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MONO-ORTHO AND NON-ORTHO SUBSTITUTED PCBS IN ARCTIC RINGED SEAL (PHOCA HISPIDA) FROM THE SVALBARD AREA: ANALYSIS AND DETERMINATION OF THEIR TOXIC THREAT.

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ABSTRACT

To determine the 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) of the PCBs in ringed seal, we analysed liver, blubber and kidney for non-ortho and mono-ortho substituted CBs as well as the total PCB and total DDE concentration. The blubber samples were compared with ringed seal blubber samples from another study[1,2], in which total PCB and PCDD/PCDF concentrations were measured, and the TEQ values for the PCBs in our samples were 11 times higher than the TEQ values for the PCDDs/PCDFs in the other seal samples. Comparing the 3 different seal matrices, we found no significant difference in PCB pattern; the absolute concentrations expressed per unit lipid weight were for the females in the following order : blubber ≈ liver > kidney. We found another order for the males: blubber > liver > kidney. Among the different seals, there was a good PCB-sex correlation indicating that the results of different studies, without specific background information, must be compared with great caution.

INTRODUCTION

Toxic non- and mono-ortho coplanar polychlorinated biphenyls (PCBs) as well as total PCB concentrations have been determined in several terrestrial and marine mammals[3,4]. In general, the concentrations of mono-ortho coplanar PCBs were found to be higher than the non-ortho congeners and both PCB congener types were significantly higher than the levels of toxic polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). 2,3,7,8-Tetrachloro-p-dioxin toxic equivalent (TEQ) analysis revealed that higher aquatic predators receive a greater toxic threat from 2,3,3',4,4'- and 3,3',4,4',5-pentachlorobiphenyl than PCDDs and PCDFs[5]. Oehme et al.[1,2] reported the presence of PCDDs and PCDFs in ringed seal (Phoca hispida) from Spitsbergen and expressed the concentrations as TEQ values. The objective of this study is to determine the degree of contamination with PCBs in different matrices and to evaluate the relative toxicological importance of the coplanar PCBs compared with the toxicological contribution of PCDDs and PCDFs in the ringed seal from Svalbard. In this respect, we analysed the kidney, liver and blubber of the arctic ringed seal for the total PCB concentration and for some selected non- and mono-ortho coplanar PCBs . We also quantitated the total DDE concentration in order to evaluate possible temporal trends in arctic ringed seal.

MATERIALS AND METHODS

Sample preparation

Samples of ringed seal blubber, liver and kidney were collected from Kongsfjorden (78°50'N) and Tempelfjorden (78°20'N), Spitsbergen, Norway. The seals were all sampled in March-April 1990 and all samples were transported in a frozen state and stored at -40 °C until analyzed. Sex and reproductive status of the animals are listed in Table 1.

Number	Sex	Sex status	Sampling date	Sampling place
1	male	mature	March 1990	Tempelfjorden
2	female	mature	March 1990	Tempelfjorden
3	female	juvenile	March 1990	Tempelfjorden
4	male	mature	March 1990	Tempelfjorden
5	female	pregnant	April 1990	Tempelfjorden
6	female	pregnant	April 1990	Tempelfjorden
7	male	mature	April 1990	Tempelfjorden
8	male	mature	April 1990	Tempelfjorden
9	male	juvenile	April 1990	Kongsfjorden
10	male	mature	April 1990	Kongsfjorden
11	male	juvenile	April 1990	Kongsfjorden
12	male	juvenile	April 1990	Kongsfjorden
13	female	mature	April 1990	Kongsfjorden

Table 1 : Sampling information about the ringed seals.

Total PCB and DDE determination

Extraction and cleanup. A sample (20 g liver or kidney and 10 g blubber) was cut into small pieces and placed in a 500 ml flask containing 250 ml 1 N ethanolic KOH and placed under reflux for 2 hours. When using this procedure, p,p'-DDT will be transformed to p,p'-DDE[6]. After this saponification step, 250 ml of distilled water and 100 ng of internal standard (chrysene) were added. The crude extract was partitioned twice with 100 ml hexane. The two extracts were combined and reduced to a volume of 1 ml. This concentrated extract was subjected to a Florisil cleanup procedure[7]. Florisil, 60-100 mesh, (Merck) was deactivated with 1.2 % water and 8 g were packed with hexane in an elution column (10 mm internal diameter and 200 mm height) with a 100 ml reservoir. The extract was placed on the column which was eluted with 40 ml hexane and 40 ml dichloromethane:hexane (20:80). The eluate was reduced to about 50 μl, and 5 μl were injected in the GC/MS for final analysis.

