Analysis of Hydroxylated Metabolites of PCBs (OH-PCBs) and Other Chlorinated Phenolic Compounds in Whole Blood from Canadian Inuit

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In this study, we identified the main hydroxylated polychlorinated biphenyls (OH-PCBs) and other chlorinated phenolic compounds and we determined their relative concentrations in whole blood from 13 male and 17 female Inuit from northern Quebec, Canada, and from a pooled whole blood sample from southern Quebec. We also determined concentrations of polychlorinated biphenyls (PCBs). Total OH-PCB concentrations were variable among the Inuit samples, ranging over 2 orders of magnitude (0.117–11.6 ng/g whole blood wet weight). These concentrations were equal to and up to 70 times those found for the southern Quebec pooled whole blood sample. Geometric mean concentrations of total OH-PCBs were 1.73 and 1.01 ng/g whole blood for Inuit men and women, respectively, and 0.161 ng/g whole blood for the southern population pool. There are limited data available for comparison, but the levels of OH-PCBs in Inuit are higher than those previously reported in the literature for other populations. There was a significant correlation ($p < 0.005$) between OH-PCBs and PCBs ($r = 0.84$) and both correlated significantly ($p < 0.005$) with age ($r = 0.68$ and 0.78, respectively). The ratio of OH-PCBs to PCBs was lower in Inuit (0.11) than in the southern Quebec pool (0.33). There is no apparent explanation for the difference. There was considerable variability in the congener pattern of the identified OH-PCBs. The main metabolite, 4-OH-CB109 (4-OH-2,3,3′,4′,5-pentachlorobiphenyl), constituted 12–62% of the total OH-PCBs in the samples. Pentachlorophenol (PCP) was the dominant phenolic compound in blood, constituting 46% (geometric mean) of the total quantitated chlorinated phenolic compounds. PCP concentrations in Inuit blood ranged from 0.558 to 7.77 ng/g on a wet weight basis. All but two Inuit samples had lower concentrations than the southern Quebec pool (6.29 ng/g). The possible role of OH-PCBs in mediating PCB-induced adverse effects needs to be investigated further.
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Polychlorinated biphenyls (PCBs) have been extensively studied since their discovery as environmental pollutants over 30 years ago (*1*). Their persistence in biota is well known but the mechanism of their adverse effects on biologic systems is still not completely understood. Some of their toxicity may be linked to the biotransformation products of PCBs (*2*). PCBs are biotransformed by a diverse enzyme system, the cytochrome P450 monooxygenases. Most of the known metabolic pathways involve the initial formation of hydroxylated metabolites. Even one of the more recalcitrant PCBs, CB153, is metabolized *in vitro* and *in vivo* to a number of phenolic metabolites (*3–5*). Phenolic metabolites can be excreted unchanged, as they were first discovered in the excreta of Baltic seals and Guillemots (*6*), or further conjugated with glucuronic acid or sulfate (*7*). The introduction of a hydroxyl group increases the polarity of the PCB and facilitates excretion. If the hydroxyl group is *para* to the phenyl–phenyl bond and has adjacent chlorine atoms, the structure resembles the prohormone, thyroxin (T_4) (*8*). This structural similarity allows hydroxylated polychlorinated biphenyls (OH-PCBs) to bind with high affinity to one of the thyroid hormone transport proteins, transthyretin (TTR) (*9–12*). For example, 4-OH-3,3′,4,5′-tetrachlorobiphenyl, a metabolite of CB77, has a binding affinity to TTR 4 times stronger than that of T_4 (*9*), and can disrupt thyroid hormone and retinol (vitamin A) transport (*13*). This is the presumed mechanism by which OH-PCBs are selectively retained in plasma and is a possible mechanism of PCB toxicity (*14*). This type of interaction is not limited to OH-PCBs. Pentachlorophenol (PCP) binds with 2 times the affinity of T_4 to TTR (*15*), indicating that other chlorinated phenolic compounds may also be interfering in thyroid hormone transport.

Some Inuit consume traditional foodstuffs consisting of fatty tissues from sea mammal species such as ringed seal and beluga (*16–18*). Ringed seal blubber, beluga skin, and beluga blubber in northern Canada contain average total PCB concentrations of 1,283, 145, and 5,000 ng/g wet weight, respectively (*19,20*), which is much higher than that in the diet of the general population (*21*). Thus, the Inuit population may be exposed to large doses of PCBs. In one study, mean PCB blood levels in Inuit were 30 times those of a southern population (*22*). An increased PCB body burden could result in the increased formation of metabolites due to induction of the P450 system. Increased levels of metabolites may be significant enough to disrupt thyroid hormone transport. Alterations of thyroid hormone status have been proposed as a mechanism of action by which PCBs would induce adverse neurodevelopmental effects (*23*). It is therefore of interest to study the concentrations of OH-PCB metabolites in the Inuit population as a first step to evaluate their possible implications mediating PCB-induced health effects. Hydroxylated PCBs have never been studied in the Arctic environment and little is known about levels and patterns in humans. There are only two other published studies on OH-PCB metabolite concentrations in human blood [from Sweden and Latvia (*14,24*)].

