8000 V acceleration voltage using electron impact ionization (EI) and the selected ion monitoring (SIM) mode.

3. Results and Discussion

This method was tested for method blank. Since no ^{13}C-labeled PCN congeners were commercially available, we determined the recovery of a PCN standard mixture treated with the complete clean-up method. Five cod liver samples were analyzed. The samples were taken by local fishers nearby Bergen (samples 1 and 2) and in the area of Skien/Porsgrund (samples 3, 4 and 5). The Bergen samples were taken close to a training area of the navy contaminated with PCB. The second area is known to be polluted by PCDD/PCDF and other organochlorines from a magnesium refining plant[7].

Table 1: Recovery of PCN standard solution treated with compared to theoretical values and typical method blank values.

Component	Parallel 1	Parallel 2	Theoretical	Blank
	pg/µl	pg/µl	pg/µl	pg/g
1,2,5,6 TeCN	10,7	10,0	12,5	< 4
2,3,6,7 TeCN	11,0	9,9	12,5	< 4
1,2,3,6,7 PeCN	10,0	11,0	12,5	< 6
1,2,3,5,8 PeCN	11,1	10,7	12,5	< 6
1,2,3,4,6,7 HxCN / 1,2,3,5,6,7 HxCN	24,2	23,2	25,0	< 6
1,2,3,5,6,8 HxCN	13,0	12,3	12,5	< 6
1,2,4,5,7,8 HxCN / 1,2,4,5,6,8 HxCN	25,7	24,9	25,0	< 6
1,2,3,6,7,8 HxCN	15,3	14,2	15,0	< 6
1,2,3,4,5,6,7 HpCN	12,3	11,5	12,5	< 2
1,2,3,4,5,6,8 HpCN	12,0	10,4	12,5	< 2
Recovery of ^{13}C-PCDD/F in %	35 - 45	78 - 95	-	55 - 58

Table 2: PCN concentration of five cod liver samples form southern Norway. Concentrations are given in pg/g wet weight.

Compound	Sample 1 pg/g	Sample 2 pg/g	Sample 3 pg/g	Sample 4 pg/g	Sample 5 pg/g
1,2,5,6 TeCN	16,3	50,8	17,3	17,2	52,3
2,3,6,7 TeCN	139	85,0	52,0	63,9	81,3
1,2,3,6,7 PeCN	31,1	43,5	20,6	44,8	15,9
1,2,3,5,8 PeCN	25,5	22,8	11,4	61,9	21,2
1,2,3,4,6,7 HxCN / 1,2,3,5,6,7 HxCN	1675	927	36737	122519	9460
1,2,3,5,6,8 HxCN	280	325	903	4723	387
1,2,4,5,7,8 HxCN / 1,2,4,5,6,8 HxCN	1028	1121	471	3295	148
1,2,3,6,7,8 HxCN	< 10,0	<10,0	< 50,0	< 50,0	24,8
1,2,3,4,5,6,7 HpCN	108	48,3	2588	38257	461
1,2,3,4,5,6,8 HpCN	31,2	61,9	107	3004	27,8
Recovery of ^{13}C-PCDD/F in %	35 - 36	48 - 50	40 - 54	38 - 52	52 - 57
TE (PCN)	3,67	1,99	81,2	359	20,3
i-TE (PCDD/F)	11,5	16,7	292	521	100
TE (non-*ortho* PCB)	1957	2070	135	103	74,5

TE(PCN): 2,3,7,8-TCDD toxicity equivalent of PCN according A. Hanberg et.al.[3] with TEF(1,2,3,5,6,7-HxCN) = 0,002 and TEF(1,2,3,4,5,6,7-HpCN) = 0,003.
i-TE(PCDD/PCDF): 2,3,7,8-TCDD toxicity equivalent of PCDD/F according NATO/CCMS(1989).
TE(non-*ortho* PCB): 2,3,7,8-TCDD toxicity equivalent of non-*ortho* PCB (PCB-77, PCB-126 and PCB-169) according to Ahlborg et.al. (1993)[8].

The concentrations of a PCN standard mixture treated by the complete clean-up method is in good agreement with the theoretical concentrations. It verifies that the PCN congeners are following the ^{13}C-labeled PCDD/PCDF standards. We used therefore the same internal standard mixture both for PCDD/PCDF and PCN determination. PCN concentration in method blanks are below the limit of detection.

Results of the congener specific determination of PCN of five cod liver samples are given in table 2. All five samples were also analyzed for PCDD/PCDF and non-*ortho* PCB. For these compounds only results reduced to the toxicity equivalent are reported here. The recovery rate of ^{13}C-PCDD/F for the PCN clean-up is in general lower (between 30 and 60 %) than for our standard PCDD/PCDF method (70 -90 %). This is probably due to additional manual steps during sulfuric acid clean-up, which can be optimized.

The toxic hexa- and heptachloro PCN congeners 1,2,3,4,6,7/1,2,3,5,6,7-HxCN and 1,2,3,4,5,6,7-HpCN have the highest concentrations of all PCN congeners found in cod liver. The same HxCN congeners were labeled HxCN(a) in a Swedish report on PCB and PCN in biota[9]. The concentration in cod liver from the Swedish south coast (2,0 ng/g) is about the same as in sample 1 and 2 (0,9 - 1,7 ng/g). However, in the samples from the Skien/Porsgrund area considerable higher PCN concentrations were found, with a concentration of 1,2,3,4,6,7/1,2,3,5,6,7-HxCN between 9 and 123 ng/g. The gradient for the concentration of PCN is in accordance with the gradient found for PCDD/F indicating a point source in the inner part of this fjord area[10].

In sample 4 more than 35 % of the total dioxin-like toxicity (i-TE + TE(non-*ortho* PCB) + TE(PCN)) originates from only two PCN congeners. This is a higher contribution of PCN than earlier found in fish samples from Swedish lakes (0,3 - 13 %)[9]. For the samples 1 and 2 taken nearby Bergen in an area with a known PCB contamination, it is not very surprising that the total dioxin-like toxicity is completely dominated by the non-*ortho* PCB.

Acknowledgement: The help of Binh T. Truong with method development is greatly appreciated.

4. References

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