GC/MS determination. The samples were analyzed on a Hewlett-Packard 5988 A GC/MS employing electron impact ionization mode at 70 eV using selected ion monitoring (SIM). The GC was equipped with a 60 m long x 0.25 mm internal diameter fused silica DB-5 column with a film thickness of 0.25 micron provided with a 1 m deactivated fused silica retention gap. The splitless injection mode was held for 1 minute after injection at an oven temperature of 60 °C on a split/splitless injector at 250 °C. Temperature programming started with a rate of 20 °C/min until 200 °C and continued at a rate of 1 °C/min until 275 °C. The carrier gas was helium and the transfer line was kept at 280 °C. A SIM-table (Selective Ion Monitoring) was set up for GC/MS identification and was based on the elution order of all 209 PCB congeners[8]. We established 5 SIM groups separated by 4 group switch times, defined by PCB # 104, # 77, # 128 and # 208 (numbers according to Ballschmitter & Zell[9]) so not all ions had to be checked in every group. The retention time, the masses and the ratio of the confirmation ion intensity to the quantitation ion intensity in

comparison with the expected ratio for each level of chlorination were used as the identification criteria. The average experimental relative deviation of the theoretical ratios for the chlorinated PCBs was 6.4 %. For the trichloro- through the heptachloro PCB congeners, at least one ion in the $(M + 70)^+$ ion cluster was examined to verify that no coeluting PCB congener containing two additional chlorines were present. For the dichloro through the octachloro congeners, at least one ion in the $(M + 35)^+$ ion cluster was examined to verify that no coeluting PCB congeners containig one additional chlorine was present, and for all congeners starting from the dichloro, at least one ion in the $(M - 70)^+$ ion cluster must be present. For the GC/MS quantitation of the total PCB concentration, a calibration curve was set up with eight pure PCB isomers (# 5, # 29, # 50, # 87, # 154, # 188, # 200 and # 209)[10] instead of using the commercial mixtures of Arochlors, Kanechlors or Clophens. After qualitative determination was accomplished, the quantitation ion areas of all identified congeners within each homologue isomer series were summed and the total concentration of each homologue series was calculated using the appropriate homologue response factor. The mono-ortho PCB congeners and total DDE were quantitated using their respective pure standards for calibration.

Non-ortho PCB determination

Extraction and cleanup. The same procedure is followed for the extraction as with the total PCB determination except for the internal standard. Instead of chrysene, we added 20 ng of C^{13} labelled pure isomers of PCB # 77, # 126 and # 169 (Cambridge Isotope Laboratories) as internal standards. The non-ortho PCBs were separated from the bulk of the PCBs in the crude extract by means of carbon chromatography[11]. Active carbon (Wako Pure Chemical Industries Ltd.) was heated at 100 °C in an oven at reduced pressure and then stored in a desiccator. The elution column (5 mm internal diameter and 40 mm height) with a 100 ml reservoir was packed with 125 mg active carbon in hexane. The extract was added to the column and initially eluted with 100 ml of dichloromethane:hexane (20:80) at one drop per second flow rate using a positive pressure. This fraction was rejected and elution proceeded with 100 ml ethylacetate:benzene (50:50). This second eluate was concentrated to 100 μl and made up with 5 ml hexane, and was vortexed with 5 ml 5 % fuming sulfuric acid in concentrated sulfuric acid. The two layers were centrifuged at 6000 rpm for 10 minutes. The upper hexane layer was reduced to 50 μl of which 5 μl were injected in the GC/MS.

Lipid content determination

About 5 g sample was taken (exact weight must be known) and mixed in a mortar with a 10 times higher weight Na_2SO_4. This mixture was packed onto a column and eluted with 50 % dichloromethane in hexane. After evaporating and drying, the lipid content was determined gravimetrically.