Materials and Methods

During fall 1992, 499 Inuit adults living in Nunavik (Northern Quebec, Canada) participated in the Santé Québec Health Survey. After they signed an informed consent form, we drew a 30-mL blood sample by venous puncture for organochlorine determination. We randomly selected 30 subsamples of these 499 samples for chlorinated phenolic compound and additional PCB residue analysis. The Laval University Medical Centre (Sainte-Foy, Québec) donated a southern Quebec general population sample of pooled whole blood for comparison to the Inuit population.

The chosen nomenclature for numbering hydroxylated PCBs or their derivatized

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analogs, methoxylated PCBs, is slightly different from general International Union of Pure and Applied Chemistry (IUPAC) guidelines (*25*). The OH- or MeO- groups are not given numbering priority. Rather, the chlorine pattern on the biphenyl rings determines the congener number according to the IUPAC PCB numbering rules (*25*), with corrections by Guitart et al. (*26*), and the OH- or MeO- groups are numbered thereafter. This facilitates direct structural comparison with PCBs because the IUPAC numbering system is so well established and familiar.

We used the following $^{13}C_{12}$-labeled standards [acquired from Wellington Laboratories (Guelph, Ontario, Canada)] as recovery internal standards for OH-PCB determination: 4′-OH-CB120 (4′-OH-2,3′,4,5,5′-pentachlorobiphenyl); 4′-OH-CB159 (4′-OH-2,3,3′,4,5,5′-hexachlorobiphenyl); 4′-OH-CB172 (4′-OH-2,2′,3,3′,4,5,5′-heptachlorobiphenyl); and 4-OH-CB187 (4-OH-2,2′,3,4′,5,5′,6-heptachlorobiphenyl). We purchased PCP ($^{13}C_6$) from Cambridge Isotope Laboratories (Andover, MA) and used it for PCP quantitation. We added a synthesized performance standard, 4′-Me-4-MeO-2,3,3′,5,6-pentachlorobiphenyl, to all phenolic compound fractions before mass spectral analysis. We used labeled PCBs ($^{13}C_{12}$-CB28, -52, -118, -153, -180, and -194) as recovery standards and $^{13}C_{12}$-CB138 as the performance standard for PCB analysis by the external standard method. The $^{13}C_{12}$-PCB standards were purchased from Cambridge Isotope Laboratories.

Whole blood samples (mass range 1.54–5.76 g) were spiked with $^{13}C_{12}$ recovery standards before extraction. The standards consisted of a PCB mixture ($^{13}C_{12}$-CB28, -52, -118, -153, -180, and -194; 10 µL, 2.5 ng/µL), a OH-PCB mixture ($^{13}C_{12}$-4′-OH-CB120, 4′-OH-CB159, 4′-OH-CB172, and 4-OH-CB187; 20 µL, 100 pg/µL), and PCP ($^{13}C_6$; 100 µL, 100 pg/µL). The samples were then extracted using the Wallenberg plasma extraction method, as described elsewhere (*14,27*), with the following modifications. The organic phase from the potassium hydroxide partitioning, which contains the neutrals, was reduced in volume and applied to a Florisil column (8 g, 1.2% deactivated). The PCBs were collected in one fraction of 75 mL dichloromethane (DCM):hexanes (1:1) then reduced in volume and applied to a silica/sulfuric acid (3 g, 22%) column to remove coextracted biogenic components. The PCBs were eluted in 50 µL DCM:hexanes, reduced to final volume (50 µL) by rotoevaporation, and spiked with performance standard ($^{13}C_{12}$; CB138, 5 µL, 2.0 ng/µL) for mass spectral analysis.

The partitioned phenolic compounds were acidified and back extracted with hexanes, dried over sodium sulfate, and derivatized with diazomethane. We generated diazomethane as needed from nitrosomethylurea precursor, as described elsewhere (*28*). The derivatized compounds were then cleaned up on a silica/sulfuric acid (5 g, 22%) column and eluted with 50 mL DCM:hexanes. We reduced the samples in volume by rotoevaporation and brought the samples down to final volume (25 µL) by a gentle stream of nitrogen. All samples were then spiked with a performance standard (4′-Me-4-MeO-2,3,3′,5,6-pentachlorobiphenyl, 5 µL, 200 pg/µL) before analysis.

