Analysis of hydroxylated metabolites of PCBs (OH-PCBs) in whole blood from Canadian Inuit

Courtney D. Sandau*, Pierre Ayotte**, Éric Dewailly**, Åke Bergman†, Eva Klasson-Wehler†, Ross J. Norstrom‡*

* Centre for Analytical and Environmental Chemistry, Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6
** Centre de Santé de Québec, CHUQ, Beauport, Québec, Canada, G1E 7G9
† Department of Environmental Chemistry, Stockholm University, S-106 91 Stockholm, Sweden
‡ Environment Canada, National Wildlife Research Centre, Canadian Wildlife Service, Hull, Québec, J8Y 1V9

Introduction

Due to traditional diets, which consist of marine species with high lipid content, such as ringed seal, bearded seal and beluga, the Inuit population living in Arctic Quebec are exposed to large amounts of organochlorine contaminants (1). PCB levels in Inuit pooled plasma samples were recently found to be 30x greater than those from the southern population (2). An increased PCB body burden could result in the formation of more metabolites due to the induction of the hepatic cytochrome P450 monooxygenase system. Hydroxy-PCB metabolites are potential endocrine disruptors as they have been shown to compete for the thyroid hormone binding site on transthyretin (TTR) in rats (3). This binding may result in the selective accumulation of HO-PCBs in blood (4). Therefore, it is of interest to quantitate HO-PCBs in Inuit whole blood and compare the results to a southern control population.

Materials and Methods

Samples: Human whole blood samples were taken as part of a Santé Quebec Inuit Health survey from communities in Nunavik, in Arctic Quebec. The control sample of pooled whole blood was donated by Le Centre Hospitalier de L'université Laval in Sainte-Foy, Quebec.

Chemicals: The following $^{13}C_{12}$ standards obtained from Wellington Laboratories (Guelph, Ontario) were used as internal standards: 4-HO-CB61, 4-HO-CB120, 4-HO-CB159, 4-HO-CB172, and 4-HO-CB187 ; $^{13}C_6$ PCP was also included.

The following standards were used for peak identification: 4'-MeO-CB120, 4'-MeO-CB108, 4-MeO-CB188, 3-MeO-CB153, 4-MeO-CB146, 3'-MeO-CB138, 4'-MeO-CB130, 4'-MeO-CB187, 4'-MeO-CB175, 4'-MeO-CB159, 3'-MeO-CB180, 4'-MeO-CB172 and 4-MeO-CB193.

Analytical Procedure: Conventional liquid:liquid extraction was adapted from the methods described by Bergman *et al.* (4) and Klasson-Wehler *et al.*(5). Briefly, whole blood or plasma (5 g) was spiked with internal standards, denatured with HCl (1M) and 2-propanol (3 mL) and extracted 3 times with 6 mL hexane:methyl-*tert*butyl ether(MTBE)(1:1). Collected organic phases were reduced in volume by roto-evaporation, diluted with 10 mL dichloromethane(DCM):hexane (1:1) and run on Gel-Permeation Chromatography to remove the lipids. The collected organic phase was partitioned with 1 M KOH (50% ethanol). The combined basic KOH aqueous phase (phenolic fraction) was acidified with concentrated sulfuric acid and back extracted with 1:1 hexane:MTBE. The collected organic phase was reduced in volume and derivatized with diazomethane and applied to an alumina column (Fisher Brand, neutral, 3.0 g, deactivated 2.3 % with water). The analytes were eluted with 25 mL 10% DCM:hexane. The collected organic phase was brought down to analysis volume with roto-evaporation. Average recoveries of the internal standards were 78% (± 15% SD)

Instrumentation: ECNI-GC-MS (electron capture negative ionization-GC-MS) was performed on a 5890A Series II gas chromatograph equipped with an HP 7673A automatic injector and a Hewlett Packard 5988A mass spectrometer. Injections were 2 μL made in splitless mode. The GC was fitted with a DB-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were 250 °C and 280 °C. The GC temperature program was as follows: initial temperature held for 2 min at 80 °C, 10 °C/min to 250 °C, hold 5 min and 5 °C/min until 300 °C.

Results and Discussion

Ten samples of human whole blood where analyzed for the presence of HO-PCBs. These Nunavik samples included both males and females who varied in age from 18 to 72. A large pooled sample that represented a less contaminated population from southern Quebec was analyzed as a control.

Over 25 HO-PCBs were quantitated using $^{13}C_{12}$ labelled internal standards. Only 3 compounds matched the internal standards so group response factors were used to semi-quantitate the unidentified compounds. Due to a lack of authentic standards, most compounds remain unidentified and can only be characterized by chlorination group and by retention time (see Figure 1). A retention time index was used based on referencing the retention times of all compounds to the retention times of PCP and 4HO-CB187 which are present in all samples analyzed and are also added as $^{13}C_{12}$ internal standards [$RI = (T_x - T_{PCP}) / (T_{4HO\text{-}CB187} - T_{PCP})$]. The main peaks found in the human whole blood are listed in Table 1. The HO-PCB pattern was similar for all samples analyzed. The average ratio of the three main metabolites in the northern population - 4HO-CB109, 4HO-CB146 and 4HO-CB187 to the total HO-PCB concentration was very similar to the southern population with the three main peaks making up 23%, 7% and 29% of the total, respectively. In the control sample, the ratios were 26%, 6%, and 30%. The ratio of 4HO-CB109 and 4HO-CB146 to the main metabolite, 4HO-