RESULTS AND DISCUSSION

General findings

Total PCB and DDE concentrations in the different matrices are presented in table 2, and expressed per unit wet weight and also per unit extractable lipid weight. Figure 1 shows a clear differentiation in residue levels between the mature male and female seals; analysis of variance confirms that concentrations in male seals are significantly higher than in female seals ($P < 0.05$). This probably occurs because males have no pathway other than slow metabolic degradation by which to excrete PCBs. In female seals, concentrations are relatively low because they have the ability to excrete lipids and its associated xenobiotics during lactation. The residue burden can also being transferred

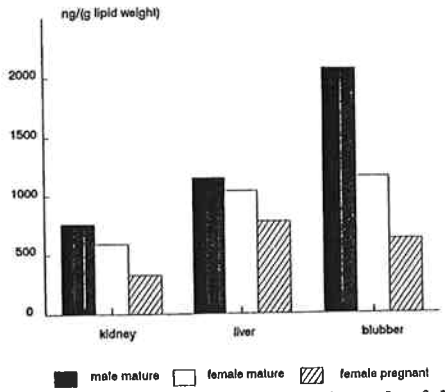

Figure 1 : Total PCB concentration in seals of different sex and reproductive status

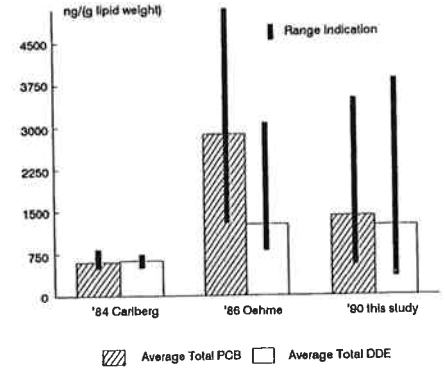

Figure 2 : Total PCB and DDE concentrations of different studies.

to the fetus during its development. Figure 2 shows the results of three studies all concerning the analysis of PCBs and DDE in ringed seal from Spitsbergen. Total PCB levels in ringed seal blubber, sampled in 1986, were reported by Oehme et al.[2] and varied between 1270 and 5050 ng/(g lipid weight). These figures are estimates and were calculated on the basis of the presence of PCB # 153 and # 138 in the seal blubber as if they were present in an Arochlor 1260 mixture. We find the concentration in seal blubber to be ranging between 539 and 3460 ng/(g lipid weight), being about half the concentration found by Oehme et al. in the 1986 samples. On the other hand, ringed seal blubber sampled in Hornsund, Svalbard, in September 1984 and analysed by Carlberg et al.[12] showed total PCB levels between 490 and 820 ng/(g lipid weight). The minimum values are comparable with our findings but our maximum value is about 4 times higher.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	<x>	STD*
							Liver#								
% lipids	3.70	2.98	2.34	2.46	3.02	3.6	3.49	3.20	3.43	3.22	3.32	3.59	2.75	3.16	0.42
PCB	497	799	1179	1354	702	831	605	434	1213	2339	699	1019	1502	1013	498
DDE	192	283	632	-&	-	-	-	175	-	977	-	-	628	481	291
PCB/DDE	2.59	2.82	1.86	-	-	-	-	2.47	-	2.39	-	-	2.39	2.42	0.29
							Blubber#								
% lipids	95.5	95.8	95.6	96.7	95.0	97.9	97.8	96.0	94.8	92.7	96.1	92.8	98.4	95.8	1.7
PCB	1248	1208	2115	3460	539	711	942	2439	1378	2624	921	759	1061	1493	853
DDE	1288	1305	2513	3790	332	662	880	2578	1116	1547	531	364	513	1340	1000
PCB/DDE	0.97	0.93	0.84	0.91	1.63	1.07	1.07	0.95	1.23	1.70	1.74	2.08	2.07	1.32	0.44
							Kidney#								
% lipids	2.18	2.46	2.40	2.03	1.88	2.21	2.36	2.14	2.35	1.97	2.29	2.39	2.10	2.21	0.17
PCB	591	618	429	995	213	430	424	771	464	1096	275	305	562	549	257
DDE	252	260	200	443	59	109	140	347	119	462	83	79	138	207	132
PCB/DDE	2.35	2.38	2.15	2.25	3.61	3.95	3.03	2.22	3.90	2.37	3.31	3.86	4.07	3.03	0.74

#concentrations in ng/(g lipid weight) * *STD : standard deviation* & *not analysed*

Table 2 : Total PCB and DDE concentration and their ratio in the 3 matrices.

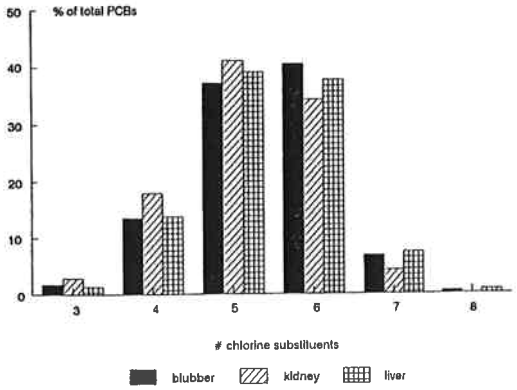

Figure 3 : Relative isomeric composition in the different matrices.