Analyses were completed on a Hewlett Packard 5890A Series II gas chromatograph Hewlett Packard, Atlanta, GA) equipped with an HP 7673A automatic injector and a Hewlett Packard 5988A mass spectrometer. We used helium as the carrier gas; we set the head pressure at 80 kPa. All injections (2 µL) were made in splitless mode onto a DB-5 column [(5%-phenyl)-methylpolysiloxane, 30 m × 0.25 µm i.d., 0.25-µm film thickness; J&W Scientific, Inc., Folsom, CA). The injector temperature was set at 250°C. We performed mass spectrometry with selected ion monitoring in electron capture negative ionization (ECNI) mode using methane (99.99% pure) as the reagent gas for the chlorinated phenolic fraction. The reagent gas source pressure was 2.5×10^{-4} torr at the inlet and the source temperature was 140°C. The GC temperature program for the derivatized phenolic compounds was as follows: 80°C for 2 min, ramp 10°C/min to 250°C, hold for 5 min, then ramp 5°C/min to 300°C. We analyzed the phenolic compound fraction using ECNI because of the presence of residual biogenic material that interferes with the analysis in electron impact (EI) mode. The PCB fraction was free of biogenic contamination, allowing analysis of the fraction using selected ion monitoring in EI mode. The electron energy was 70 eV and the source temperature was 200°C. The GC temperature program for the PCB analysis was 100°C for 3 min, 10°C/min to 180°C, and then 2.5°C/min to 280°C.

Because analysis was carried out on methylated OH-PCBs, we used a standard mixture of MeO-PCBs. The MeO-PCB mixture included 4′-MeO-2,3′,4,5,5′-pentachlorobiphenyl (4′-MeO-CB120); 4′-MeO-2,3,3′,4,5′-pentachlorobiphenyl (4′-MeO-CB107); 4-MeO-2,2′,3,4′,5,6,6′-heptachlorobiphenyl (4-MeO-CB188); 3-MeO-2,2′,4,4′,5,5′-hexachlorobiphenyl (3-MeO-CB153); 4-MeO-2,2′,3,4′,5,5′-hexachlorobiphenyl (4-MeO-CB146); 3′-MeO-2,2′,3,4,4′,5′-hexachlorobiphenyl (3′-MeO-CB138); 4′-MeO-2,2′,3,3′,4,5′-hexachlorobiphenyl (4′-MeO-CB130); 3′-MeO-2,2′,3,4′,5,5′,6-heptachlorobiphenyl (3′-MeO-CB187); 4′-MeO-2,2′,3,3′,4,5′,6-heptachlorobiphenyl (4′-MeO-CB175); 4′-MeO-2,3,3′,4,5,5′-hexachlorobiphenyl (4′-MeO-CB159); 3′-MeO-2,2′,3,4,4′,5,5′-heptachlorobiphenyl (3′-MeO-CB180); 4′-MeO-2,2′,3,3′,4,5,5′-heptachlorobiphenyl (4′-MeO-CB172); and 4-MeO-2,3,3′,4′,5,5′,6-heptachlorobiphenyl (4-MeO-CB193). The MeO-PCBs were supplied by Å. Bergman (Wallenberg Laboratories, Stockholm, Sweden), and synthesized as described elsewhere (*29*). We used the MeO-PCBs for quantitation by the external standard method.

We used a serial dilution of the MeO-PCB mixture for quantitation. We generated response factors relative to the performance standard (RRFs) for each of the compounds in the standard mixture. The RRFs were then used to quantitate all identified and unidentified OH-PCBs in each sample by the external standard method, with volume correction using the performance standard. Identified compounds were quantitated using their RRF. Structures were considered confirmed if they had identical spectra and matching retention times on three GC columns with varying polarities (DB5, DB1701, and DB210; J&W Scientific, Inc.). We characterized unidentified compounds by full scan mass spectrometry to determine the chlorination pattern. The average RRFs for that chlorination pattern were used for quantitation for the unidentified compounds. The RRFs for compounds with a given chlorination and methoxy group substitution were comparable. For example, the RRFs for the *para*-methoxylated heptachlorobiphenyls were all within 10% of the average RRF. We determined recoveries using the $^{13}C_{12}$ OH-PCB standards and 4′-Me-4-MeO-2,3,3′,5,6-pentachlorobiphenyl as the performance standard. PCP was quantitated by isotope dilution using the $^{13}C_6$ internal recovery standard correcting for the 3.4% contribution of native PCP (*m/z* = 280 amu) to the main ion of the $^{13}C_6$ standard (*m/z* = 286 amu) cluster.

PCBs were quantitated on a congener-specific basis by the external standard method using an Aroclor 1242:1254:1260 (1:1:1) secondary quantitation standard solution calibrated against primary standard PCB congener solutions provided by the National Research Council of Canada (Marine Analytical Chemistry Standards Program, Halifax, Nova Scotia, Canada). We determined concentrations of congeners for which standard solutions were not available in the Aroclor mixture by GC using flame ionization detection (*30*). We purchased Aroclor from Monsanto (St. Louis, MO).

