POLYCHLORINATED NAPHTHALENE (PCN) LEVELS AND DISTRIBUTION PATTERNS IN FISH FROM THE BALTIC SEA

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Introduction

Polychlorinated naphthalenes (PCNs) have been released incautiously into the marine environment since the beginning of the 20th century when the production and use of technical formulations containing various combinations of PCNs began. PCNs are also continuously formed, and released into the environment, via diverse processes such as municipal waste incineration[1] and chlor-alkali production[2]. This group of compounds have been detected in biota samples from remote areas[3,4] and in higher trophic level marine species[5,6]. In the Baltic Sea, the ΣPCN concentrations were found at low ng/g lw in biological tissues from uncontaminated areas[7]. Concentrations of PCNs in fishes caught in the Baltic Sea have been reported in several studies[7,8,9,10,11] and the levels in some of the fishes were as follows: perch (fish from the lithoral zone), 19 ng/g lw[8]; herring (fish from the pelagic zone), 0.98-29 ng/g lw[7,9]; four-horned sculpin (fish from the benthic zone), 0.47-1.9 ng/g lw[11]. In this paper, the PCN concentrations and patterns obtained in perch (*Perca fluviatilis*), herring (*Clupea harrengus*), whitefish (*Coregonus lavaretus*), whitefish roe, and sea-trout (*Salmo trutta*) caught in the Gulf of Bothnia, northern Baltic Sea, are reported and discussed.

Methods and Materials

Fish samples

Perch and herring were caught in fishing-nets in the Gulf of Bothnia (Bothnian Bay and Bothnian Sea), northern Baltic Sea (Fig. 1). Perch were sampled at four coastal locations (HF, Harufjärden; UM, Umeå; HL, Hornslandet; GB, Gävlebukten) and herring at two open sea locations (F9 and SR5). In addition, herring were collected at three different occasions (early spring, late spring, and autumn at SR5) during a year. Whitefish and sea-trout were caught in fish-traps placed at HL. A special whitefish roe sample was also prepared and analysed. The biological tissues (whole body or roe) were initially homogenized and pooled and sub-sampled into replicates. The sub-samples were stored at –20 ºC until analysis.

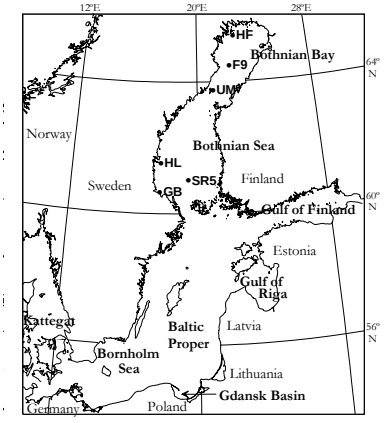

Figure 1. Sampling locations in the Gulf of Bothnia.

Extraction and cleanup

A multi-residue, non-destructive analytical method was used for the extraction, cleanup, and analysis of all the samples[12]. The homogenate derived from the fish samples was placed in pre-extracted cellulose thimbles and extracted wet in a Soxhlet apparatus, equipped with a Dean Stark

trap for collecting water. The homogenate was extracted with toluene for 24 h followed by acetone:*n*-hexane (59:41) for another 24 h. After solvent reduction, the lipid content in each sample was determined gravimetrically. Prior to extraction, a ^{13}C-labelled non-*ortho* PCB (^{13}C-PCB 126) was added as an internal standard. The cleanup was carried out by means of dialysis with semi-permeable membranes (SPMs) to reduce the bulk of the lipids using cyclopentane[13]. The dialysate was further cleaned-up by elution on a silica column with *n*-hexane and fractionated on an HPLC aminopropylsilica column[14]. A fraction from the amino-column containing dicyclic aromatic compounds was introduced onto an HPLC column containing PX-21 activated carbon[15]. This resulted in a final separation of planar PCNs from *ortho*-substituted PCBs and other interfering compounds, by gradient elution with a mixture of dichloromethane (DCM, 1%) in *n*-hexane and toluene (0-10%). PCNs were backflushed from the column with 80 mL of toluene. A tetradecane keeper (30 µL) and a recovery standard (^{13}C-labelled PCB 101) were added to the fraction containing planar compounds prior to evaporation and the final analysis.

