Concentrations Of Halogenated Phenolic Compounds In Plasma Samples From Inuit Mothers And Their Newborns In Nunavik, Canada

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Introduction

Polychlorinated biphenyls (PCBs) are one of the most intensely studied compound classes since they were discovered as environmental pollutants over 30 years ago. After banning production and use of PCBs in most countries in the late 1970s, levels in the environment peaked in the early 1980s and now seem to be declining at variable rates in most environmental compartments.[1] Nevertheless, PCBs usually dominate the burden of organohalogen contamination in human plasma samples.[2;3] While knowledge of PCBs behaviour and effects in the environment is considerable, the levels and effects of the hydroxylated PCB metabolites and other related phenolic organohalogens have not been studied to such a great extent.

PCBs are biotransformed by the cytochrome P-450 monooxygenases system and the major metabolic pathway leads to the formation of hydroxylated PCBs (HO-PCBs). Even PCB 153, known to be very persistent in the environment, is biotransformed both *in vitro* and *in vivo* to form a number of hydroxylated metabolites.[4]

In human blood, a number of HO-PCBs have been identified and some have been found at high levels.[5;6] In a study by Klasson-Wehler *et al.*[7], HO-PCB levels in human plasma were comparable to levels of PCBs. As many as 38 different HO-PCB congeners have been identified in another study by Hovander *et al.*[8]Several HO-PCBs have been shown to displace thyroxine from the thyroid transporting protein, transthyretin.[9]

Pentachlorophenol (PCP), a fungicide used mainly for wood preservation, is another major halogenated phenolic compound (HPC) found in the environment. It is still produced worldwide, though it is banned in Scandinavia, and registered only for restricted use in Canada, USA and some European countries.[10] PCP, like HO-PCBs, binds to transthyretin [11], and it has been measured at higher levels than HO-PCBs in human plasma.[6;8;12]

Due to the known bioactivity of HPCs, the need to quantify HPCs, like HO-PCBs and PCP in addition to the neutral organohalogens is evident. Thus, the aims of this paper were to modify a method already validated for the analysis of PCBs and pesticides[13-15] and to enable HPC analysis in the same low-volume plasma samples. Using this method, HO-PCB and PCP plasma levels were determined in Inuit women (and their newborns) giving birth in Nunavik (Northern Québec, Canada). We have previously reported on the high exposure of this population to organochlorines through their traditional diet that is comprised of fatty tissues from sea mammals. [16]

Material and Methods

Pregnant Inuit women from Nunavik were invited to participate in a study focusing on infant health and development. The Nunavik region is located north of the 55th parallel, about 1,500 km from Montreal and 2,000 km from the Great Lakes in the United States (Figure 1). About 7,660 Inuit live in 14 villages scattered along a 2,000-km seashore line along Hudson Bay, Hudson Strait, and Ungava Bay. The study participants were living in Puvirnituq (50%), Inukjuaq (37%), and Kuujjuaraapik (13%), the three largest communities on the Hudson Bay coast. A detailed informed consent was obtained from each participating mother. Details regarding recruitment and study population were

published previously.[15;16] The biological samples collected in the course of this study included a blood sample obtained from the umbilical cord after it was severed and a 12.5-mL blood sample obtained from each participating mother at delivery or within a few weeks thereafter.

The plasma samples were extracted on an Oasis HLB (540 mg; Waters Corp.) solid phase extraction column according to a modified method by Sandau *et al* [15]. Extraction and clean up was completed on a Zymark Rapidtrace Automated SPE workstation (Zymark Corp.). Evaporation was performed on a Labconco evaporator (Labconco Corp., Kansas City, MO). The HPCs were eluted with Dichloromethane/Methanol (9/1) in a third fraction on a pre-packed Florisil column (1g; Alltech). After evaporation to dryness the compounds were derivatised using fresh diazomethane in hexane according to a method by Sandau [17]**.** The derivatised fraction was then finally cleaned up on an activated silica/acidic silica column before evaporation, addition of recovery standard and quantification on a GC-HRMS. The following carbon-13 labelled compounds were used as internal standards; PCP, 4-HO-CB107, 3-HO-CB153, 4-HO-CB146, 3'-HO-CB138, 4'-HO-CB130, 4-HO-CB187, 4'-HO-CB172, 4-HO-CB193.

The GC was fitted with a 30 m DB-5 column (5 % phenyl-methylpolysiloxane; 0.25 mm i.d., 0.25 mm film thickness) from J&W Scientific (CA, USA). The carrier gas was helium, and all injections were 2 mL in splitless mode. The GC-HRMS was run in selected ion monitoring mode measuring two ions per compound for quantitation and positive identification.

Results and Discussion

The recovery rates of the labelled compounds were in the range of 45 – 84 % with a mean value of 68 %. Monitoring of levels in a QA/QC pool revealed less than 25 % variation in the determined levels. The maternal plasma levels (n=36) and cord plasma levels (n=48) are presented in Table 1. Of the maternal and cord samples, there were 14 matched pairs and these levels are presented in Table 2.

