BIOACCUMULATION OF DIOXINS, COPLANAR PCBs, PCNs, R-PCNs, R-PCPHs AND R-PCBBs IN FISH FROM A PULP MILL RECIPIENT WATERCOURSE

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Introduction. Certain isomers of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are known to be highly toxic to animals: 2,3,7,8-substituted PCDDs and PCDFs and non-ortho substituted PCBs are the most potent congeners. Polychlorinated naphthalenes (PCNs) also have toxicological significance. Hexa-CNs have been indicated to be as active inducers of MFO enzymes as coplanar PCBs[1]. Other planar aromatic chlorocompounds or compounds which can take planar form exhibiting similar chemical and physical properties might also be included to the class of dioxin-type toxic compounds. Many chlorocompounds formed in chlorine bleaching of pulp could be such toxicants, e.g. alkyl polychlorophenanthrenes (R-PCPHs) and alkyl polychloronaphthalenes (R-PCNs)[2-4] or alkyl polychlorodibenzofurans (R-PCDFs)[5]. Some isomers of alkyl polychlorobibenzyls (R-PCBBs; polychloro-1,2-diphenylethanes), which occur at tens of ng/l levels in pulp mill effluents[6], also could possess toxic properties.

Residue analyses of organochlorines in fish have shown that fish accumulate toxic compounds like PCDDs and PCDFs. The accumulation of PCDDs and PCDFs has been reported to be isomer-selective to most toxic congeners[7,8]. Because the consumption of freshwater fish is relatively high in Finland, the information of levels of toxic compounds in fish is important. Therefore, fish caught near pulp mills and downstream from mills have been analyzed for the levels of PCDDs, PCDFs, coplanar PCBs, PCNs and some pulp mill originating chlorocompounds.

Materials and methods. Fish caught in the vicinity of three pulp mills have been investigated during years 1990-1992. Latest samples were from South-East Finland from two sites in River Kymijoki and were taken in the fall 1991. The sampling site 1 (K) was near the effluent pipe of a pulp mill and the sampling site 2 (S) 35 km downstream from pulp mills. Previously, two other recipient watercourses in Central Finland were studied[6].

Fish species used as food were chosen for analyses. Fish were delivered frozen to laboratory, where they were measured and weighed. Large fish were analyzed as individuals, but small fish were analyzed as composite samples of four individuals.

Sample data of fish from River Kymijoki are presented in Table 1.

Table 1. Sample data of River Kymijoki fish (in parenthesis number of individuals in the combination sample).

Code	Species	Sampling place	Weight g	Length cm
KPI	Pike	1	2338	70
KPE (n=4)	Perch	1	89-161	20-24
SPI 1	Pike	2	1177	58
SPI 2	Pike	2	1964	64
SPI 3	Pike	2	3810	79
SBR 1	Lake bream	2	777	42
SBR 2	Lake bream	2	221	27
SPE (n=4)	Perch	2	84-151	20-23

Fillets of muscle were freeze-dried before extraction, which was done in Soxhlet-device with organic solvent mixture for 6 hours[6]. Internal standards (^{13}C-labeled 2378-TCDD, 2378-TCDF, 123478-HxCDD, OCDD, and ^{13}C-labeled PCB 77, 126 and 169) were added before extraction.

Extracts were shaked with conc. sulfuric acid and fractionated using basic alumina and activated charcoal columns[6]. The dioxin fractions were analyzed by GC/MS in SIM mode. GC/MS was done both with LRMS and HRMS: quantitative analyses were mainly done with LRMS instrument and HRMS was used for verification. LRMS was performed with a HP mass selective detector model 5970. HRMS instrument used was a VG AutoSpec.

C_4-PCPHs (P=1-4) and C_{2-3}-PCNs (P=1-2) which are found in pulp mill wastes[2-4] were included to the analyzed compounds of River Kymijoki fish. Unlike other studied compounds, C_5-PCBBs (P=1-4) were analyzed after alumina column fractionation before activated carbon clean-up because the synthetized model substances of C_5-PCBBs had been observed to elute mainly in the first fraction in carbon column[4].

Results and discussion. Levels of 2378-TCDF and coplanar PCBs in Kymijoki fish are presented in Table 2.

Table 2. 2378-TCDF, PCB 77 and PCB 126 in fish of River Kymijoki (pg/g fresh weight).

Sample	2378-TeCDF	PCB 77	PCB 126
KPI	12	11	4
KPE	2	8	2
SPI 1	6	73	4
SPI 2	8	52	4
SPI 3	9	45	4
SBR 1	<2	21	2
SBR 2	3	61	2
SPE	3	58	5

In contrary to mussels incubated previously in River Kymijoki[6,9,10], 1234678-HpCDF and OCDF were not observed in the present samples, except in the pike KPI, where 1234678-HpCDF was measured 12 pg/g fresh weight. The absence of HpCDF and OCDF congeners in fish compared to their occurrence (OCDF up to 70 ng/g fat) in incubated mussels refers to different accumulation and metabolism of PCDFs in mussels and in fish. The low dietary uptake in fish of highly chlorinated PCDDs/PCDFs has been suggested to be due their molecular configuration[11].

The main PCDF isomer generally found was 2378-TCDF. Its source could be pulp chlorobleaching. 12378-PeCDF could not be determined due to interfering polychlorinated diphenylethers (PCDEs). PCDEs are usually detected in samples from Baltic Sea[12-14] and they were not earlier observed to disturb PCDF analysis of freshwater fish. In tests with standards, present activated basic alumina (1 g column of alumina from ICN Biomedicals) proved to be inadequate for the separation of PCDFs and PCDEs. Properties of new basic alumina, as also properties of new activated charcoal, in fractionation of PCDEs seemed to be different from materials used earlier. Therefore, levels of PCDFs and PCDEs in some present samples will be reanalyzed with improved methods. Also the origin of PCDEs will be studied further.

PCB 77 was the most abundant non-ortho substituted PCB congener in all fish samples studied. Fish from the sampling site 2 contained slightly elevated levels of PCB 77 compared to fish collected upstreams. In Central Finland, coplanar PCB levels in fish were similarly low[6]. High levels of coplanar PCBs in Finnish freshwater fish have so far only been measured in fish from Lake Kernaala, which is heavily polluted by a PCB leakage[14].

Unlike fish from Central Finland[6], the River Kymijoki samples were measured only for hexachloroisomers of PCNs. HxCNs occurred at highest about 10 pg/g fresh weight.

Concentrations of alkyl chloroaromatics of pulp mill origin were also low in fish. A possible trace of C_5-monoCBB was found about 5 pg/g fresh weight (1 ng/g fat) in the pike KPI and in the perch KPE. In fish caught near pulp mills in Central Finland, C_5-PCBBs were also at low level (few pg/g fresh weight)[6]. Mussels incubated in River Kymijoki contained C_5-PCBBs (P=1-4) about 26 ng/g fat[6].

Fish from the sampling site 1 were not observed to have accumulated C_{2-3}-PCNs or C_4-PCPHs. C_4-monoCPHs in fish near pulp mills were estimated to be below 0.2 ng/g fat. Compared to this, C_4-PCPHs (P=1-4) in incubated mussels at the level of about 90 ng/g fat[4] are significantly higher.

The uptake of R-PCPHs, R-PCNs and R-PCBBs in fish could be selective like the uptake of PCDDs and PCDFs as these are non-detectable or occur at low levels in fish. The accumulation of fish having higher fat content might be different. Anyhow, the present results and earlier observations so far indicate that freshwater fish species in Finland contain only near background levels of PCDDs, PCDFs, coplanar PCBs and PCNs.

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