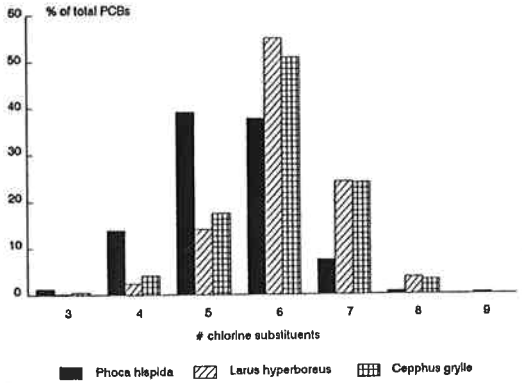

Figure 4 :Comparison of the isomeric PCB composition of two seabird species and the ringed seal

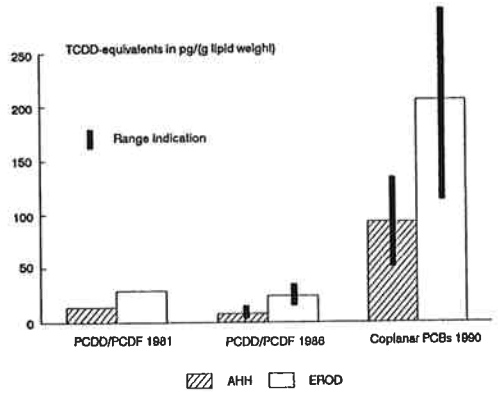

Figure 5 : Comparison of TEQs of PCDDs, PCDFs and PCBs in the different ringed seal blubber studies.[2,20]

Since no data is available about the age, sex or condition of the seals caught in 1984, it is difficult to assess this wider concentration range. The liver samples from 1984 analysed by Carlberg et al.[12] had a total PCB content between 370 and 600 ng/(g lipid weight). We find the liver concentration to be between 497 and 2339 ng/(g lipid weight). Again the minimum value is of the same order of magnitude and the maximum value in our study is about 4 times higher. We also find total PCB concentrations in kidney to be ranging between 213 and 1096 ng/(g lipid weight) and this is two to three times lower than in the liver and blubber samples. We found no other reported data of kidney concentrations to compare our results with.

Total DDE concentrations in the 1984 samples from Svalbard ranged between 140 and 290 ng/(g wet weight) in liver and between 510 and 750 ng/(g wet weight) in blubber. Oehme reported in his 1986 ringed seal blubber samples a concentration range from 787 to 3024 ng/(g lipid weight). The average ΣPCB/ΣDDE ratio in blubber almost doubles from 0.96 in 1984 to 1.73 in 1986. We find an intermediate average ratio of 1.32 in blubber. This general growing trend could be caused by a faster excretion of DDT and metabolites than PCBs. The adverse trend was observed by Addison et al.[13] who reported that PCBs had declined more than DDT group residues in arctic ringed seals from Holman Island in the North-West Territories between 1972 and 1981. The average ΣPCB/ΣDDE ratio dropped from 3.34 in 1972 to 1.71 in 1981. Apart from the difference in sampling date, this difference between Svalbard and the Canadian Arctic is probably a consequence of different geographical origin and pathways of transport of the anthropogenic pollutants.