Levels of HPCs in the maternal and cord plasma were comparable indicating that these compounds cross the placenta barrier. Further, major HO-PCBs and PCP in the 14 paired cord and maternal samples were all highly correlated (r^2>0.7). For estimation of correlation coefficients the log transformed values were employed. Using a paired sample t-test it was found that for several of the congeners there was a significant difference in the levels. 4-HO-CB107, 4-HO-CB187 and 4-HO-CB202 were found at significantly higher levels in maternal samples, whereas 4-HO-CB163, 4'-HO-CB172 and 4-HO-CB193 were all found at significantly higher levels in the cord blood samples.

Table 1: Maternal and cord plasma levels (pg/ml plasma) of HPCs.

	Cord plasma (n=48)				Maternal plasma (n=36)				Matched pairs (n=14)
Compounds	**GM**	**AM**	**MIN**	**MAX**	**GM**	**AM**	**MIN**	**MAX**	
PCP	1087	1210	300	2913	966	1044	241	2898	r^2=0.82
4-OH-HpCS	19	26	3	121	29	34	3	92	r^2=0.62
4'-HO-CB101/120	3	4	2	13	3	4	2	9	r^2=0.54
3-HO-CB118	4	6	1	27	5	9	1	51	r^2=0.83
4-HO-CB107	18	23	5	83	35	44	3	133	r^2=0.94
3-HO-CB184	1	2	1	12	2	2	1	8	r^2=0.70
3-HO-CB153	26	37	7	136	32	48	5	248	r^2=0.95
4-HO-CB146	56	74	13	453	68	85	11	312	r^2=0.96
4'-HO-CB127	2	3	2	16	2	2	2	4	r^2=0.50
3-'HO-CB138	24	33	6	120	21	28	2	115	r^2=0.95
4'-HO-CB130	4	6	2	51	4	6	2	31	r^2=0.14
4-HO-CB163	16	20	3	68	11	13	3	66	r^2=0.64
4-HO-CB178	2	3	1	26	2	4	1	48	r^2=0.28

3'-HO-CB183/ 4'-HO-CB175	2	2	1	12	2	2	1	7	r^2=0.65
4-HO-CB187	41	54	13	259	62	80	15	461	r^2=0.94
4-HO-CB202	6	9	1	62	9	14	1	99	r^2=0.88
2'-HO-BDE75	4	6	3	36	3	4	3	14	r^2=0.22
4'-HO-CB201	1	2	1	7	2	3	1	11	r^2=0.60
3'-HO-CB180	2	3	1	19	3	5	1	25	r^2=0.24
4'-HO-CB172	15	21	3	80	12	16	3	72	r^2=0.94
4-HO-CB193	6	11	2	82	4	7	2	69	r^2=0.61
4'-HO-CB199	11	19	1	190	14	20	4	171	r^2=0.97
4,4'-HO-CB202	1	2	1	65	1	1	1	1	r^2=0.65
4-HO-CB208	4	8	1	72	5	9	1	68	r^2=0.87
sum HO-PCBs	301	371	101	1544	356	429	86	1600	
sum major HO-PCBs	239	309	65	1358	290	363	50	1517	
% major of sum all	79	80	59	91	81	82	58	95	

Sum of major HO-PCBs consists of 107, 153, 146, 138, 163, 187, 202, 172, 193, 199, 208.

AM – arithmetic mean, GM – geometric mean

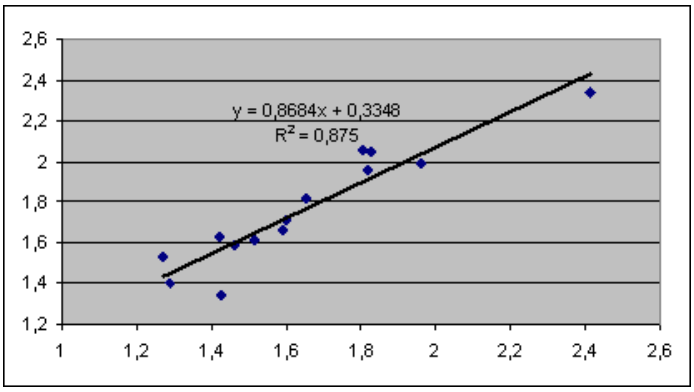

Figure 1 : Correlation of log-transformed 4-HO-CB187 plasma concentrations in maternal and cord plasma matched pairs (n=14).

As can be seen from the table these differences are small but nevertheless significant. Looking at the ratio of HO-PCBs to PCBs reveals that the mean ratio was considerably higher in the cord blood samples (sumHO-PCBs/sumPCBs=0.4) compared to the maternal samples for which the mean ratio was 0.14. This difference is mainly explained by the lower PCB concentrations in cord blood samples compared to maternal samples. The elevated HO-PCB levels measured in umbilical cord plasma samples are a cause of concern because of the known thyroid disrupting properties of these compounds.

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