PCBs were previously determined in these samples by the Québec Toxicology Centre (Sainte-Foy, Québec, Canada) using

plasma as the tissue for analysis. Because of Aroclor 1254 contamination in one of our rotoevaporators, 15 of the 30 whole blood samples were irreclaimable for PCB quantitation. We compared the 15 whole blood samples not contaminated with Aroclor 1254 to the previous plasma analyses. Concentrations of all previously quantitated congeners (CB28, CB52, CB99, CB101, CB105, CB118, CB128, CB138, CB153, CB156, CB170, CB180, CB183, and CB187) and the sum of congeners in plasma were highly correlated ($r > 0.97$) to the whole blood concentrations using least-squares regression analysis. The regression analysis for CB153 and total PCBs are given as examples in Figure 1. Thus, the previous PCB plasma concentrations for the 15 samples not quantitated in our lab were adjusted by applying a correction factor from the regression analysis. We used the adjusted PCB concentrations and the values determined in the 15 whole blood samples in the present study for comparison to OH-PCB concentrations and for subsequent statistical analysis. Aroclor contamination had no effect on the quantitation of OH-PCBs in the contaminated samples because PCBs elute earlier and have no fragment ions that interfere with the detection of OH-PCBs.

All solvents were residue analysis grade and were purchased from EM Science (Gibbstown, NJ). Florisil (Pesticide Analysis Residue grade, 60–100 mesh) was purchased from BDH, Inc. (Toronto, Ontario, Canada). Merck Silica gel (Grade 60, 70–230 mesh, 60Å) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). We purchased trace-metal-grade sulfuric acid from Fisher Scientific (Pittsburgh, PA).

Results

Whole blood recoveries of OH-PCBs were variable, ranging from 50 to 105%. The mean recoveries for $^{13}C_{12}$- 4′-OH-CB120, -4′-OH-CB159, -4′-OH-CB172 and -4-OH-CB187 were 82, 69, 72, and 76%, respectively. This was the first study to use $^{13}C_{12}$-labeled OH-PCBs for accurate OH-PCB quantitation. PCB recoveries had a slightly lower mean recovery of 60%. All PCB and OH-PCB concentrations were consequently recovery corrected.

An example chlorinated phenolic ECNI-MS full scan chromatogram is given in Figure 2. The major peaks are identified when known. The OH-PCB fraction in Inuit whole blood contained > 30 congeners, of which 11 were positively identified with authentic standards. Positive identification is based on identical mass spectra using full scan ECNI mass spectrometry and matching retention times using three GC columns with varying polarities (results not shown). All identified congeners are listed in Table 1. The congeners constituted between 59 and 81% of the total OH-PCBs in the samples (mean = 70%).

We did not determine the lipid content of the whole blood samples. Therefore, all concentrations hereafter are expressed on a whole blood wet weight basis. The chemical residue data were not normally distributed. Log-transformed data approached normal distribution; therefore, log-transformed data were used in all statistical analyses and geometric mean values were used unless otherwise stated. The geometric mean concentrations in men and women of all identified congeners and their range of values are listed in Table 1. Women consistently had lower geometric mean concentrations of all the chlorinated phenolic compounds quantitated, but the difference was statistically significant between men and women using Student's t-test only for PCP.

The concentrations in the pooled sample are equivalent to arithmetic mean concentrations. The arithmetic mean of the total OH-PCBs in Inuit whole blood was 1.89 times greater than the geometric mean. Therefore, the difference in geometric mean values between populations is probably an overestimate of the geometric mean and the differences in concentrations are even greater than indicated in Table 1.

PCP was the main chlorinated phenolic compound in the Inuit whole blood. It contributed between 14 and 89% of the total chlorinated phenolic compounds quantitated. The concentration of PCP ranged from 0.558 to 7.77 ng/g with a geometric mean of 2.02 ng/g wet weight. The pooled whole blood sample from the southern population had higher PCP levels than all but two Inuit samples analyzed, with a concentration of 6.29 ng/g. PCP composed 97% of the total quantitated chlorinated phenolic compounds in the southern population.

The major OH-PCB congeners varied considerably among individuals. The mean ratios of the five main congeners to total OH-PCBs are shown in Figure 3, with bars representing the range. The main congener identified in Inuit whole blood (21 of 30 samples) was 4-OH-CB109, with a geometric mean concentration of 0.266 ng/g and a range of 0.015–2.55 ng/g. This congener is probably a mixture of two congeners, 4-OH-CB109 (4-OH-2,3,3′,4′,5-pentachlorobiphenyl) and 4′-OH-CB107, because they coelute and because separation was not achieved with the DB-5 column used in this study. This peak was quantitated using only 4′-OH-CB107 as the quantitation standard because 4-OH-CB109 was not commercially available. The peak is assumed to be predominantly 4-OH-CB109 because it is the dominating compound of the mixture in human plasma at a 5:1 excess (*14,31*). However, considering pattern variability this would need to be confirmed for each sample in the future.