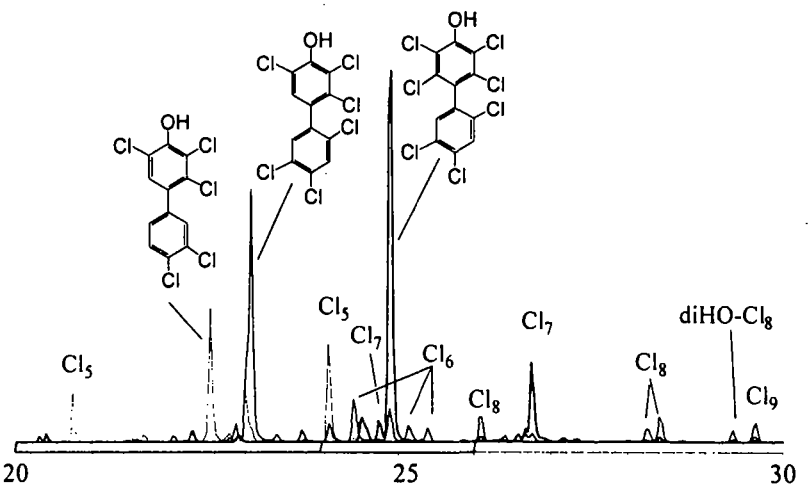

Figure 1 - GC-MS(ECNI) chromatogram of the HO-PCBs in the phenolic fractions taken in SIM mode. The peak identities are given when known. Unidentified peaks are listed by number of chlorines.

CB187, in the northern samples also did not differ from the southern population. This implies that similar congeners are making up the total amount of retained HO-PCBs and that body burdens or diet are not affecting the pattern of HO-PCBs.

The total HO-PCB concentration varied amongst samples and between populations. The northern population had 4-100x the levels of HO-PCBs compared to the control sample. The concentration of HO-PCBs was found to correlate well with age (r^2= 0.96, p = 0.0001) (see Figure 2 - left panel). The sum of PCB congeners CB138 and CB153 in these same samples is similarly correlated with age (r^2= 0.90, p = 0.002). This is the first time that HO-PCBs concentrations have been shown to correlate with age.

A significant relationship between HO-PCBs and the sum of CB153 and CB138 was

Table 1 - Concentration (pg/g) of the main HO-PCBs found in human whole blood.

Samples	Control	A	B	C	D	E	F	G	H	I	J
Age	-	18	20	27	34	35	-	-	45	66	72
% Lipid	1.28	n/a	1.98	1.20	n/a	1.51	n/a	n/a	1.36	1.43	n/a
4HO-CB109	0.9	7.8	2.6	23.3	15.5	6.7	9.7	21.1	39.9	47.1	40.4
Cl_6 (RI - 0.70)	0.1	1.4	0.3	3.3	2.4	3.1	1.9	4.3	6.9	22.9	30.0
4-HO-CB146	0.2	1.4	0.6	4.9	1.7	3.5	2.6	4.0	11.1	34.5	30.9
Cl_4 (RI - 0.79)	0.2	2.6	0.7	6.3	4.3	4.9	4.0	6.2	13.1	38.5	58.2
mixture Cl_7 (RI - 0.83)	0.1	0.7	0.2	1.0	0.8	1.4	1.3	3.0	4.2	8.8	10.2
4-HO-CB187	1.0	9.0	4.2	14.6	9.1	11.6	18.9	22.6	54.5	106.6	94.5
Cl_8 (RI - 0.95)	n.d.	0.1	0.2	0.9	0.2	0.6	0.6	0.4	2.7	6.9	4.4
Cl_7 (RI - 1.00)	0.2	2.5	1.4	2.0	2.8	1.9	2.4	6.8	7.2	17.4	27.3
Cl_8 (RI - 1.13)	0.2	0.1	0.2	1.5	0.2	0.8	0.6	0.9	4.0	7.1	8.4
Total HO-PCBs	**3.5**	**29.6**	**12.9**	**67.4**	**45.3**	**40.1**	**54.1**	**80.0**	**160.5**	**331.4**	**344.2**

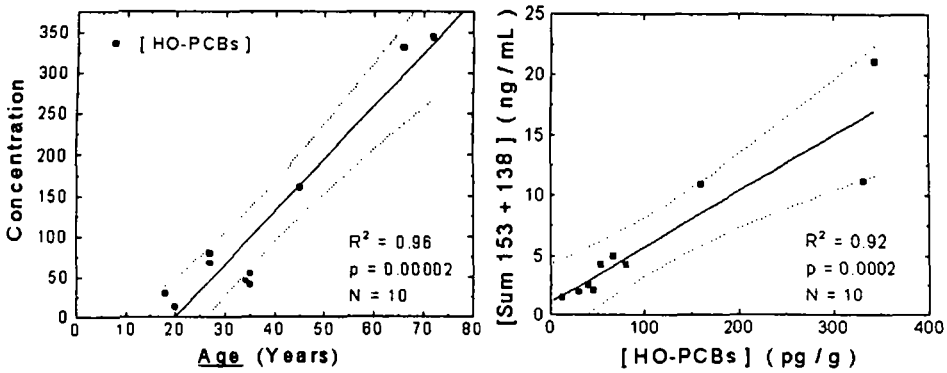

Figure 2 - (Left panel) Concentration of HO-PCBs (pg/g) versus age (years) of individual. (N=10). (Right panel) Correlation between the sum of CB153 + CB138 (ng/mL) and HO-PCBs (pg/g). (N=10) [CB138 and CB153 concentrations were taken from Santé Quebec Inuit Health Survey (see reference (2))].

also shown for the Inuit samples (r^2=0.92, p = 0.001) (See Figure 2 - right panel). The relationship between PCBs and their metabolites is probably a direct relation between amount of available precursor PCB and the production of metabolites. Thus, since PCBs increase with age, the production of metabolites concomitantly increases.

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