	Absolute concentration (ng/(g lipid weight)			relative concentration (%)		
PCB #	kidney	liver	blubber	kidney	liver	blubber
31	4.2	9.0	4.1	0.8	0.9	0.3
28	8.8	26.8	13.1	1.6	2.6	0.9
52*	28.2	71.2	51.6	5.1	7.0	3.5
49	7.5	13.6	12.8	1.4	1.3	0.9
75	9.0	14.4	16.5	1.6	1.4	1.1
44	1.7	6.6	4.6	0.3	0.6	0.3
61*	21.8	35.7	44.0	4.0	3.5	2.9
70	5.7	8.5	7.8	1.0	0.8	0.5
66*	13.2	24.8	19.8	2.4	2.4	1.3
55	6.6	12.8	10.3	1.2	1.3	0.7
101*	53.8	67.2	112.2	9.8	6.6	7.5
97	0.6	1.4	2.2	0.1	0.1	0.1
87	9.2	14.0	18.7	1.7	1.4	1.3
110*	14.3	18.1	21.6	2.6	1.8	1.4
107/108	1.3	2.9	6.4	0.2	0.3	0.4
118*	49.1	57.1	107.7	8.9	5.6	7.2
114	< DL	0.3	3.0	< DL	0.0	0.2
105*	20.8	27.4	40.4	3.8	2.7	2.7
149*	11.8	24.8	20.7	2.1	2.5	1.4
153*	94.6	188.4	279.6	17.2	18.6	18.7
168	0.7	2.6	3.4	0.1	0.3	0.2
141	1.3	4.7	5.5	0.2	0.5	0.4
137	0.7	28.7	5.5	0.1	2.8	0.4
138*	66.8	114.8	187.7	12.2	11.3	12.6
158	3.6	6.6	9.6	0.7	0.7	0.6
128/162	4.0	7.9	12.2	0.7	0.8	0.8
156	3.9	6.8	9.1	0.7	0.7	0.6
157	< DL	1.8	2.9	< DL	0.2	0.2
178	0.6	< DL	5.3	0.1	< DL	0.4
187	7.8	47.3	23.8	1.4	4.7	1.6
183	2.7	19.1	14.3	0.5	1.9	1.0
181	< DL	1.0	0.2	< DL	0.1	0.0
177	< DL	2.1	2.1	< DL	0.2	0.1
171	< DL	2.3	1.5	< DL	0.2	0.1
172	< DL	1.9	2.1	< DL	0.2	0.1
180*	13.2	43.4	42.4	2.4	4.3	2.8
170	2.9	13.3	15.3	0.5	1.3	1.0
202	< DL	4.5	2.9	< DL	0.4	0.2

Table 3 : PCB congener specific concentrations (averaged over the 13 seal samples) in three different matrices (non-ortho PCBs are not included). Relative concentrations are related to the average total PCB concentration of a certain matrix. The PCBs used for analysis of variance of the 11 most abundant congeners are indicated with an asterix

Congener specific results

Table 3 shows a listing of 38 PCB congeners, with their respective concentrations, which could be identified in the seal samples. These 38 congeners constitute 92 % of the total PCB concentration found in liver, 77 % in blubber and 86 % in kidney. On average, we found 50 PCB congeners to be present in the gas chromatograms with a signal-to-noise ratio ≥ 6. Table 4 shows that there is a significant difference between the concentrations of the individual congeners in the different matrices when performing a two-way analysis of variance (ANOVA) using all 38 congeners. Transforming the absolute concentrations to relative concentrations (relative to the total PCB concentration), the difference between the different matrices is significant only at the 5 % level. These results are visualised by figure 3, in which the total PCB concentration is expressed as a function of isomeric groups. Apparantly, there is a slight tendency for more lower chlorinated (tetra- and penta-CB) PCBs and less higher chlorinated (hexa- and hepta-CB) in the kidney samples compared to the liver and blubber samples. This can be explained by the fact that the absolute

concentration in the kidney samples is much lower as in the blubber and liver samples. Therefore, some higher chlorinated biphenyls, which have a lower response factor on the GC/MS than the lower chlorinated biphenyls, fall below the limit of detection and cannot be seen on the chromatogram anymore although they are presumably present. Taking into account the above, we can state that, visually, the same pattern emerges for the three different matrices which clarifies table 4 a little more.

	Expressed as absolute concentration	Expressed as relative concentration using all 38 congeners	n_1,n_2 (degrees of freedom)
F_r (variation in rows)	11.75^{**}	80.48^{**}	37,74
F_c (variation in columns)	8.78^{*}	3.24^{*}	2,74
	using the 11 most abundant congeners		
F_r (variation in rows)	9.79^{*}	73.11^{*}	10,20
F_c (variation in columns)	7.67^{**}	1.52	2,20

* : $P<0.05$; ** : $P<0.01$

Table 4 : Two-way ANOVA of PCB congener specific analysis in three different matrices as listed in table 3.