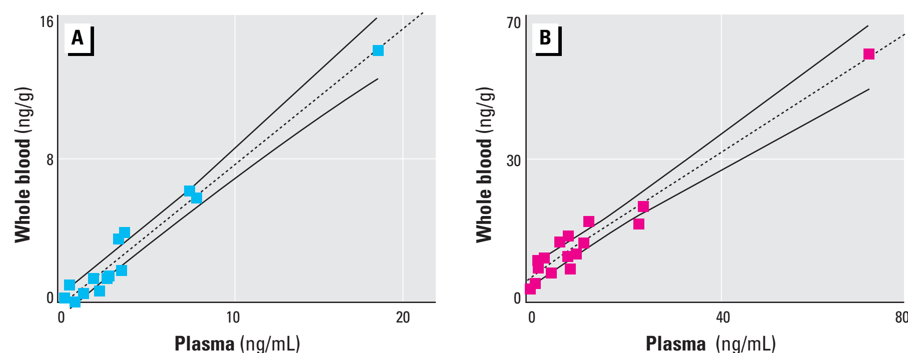

Figure 1. The regression analysis of (*A*) CB153 and (*B*) total PCBs, showing that the concentrations previously determined in plasma are significantly correlated to those in whole blood determined in this study. The correction factor was developed by regression of the whole blood concentration versus the plasma concentration from the previous analyses. The equation for the least-squared regression can then be used to adjust the plasma values for the samples into whole blood equivalents. This was done for each congener independently and for the sum of all quantitated PCBs. (*A*) $r = 0.97$, $p < 0.005$; $y = 0.76x - 0.23$. (*B*) $r = 0.98$, $p < 0.005$; $y = 0.74x + 5.7$.

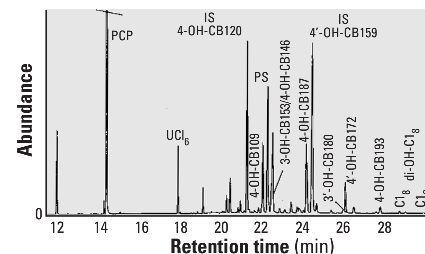

Figure 2. GC-MS (ECNI) SIM chromatogram of the OH-PCBs and other chlorinated phenolic compounds from Inuit whole blood analysis. PCP was truncated to fit on the scale shown. The chromatogram can only be used as a qualitative graph because each compound fragments differently and the response varies for compounds with different chlorination patterns. For this sample, only two internal standards (IS) and the performance standard (PS) were added for illustrative purposes.

In eight of the samples, 4-OH-CB187 was the dominating congener. It ranged from 0.022 to 2.26 ng/g with a geometric mean of 0.202 ng/g for all samples. This was the main metabolite in plasma determined in previous studies (*14,32,33*). The main metabolite in the remaining sample was 4-OH-CB146. The geometric mean concentration for 4-OH-CB146 in all samples was 0.149 ng/g and ranged from 0.010 to 1.88 ng/g. The metabolite may be formed from CB146 via a direct oxygen insertion or from CB153 or CB138 via a National Institutes of Health (NIH) shift of Cl in the hydroxylation step. If a metabolite is formed by a direct oxygen insertion, it would retain the precursor PCB number. If the metabolite is produced via an NIH shift of Cl, the metabolite PCB number will be different from that of the parent PCB.

Total quantitated OH-PCBs ranged from 0.117 to 11.6 ng/g with a geometric mean value of 1.27 ng/g. Total OH-PCBs in individuals were equal to and up to 70 times that of the southern Quebec pooled sample (0.161 ng/g). Geometric mean CB153 and geometric mean total PCB concentrations in combined Inuit samples were 2.37 ng/g (range 0.263–13.9 ng/g) and 15.2 ng/g (range 1.19–65.9 ng/g), respectively. Concentrations in the southern Quebec pooled sample were 0.074 ng/g for CB153 and 0.488 ng/g for total PCBs. The mean ratio of total OH-PCBs to total PCBs was 0.11 (range 0.02–0.45) for combined Inuit and 0.33 for the southern Quebec pooled sample, demonstrating the importance of OH-PCBs in the total PCB-related compounds in blood. The concentration of the five main OH-PCB metabolites relative to total OH-PCBs and CB153 for both Inuit and the southern Quebec pooled sample are shown graphically in Figure 3.