HRGC-HRMS analysis

The extracts (3 µl) were injected in splitless mode on a Hewlett Packard 5890 high-resolution gas chromatograph system coupled to a VG Analytical 70-250S double focusing high-resolution mass spectrometer (HRGC-HRMS). PCN separation was performed on an Rtx-5 capillary column (60 m, 0.32 mm i.d., 0.25 µm film thickness) using the following temperature program: 180 ºC (2 min), 20 ºC/min to 200 ºC, then 4 ºC/min to 300ºC (held for 15 min). Electron ionisation was used at 35 eV and the HRMS operated at a mass resolution of 8000. The detection of PCN ions was carried out in SIM-mode and the two most abundant ions in the molecular ion chlorine distribution cluster for each PCN homologue (tetra- through hepta-CNs) were monitored. The identification of PCNs was based on retention data quoted in the literature[16] and using a Halowax 1014 mixture.

Results and Discussion

PCN concentrations in fish from the Baltic Sea

Average PCN concentrations, PCN-TEQs, number of samples, and content of lipids in the analysed fish samples are presented in Table 1. The PCN-TEQ calculations were based on relative potencies (REPs) of individual congeners reported in the literature[17,18,19]. The results show that the average concentrations of ΣPCNs were highest in the sea-trout (3.0 ng/g lw) and lowest in the perch from the northern locations (0.22-0.25 ng/g lw). The average ΣPCN concentration in the perch samples from Gävlebukten (GB) was elevated (1.2 ng/g lw), indicating a possible source in this area. Significantly higher ΣPCN concentrations have been reported in perch caught in the Gdansk Basin (19 ng/g lw)[8]. These results suggest heavier PCN pollution in the southern part of the Baltic Sea. The ΣPCN concentrations detected in the herring samples (0.41-0.58 ng/g lw) were relatively moderate but twice as high as in the perch from the northern locations. No significant spatial concentration variations were observed in herring caught in the Bothnian Bay compared to the herring caught in the Bothnian Sea. Furthermore, the ΣPCN concentrations in herring from the Gulf of Bothnia were lower than reported levels in herring caught at different locations in the Baltic Sea (0.98-29 ng/g lw)[7,9]. Herring sampling in early spring, late spring, and autumn enabled investigation of within year variations of PCN concentrations. The results show a fairly constant ΣPCN concentration in herring (Table 1). PCN concentration and PCN-TEQ in the whitefish roe (2900 and 0.68 pg/g lw, respectively) was approximately four times higher than in whole body whitefish (660 and 0.19 pg/g lw) indicating a high transfer rate of PCNs from fish to roe.

PCN congener patterns

PCN congener patterns obtained from fish samples collected in the Gulf of Bothnia are shown in Figure 2. In general, the PCN distribution pattern in the fishes were similar and the dominating PCN congeners were as follows: TeCN 33/34/37, 44/47, 28/43/45, 35/39, and 38/40/48; PeCN 52/60, 58, and 61; HxCN 66/67, 64/68, 69, and 71/72. The dominance of PCN 52/60 and 66/67

Table 1. Average PCN levels (pg/g lw) in fish samples collected in the Gulf of Bothnia[a].