If the ANOVA is performed using only the most abundant congeners, the resulting F_c value indicates a clear resemblance between the different matrices. Boon et al[14] also reported that the relative pattern of PCBs in different organs of harbour seals were virtually identical. This leads to the conclusion that although different animals within one species can show different levels of contamination (depending on parameters like age, sex, blubber thickness ...), the relative congener composition is constant for all the animals no matter what organ is examined. This relative PCB composition seems to be typical for each animal. In figure 4, the relative PCB composition according to isomer groups for the ringed seal is compared to two seabird species from Svalbard, the glaucous gull and the black guillemot[15]. The higher chlorinated biphenyls (hexa-, hepta- and octa-CB) are clearly more abundant in the 2 seabird species than in the seal, where the lower chlorinated biphenyls (tetra- and penta-CB) are relatively more abundant. The use of the isomeric group composition is less specific than the congener composition, but it serves well the purpose of indicating the difference between the seabirds and the seal. This shows that there is a clear difference in PCB pattern in different branches of the arctic food chain.

Toxicity levels

	Kidney[&]	Liver[&]	Blubber[&]
# 77	<DL[*]	390	102
# 126	<DL	781	191
# 169	<DL	<DL	<DL

[&] *concentrations in pg/(g lipid weight)* [*] *<DL : below limit of detection*

Table 5 : Average concentrations for the most toxic non-ortho substituted PCBs in the three different matrices

Table 5 lists the concentrations of the 3 most toxic non-ortho substituted PCBs. The liver and kidney results were obtained using pooled samples. PCB #126 is most abundant in the liver and blubber samples. This is in accordance with the relative concentrations in the 2 seabird species mentioned above, and with a recent study of Kannan et al.[16]. To evaluate toxicity levels for these coplanar PCBs, we used toxic equivalence factors (TEFs). The TEFs, derived from the normalized potencies of induction relative to 2,3,7,8-TCDD, for the PCDDs and PCDFs are taken from the data of Safe et al.[17] who measured induction potencies of various congeners to half maximally induce

AHH (Arylhydrocarbonhydroxylase) and EROD (Ethoxyresorufine-O-deethylase) in rat hepatoma cell line.

Reference	Matrix	TEQ (AHH-basis)	TEQ (EROD-basis)
		PCDDs and PCDFs	
Carlberg & Bøler 1985	blubber	13.9	29.1
Oehme et al. 1990	blubber	8.2 (3.9 - 15.3)	24.2 (15.1 - 35.7)
		non- and mono-ortho substituted PCBs	
this study	liver	258	555
this study	blubber	92 (51 - 135)	206 (114 - 293)
this study	kidney#	14.9 (4.0 - 29.3)	28.9 (8.5 - 56.1)

non-ortho substituted PCBs not included

Table 6 : TEQ values of PCDDs, PCDFs and PCBs originating from different studies

The TEF for the coplanar PCBs are taken from a review article of De Voogt et al.[18]. These TEF values are used to define the 2,3,7,8-TCDD toxic equivalent (TEQ) value of a corresponding congener[4,11,19]. This defenition is merely the multiplication of the concentration of a certain PCDD, PCDF or coplanar PCB with its respective TEF and is expressed as (pg 2,3,7,8-TCDD)/g. We calculated the TEQ values for the coplanar PCBs on the basis of PCB #77, #126 and # 169 (non-ortho substituted) and PCB #105, #114, #118, #156 and #157 (mono-ortho substituted). Table 6 lists the TEQ values of coplanar PCB congeners and of PCDDs and PCDFs for the ringed seal. Compared to the 1986[2] and the 1981[20], PCDD and PCDF TEQ values in seal blubber, TEQ values for the coplanar PCBs are 11 times and 7 times higher respectively (figure 5). An attempt was made to analyse the PCDDs and PCDFs in our 1990 samples, but the concentrations all fell below our limit of detection of 50 pg/(g wet weight). In trying to make corrections for the difference in sampling date in order to evaluate the results of the other studies on ringed seals, we have to take into account that the source of PCBs and the source of PCDDs and PCDFs only overlaps. PCDDs and PCDFs can enter the arctic food chain as contaminants of PCB formulations and thus correlated with the PCB concentration, but they can also originate from many other sources like from municipal waste incinerators, having no correlation with the PCB concentration at all. It is so that, if we assume that PCDD and PCDF concentrations are fully correlated with PCB concentrations (which is a very rough assumption), the theoretical average TEQ value of PCDDs and PCDFs in our 1990 samples would be about half the TEQ value of the 1986 samples. This means that the TEQ value for the coplanar PCBs would be about 22 times higher instead of 11 times.

CONCLUSIONS

In spite of all the possible geographical and temporal trends in dioxin and furan concentrations, we think that the TEQ difference between PCBs on one hand and PCDDs and PCDFs on the other is so high that it is safe to state that PCBs pose a greater toxic threat than the PCDDs and PCDFs.

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