Plots of log-normalized concentrations versus age are shown in Figure 4. Both PCBs ($r = 0.78$) and OH-PCBs ($r = 0.68$) were significantly ($p < 0.005$) correlated with age. As seen by the similar slopes, total OH-PCBs and total PCBs were also significantly correlated ($r = 0.84$, $p < 0.005$).

Table 1. Concentration of PCP and hydroxylated metabolites (ng/g whole blood wet weight × 10^3) in Inuit whole blood.[a]

	Males ($n = 13$)			Females ($n = 17$)			Southern
		Range			Range		population
	GM	Min	Max	GM	Min	Max	pool
Age (years)	38	18	66	38	18	72	–
PCP	2,740	1,350	7,770	1,590	558	7,510	6,290
4´-OH-CB120	12	3	67	7	< 1	39	3
4-OH-CB109[b]	314	39	2,550	234	15	1,470	25
3-OH-CB153[b]	85	5	537	49	4	830	2
4-OH-CB146[b]	219	10	1,750	111	10	1,880	8
3´-OH-CB138	37	3	225	20	2	401	2
4´-OH-CB130	15	1	506	4	< 1	47	< 1
3´-OH-CB187	17	1	213	11	< 1	167	< 1
4-OH-CB187[b]	293	22	1,840	152	27	2,260	31
3´-OH-CB180	17	2	134	8	1	239	< 1
4´-OH-CB172[b]	74	5	443	38	4	740	11
4-OH-CB193	41	3	659	26	2	95	18
Sum congeners[b]	1,040	88	6,740	614	59	7,070	76
Sum identified	1,210	119	7,520	712	87	8,060	82
Sum all OH-PCBs	1,730	162	10,100	1,010	117	11,600	161
Sum PCBs	12,900	2,070	65,900	7,940	1,190	38,100	488
CB-153	3,120	460	13,900	1,960	263	9,510	74

Abbreviations: GM, geometric mean; max, maximum; min, minimum.
[a]Total OH-PCBs, sum five main congeners, and sum identified congeners are given for comparison with previous studies.
[b]Sum five main congeners: 4-OH-CB109 + 3-OH-CB153 + 4-OH-CB146 + 4-OH-CB187 + 4´-OH-CB172.

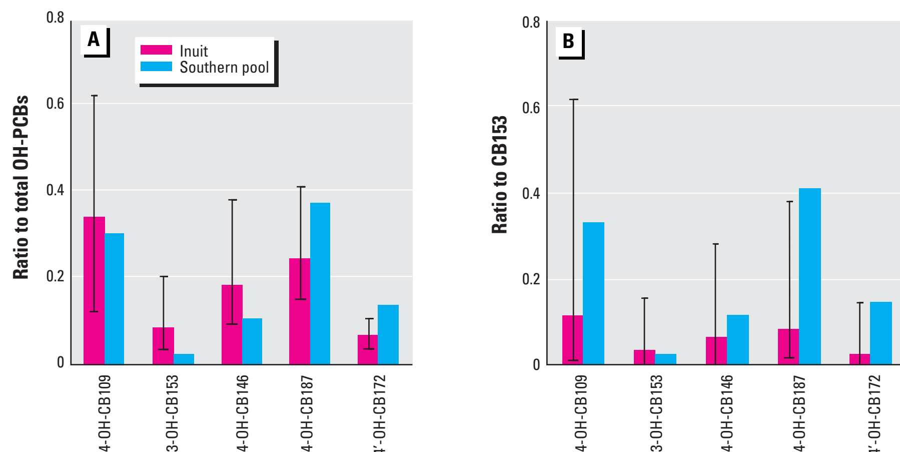

Figure 3. Ratio of the five main metabolites to total OH-PCBs and to CB153. Mean ratios are given for combined male and female Inuit samples, and error bars represent the range.

Discussion

The only human blood analyzed previously for OH-PCBs and other chlorinated phenolic compounds was plasma from the Swedish population (*14,31*). PCP was described as the dominant compound in these studies, but levels were not given. The geometric mean PCP concentration in the Inuit samples was 2.02 ng/g, approximately 3 times lower than in the southern Quebec pooled sample. Geyer et al. (*34*) measured PCP in two different general populations in Germany and found average concentrations of approximately 20 ng/g wet weight. The study did not indicate if the samples analyzed were whole blood or plasma, so the comparison of levels with the present results may be incorrect. However, a recent study documented levels of PCP in men from Sweden and Latvia between 170 and 1,800 ng/g on a plasma lipid weight basis (*24*). When the Swedish data are approximated to plasma equivalents, these levels are the same order of magnitude and have a similar range as those for the Inuit and the southern Quebec pooled whole blood samples. The plasma equivalent was estimated by assuming that whole blood is approximately half plasma by weight and that the average plasma lipid levels in the population are approximately 1%.