Type of sample	**Perch**				**White-fish**	**Whitefish roe**	**Herring**				**Sea-trout**
Location[b]	**HF**	**UM**	**HL**	**GB**	**HL**	**HL**	**SR5(es)**	**SR5(ls)**	**SR5(a)**	**F9(a)**	**HL**
PCN											
42	1.0	2.1	0.6	5.5	5.6	66	4.5	1.9	0.50	2.4	310
33/34/37	17	17	23	96	58	240	37	27	7.7	21	110
44/47	13	9.6	16	76	33	160	36	34	22	28	100
36	2.7	6.0	6.8	5.2	15	28	4.4	4.7	2.3	2.7	13
28/43/45	18	11	21	47	28	120	24	21	14	13	49
27/29	4.6	2.2	6.1	6.6	5.3	28	7.5	9.0	2.5	4.0	10
30/32	2.5	3.3	2.9	3.2	3.0	13	5.7	5.9	3.3	3.5	13
35/39	16	8.7	18	26	20	79	33	34	25	17	70
38/40/48	20	12	22	23	21	73	21	15	14	15	27
46	8.5	4.1	10	7.9	12	40	7.8	8.9	6.3	9.4	18
31	0.20	0.90	0.25	n.d.	n.d.	n.d.	n.d.	1.3	0.18	0.50	n.d.
41	1.9	2.0	0.52	0.45	1.2	1.2	0.61	1.0	n.d.	n.d.	n.d.
ΣTeCNs	**100**	**78**	**130**	**300**	**200**	**850**	**180**	**160**	**97**	**110**	**720**
52/60	4.4	45	5.2	170	140	950	23	27	19	27	1100
58	6.1	6.7	3.5	20	13	87	2.8	1.6	1.3	n.d.	99
61	23	26	14	110	120	460	81	100	72	130	560
50	3.8	2.6	3.1	11	5.3	12	5.2	6.6	2.9	5.8	5.0
51	5.5	2.9	3.2	11	3.6	11	3.4	4.4	3.4	3.6	7.0
54	4.6	6.2	3.4	5.9	8.8	33	18	23	22	20	40
57	11	9.2	8.2	26	11	30	15	16	11	20	25
62	13	9.4	9.2	37	13	34	18	26	18	23	31
53/55	13	7.5	9.7	33	20	54	19	24	19	16	32
59	13	9.4	5.8	19	19	56	17	21	17	16	33
49	n.d.	n.d.	0.15	1.3	1.0	1.8	1.8	2.9	2.4	0.70	n.d.
56	0.33	0.095	n.d.	0.090	0.42	0.76	n.d.	n.d.	n.d.	n.d.	n.d.
ΣPeCNs	**97**	**120**	**66**	**440**	**350**	**1700**	**200**	**250**	**190**	**260**	**1900**
66/67	6.5	20	0.75	140	47	190	6.0	29	13	16	220
64/68	5.1	4.7	2.2	19	11	36	9.5	20	13	27	37
69	7.3	7.1	7.1	24	20	58	21	36	49	37	63
71/72	14	5.8	8.6	110	13	37	17	25	23	26	33
63	1.8	0.55	0.75	5.1	2.0	4.0	2.7	4.8	4.0	4.3	3.1
65	1.7	0.43	1.6	5.5	2.9	7.2	1.7	3.1	1.2	1.6	1.4
70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ΣHxCNs	**36**	**39**	**21**	**310**	**96**	**330**	**58**	**120**	**100**	**110**	**360**
73/74	8.7	3.5	3.3	120	7.9	14	19	42	27	19	18
ΣHpCNs	**8.7**	**3.5**	**3.3**	**120**	**7.9**	**14**	**19**	**42**	**27**	**19**	**18**
ΣPCNs	**250**	**240**	**220**	**1200**	**660**	**2900**	**460**	**580**	**410**	**500**	**3000**
PCN-TEQ	0.055	0.077	0.029	0.55	0.19	0.68	0.093	0.21	0.18	0.18	0.76
No. of samples	4	2	1	2	5	1	4	4	3	2	1
Lipids (%)	10	8.0	15	11	7.6	19	24	25	22	33	20

[a] n.d., not detected
[b] HF, Harufjärden; UM, Umeå; HL, Hornslandet; GB, Gävlebukten; SR5, open sea station in Bothnian Sea; F9, open sea station in Bothnian Bay; es, early spring; ls, late spring; a, autumn.

was not so pronounced in the herring samples and this different pattern has earlier been reported in the literature[9]. Previous studies have shown that the PCN congener pattern shifts from a sediment-like pattern to a non-sediment-like pattern, similar to the patterns obtained in this study, when moving from the bottom to the top of a food chain[9,11]. Biological processes seem to alter the composition and PCN pattern in predators such as fish higher up in the food chains. The difference in PCN patterns may reflect a congener-specific rapid excretion, intestinal absorption, and/or metabolic transformations in the marine species. The PCN congener patterns in the whitefish and roe were similar, demonstrating a non- congener-specific transfer of PCNs from fish to roe.