Although the concentration of PCP is lower in Inuit than that measured in the southern Quebec pooled whole blood sample, it may still play a role in the disruption of thyroid hormone transport. PCP binds to TTR with twice the affinity of the native

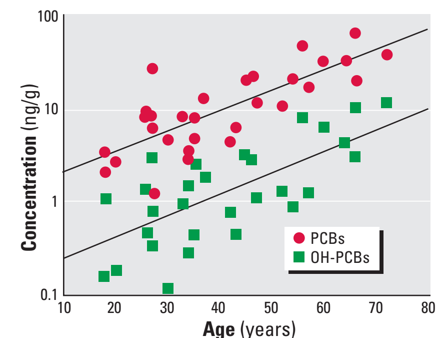

Figure 4. Log-normalized concentrations versus age (in years) for total PCBs and total OH-PCBs. PCBs, $Y = 0.022x + 3.10$; $r = 0.78$; $p < 0.005$. OH-PCBs, $Y = 0.023x + 2.17$; $r = 0.68$; $p < 0.005$.

hormone (*15*) and is responsible for 14–89% of the total phenolic compounds quantitated. Geyer et al. (*34*) calculated the half-life of PCP as approximately 19 days and concluded that the German cohort's calculated intake of PCP exceeded the elimination rate and approximated a bioconcentration factor of 1.4 for blood. This is likely due to binding with TTR and explains the high levels of PCP found in blood. Considering the significance of PCP in the chlorinated phenolic fraction, further investigation of PCP and its possible effects on thyroid hormone transport is required, especially for southern populations, where levels of PCP far exceed OH-PCBs and other chlorinated phenolic compounds.

Another major compound in the Inuit whole blood chromatogram is an unidentified chlorinated phenolic compound that elutes just before the OH-PCBs. The major ion cluster for this compound, using the given mass spectrometry conditions, has an isotope pattern indicative of a hexachlorinated compound. Therefore, the levels were approximated using the Cl6 response factor from the OH-PCB standard mixture. Based on approximate concentrations, the compound accounts for 1–17% of total chlorinated phenolic compounds in the Inuit. Levels occasionally exceeded those of the main OH-PCBs. Further work on the identification and accurate quantitation of this unknown compound is currently underway.

The chromatogram of the phenolic compound fraction consists of > 30 peaks that were identified as hydroxylated metabolites of PCBs based on their mass spectra. These represent 20% of the total chlorinated phenolic compounds, on average, in Inuit whole blood (range 2–56%). OH-PCBs constitute a significantly more important proportion of the total chlorinated phenolic compounds in Inuit as compared to the general population pooled sample, in which OH-PCBs represent only 1.2% of the estimated total.

The mean concentration of the five main OH-PCBs (1.47 ng/g) in Inuit samples is slightly higher than that of a Swedish general population cohort (1.0 ng/g) (*31*). The Swedish data have been adjusted to whole blood wet weight equivalents. It was expected that levels of OH-PCBs in Inuit would be higher than those found because the mean PCB levels in plasma were 3 times that of this particular Swedish population, and an average of 30 times (range 2.5–133) that of the southern Quebec pooled sample. The range of the sum of these five congeners in the Swedish study (0.35–1.65 ng/g) is small as compared to concentrations in the Inuit population, which range from 0.059 to 7.07 ng/g plasma wet weight levels of OH-PCBs. The higher levels of OH-PCBs are likely due to the higher levels of parent PCBs. OH-PCBs were significantly correlated with PCBs ($r = 0.84$, $p < 0.005$), as expected.

The strong linear relationship observed between concentrations of PCBs and their metabolites suggests that no changes in enzyme activity affecting the rate of formation or saturation of OH-PCBs binding to plasma proteins are taking place for this range of PCB exposure. The concentration of total quantitated chlorinated phenolic compounds was < 2% of the theoretical molar circulating concentration of TTR in an average human (*35*). Thus, it is not likely that the concentration of total chlorinated phenolic compounds reached the carrying capacity of the blood, assuming that Inuit have similar TTR concentrations as the published values from other populations (*35*).

Women had lower mean concentrations of all phenolic compounds quantitated than men. The generally lower levels in women may result from the loss of OH-PCBs and PCBs through lactation because both have been identified in milk (*36–38*). Both OH-PCBs and PCBs were significantly correlated with age ($r = 0.68$ and 0.78, respectively; $p < 0.05$). The increase of both PCB and OH-PCB concentrations with age may be due to the increased exposure with age (elders' preference for traditional foods), a slow excretion rate of PCBs that prevents steady state from being achieved, or both. A positive association between age and PCB levels in breast milk was reported previously in southern Quebec women (*39*) and in Inuit adults from Nunavik (*22*).