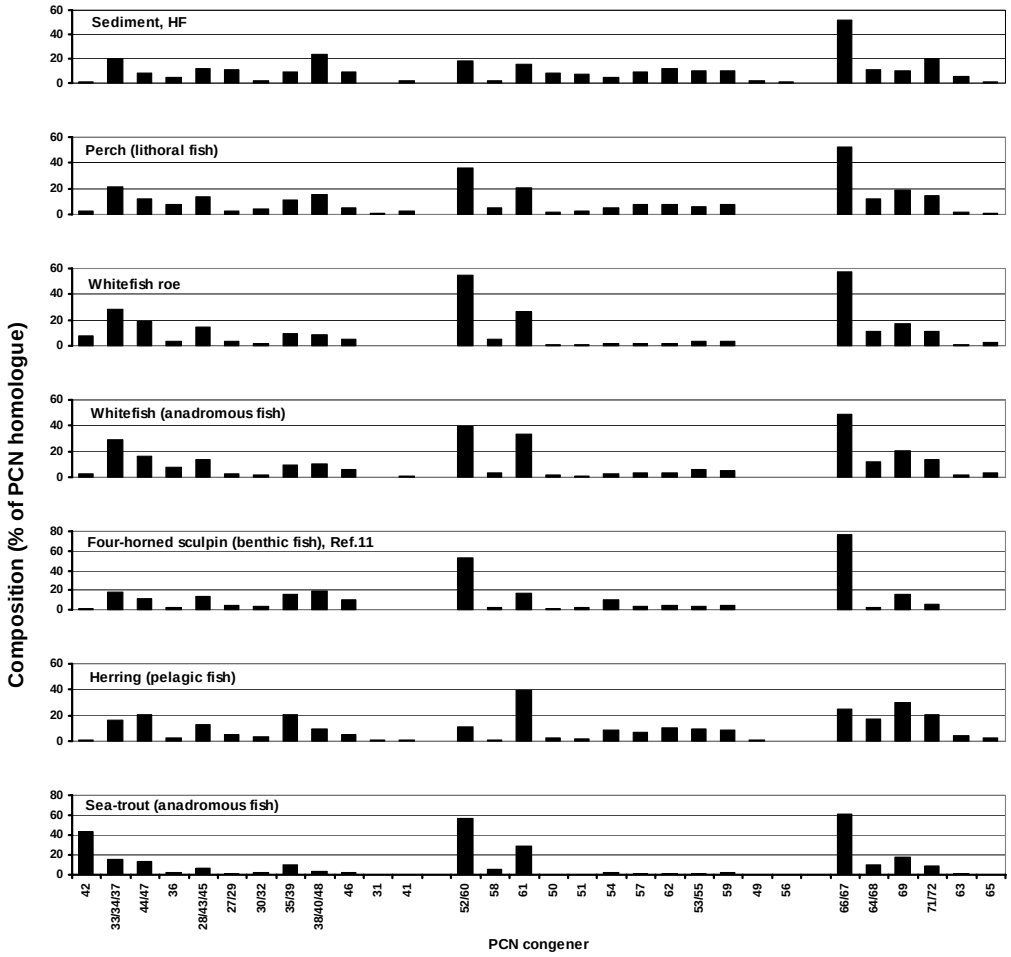

Figure 2. Composition of PCNs (% of PCN homologue) in fish samples collected in the Bothnian Bay and Bothnian Sea.

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