The concentration of omega-3 fatty acids in plasma phospholipids is a good indicator of fish intake (*40*). In the course of the Santé Québec Health Survey, polyunsaturated fatty acid analysis was performed on plasma samples obtained from 492 Inuit adults. There was a strong association between omega-3 fatty acid content in plasma phospholipids and the age of the participants, with mean (geometric) concentrations of 6.4% [95% confidence interval (CI), 5.9–6.9]; 8.7% (CI, 8.2–9.3); and 12.2% (11.5–13.0) for the 18–24 year, 25–44 year, and 45–74 year age groups, respectively (Fisher exact test: $p < 0.0001$) (*41*). Thus, an increase in the intake of traditional foods with age is indicated, and is probably partly responsible for the increase in PCBs with age. However, indications of increasing concentration of PCBs with age in other populations suggest that slow excretion of PCBs is also important.

The main OH-PCBs identified in previous studies were also the dominating congeners in the Inuit population (Figure 2). The major metabolite in 21 of the 30 samples analyzed was 4-OH-CB109. This was not the major metabolite found in the study by Bergman et al. (*14*), but it was later identified by Klasson-Wehler et al. (*31*) as the dominant OH-PCB in another study of human plasma. The probable mechanism of formation of this metabolite is direct oxygen insertion into CB-109 or an NIH shift of Cl through an arene oxide intermediate of CB-118 or CB105. CB118 and CB105 are major congeners of Aroclor mixtures, whereas CB-109 represents only a fraction of a percent of the Aroclor mixture; therefore, the NIH shift mechanism is more likely, and occurs in the mink and the mouse (*42*). The majority of metabolites were highly correlated to all PCB congeners, making it impossible to determine the most probable precursors by correlation analysis. For example, 4-OH-CB109 was significantly correlated to both CB105 and CB118 ($r = 0.67$ and 0.72, $p < 0.005$). The correlation coefficients for 4-OH-CB109 and unrelated PCBs, such as CB153 and CB187, were equally high (0.77 and 0.71, $p < 0.005$). This was true for the majority of the identified metabolites and the major PCBs in the Inuit whole blood samples.

The main metabolite in most of the remaining samples was 4-OH-CB187, which was previously identified as the major metabolite in a Swedish population (*14*). This compound was also identified as the main metabolite for the southern Quebec pooled sample and in other species including polar bears (*33*) and albatrosses (*32*). This metabolite is most likely formed from the metabolism of CB183 and/or CB187, which represent 2 and 5%, respectively, of the Aroclor 1254 mixture (*43*).

The OH-PCB pattern present in the chlorinated phenolic compound fraction in plasma is complex and it is difficult to generalize patterns in humans from such a small data set. The ratio of 4-OH-CB109 to total OH-PCBs ranged from 0.12 to 0.62 (mean = 0.34) (Figure 3). The remaining congeners in the Inuit all composed similar proportions of total OH-PCBs as compared to the southern population. The ratio of the five main congeners to CB153 in the southern pooled samples was within the range of those determined for the Inuit samples, except for 4-OH-CB187, where the southern pooled sample was higher than all of the Inuit samples.

A number of factors, which include exposure to different proportions of precursor PCBs, alteration of metabolism rates by induction of hepatic enzymes, inhibition of the metabolizing enzymes and the protein-binding specificity of the plasma, may all influence the retention of hydroxylated metabolites. Genetic diversity among individuals and populations may also influence the metabolism rates and binding specificity.

To evaluate the toxicologic significance of the phenolic fraction, the main compounds must be identified, accurately quantitated, and toxicologic studies must be undertaken to evaluate the effects of these compounds on thyroid hormone and retinol homeostasis and binding at receptor sites. PCP remains the dominating phenolic compound in Inuit whole blood, even more so for the southern Quebec pooled sample. Concentrations of PCP in most of the samples far exceeded the main OH-PCBs. Other compounds, such as the unknown chlorinated compound, will be researched further to elucidate structure and possible roles in disruption of thyroid hormone transport.

In conclusion, total OH-PCB concentrations were 11 and 33% of total PCB concentrations in Inuit and a southern Quebec pooled sample of whole blood, respectively. Both total PCB and total OH-PCB geometric mean concentrations were higher for Inuit than for the southern Quebec pooled sample and the current literature values (*31*). Increased concentrations resulted in increased OH-PCB levels in Inuit blood, but the ratio of metabolites to PCBs was not as high as that found for the southern Quebec pooled sample. Unlike PCBs, the pattern of OH-PCBs was not consistent among individuals. Three different congeners alternated as the dominant metabolite of PCBs in the 30 samples analyzed. A larger study would help elucidate the reasons for the pattern variability, the lower ratio of OH-PCBs to PCBs in Inuit, and the possible differences between the sexes. PCP was the dominant chlorinated phenolic compound in the majority of the samples and must be included in future studies that may be evaluating the effects of phenolic compounds on circulating levels of thyroid hormones and vitamin A.

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