PERSISTENT ORGANIC POLLUTANTS IN HUMAN PLASMA OF MALES FROM TWO COMMUNITIES IN NORTHERN NORWAY

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Introduction

High levels of persistent organic pollutants (POPs) have been found in environmental samples from the Arctic, which cannot be related to local sources. Therefore contamination can only be explained by long-range transport from lower latitudes described in well-documented studies.[1] In general POPs accumulate in lipid rich body tissues. The lipophilicity and resistance to biodegradation are responsible for the bioaccumulation in the food chain, and in particular the aquatic food web. Use of local and indigenous food is a common characteristic of people from Arctic communities, and a wide range of animals and plants remain important dietary and cultural resources.[2] Studies on humans of the Arctic show that levels of POPs are related to local dietary habits and geographical location. It could be shown that diets rich in marine adipose tissue causes elevated levels in humans. In this connection higher concentrations are documented in human beings living coastal compared to inland living ones.[3-5] The topic is still of interest because for many organochlorines biological half-lives of several years or longer have been reported and in addition is known that compounds like POPs can have hormone related or interfering effects on organisms. Those substances are also called endocrine disrupting chemicals (EDCs). They show estrogen or anti-androgen effects on people as well as on animals. Sperm production in males can be influenced negatively by EDCs, low sperm concentration and sperm mobility correlate with increasing EDC concentrations. Thus is hypothesized that organochlorines affect the fertility of men [6], in males with a possible coherence to testicular cancer.

Polychlorinated biphenyls (PCBs) are usually dominating in the group of organohalogen contaminants in human plasma. They are biotransformed by the cytochrome P-450 mono-oxygenases system in organisms 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 bio-transformed both *in vitro* and *in vivo* to form a number of hydroxylated metabolites.[7] HO-PCB concentrations in human plasma are in general about a factor 2 to 8 lower compared to the levels of PCBs. Higher levels of HO-PCBs are found in the liver compared to adipose tissue, but, due to transport protein binding, plasma is the preferred tissue. Several HO-PCBs have been shown to displace thyroxine (T_4) from the thyroid transporting protein transthyretin (TTR), due to their higher affinity to TTR.[8]

Pentachlorophenol (PCP), a fungicide mainly used for wood preservation, is another halogenated phenolic compounds (HPCs) found in the environment. It binds, like HO-PCBs, to transthyretin and it has been measured at higher levels than HO-PCBs in humans from Sweden[9] and Russia.[4]

p,p´-DDT, a well known insecticide and prohibited since several years, and derivates accumulate in adipose tissue and have estrogen-like effects on organisms. Carcinogenic effects on humans have not definitely been proved.

4-,HO-heptachlorostyrene (4-,HO-HpCS) is a metabolite of octachlorostyrene, which is known to be a persistent chemical and degradation processes are generally slow. Studies indicate a possibility of accumulation of 4-,HO-heptachlorostyrene in blood plasma due to hindered excretion.[10]

There are unfortunately only few studies on concentrations of POPs in men from the general population. In this project blood plasma from males, aged from 19 to 88 years, was analysed for 14 PCBs and 37 hydroxylated metabolites, DDT and 2 derivatives, PCP, 4-,HO-HpCS and selected hormones. The levels of these compounds will be presented both on awet and lipid weight basis.

Material and methods

Samples

The University Hospital of Northern Norway (UNN) organized and collected the plasma sampling of 100 male and healthy people of Kautokeino and Hammerfest by public incitement of local media. All samples were stored at -20ºC and made anonymous by UNN before dissemination and analysis.

Sample preparation

Between 0.5 to 2 mL plasma samples were extracted on an Oasis HLB (540mg; Waters Corp.) solid phase extraction cartridge according to Sandau *et al.* after adding ^{13}C-labelled standard compounds.[11] Extraction and clean up was accomplished on a Zymark Rapidtrace Automated SPE workstation (Zymark Corp.). Evaporation of the organic solvent was carried out on a Labconco evaporator (Labconco Corp., Kansas City, MO). Lipid removal and fractionating of the extracts were performed on a self-packed florisil column (1g, 100% activated) with dichloromethane/hexane (1:3) as eluent for fraction one and methanol/dichloromethane (1:4) for fraction two. Fraction one includes neutral compounds like PCBs and pesticides, whereas in fraction two hydroxy-phenolic compounds (HPCs) were collected. Fraction two was evaporated to dryness and derivatised. Diazomethane in hexane (0.5 mL for 3 h at 20ºC) was used for methylation of the HPCs. In addition a standard solution was derivatised in order to check effectiveness of methylation and by-products. After derivatisation of the HPC fraction, a last clean up step was needed prior to GC-analysis. Therefore a self-packed SPE-cartridge (100% activated silica and acidified silica, 2:7) was used and dichloromethane was employed as elution-solvent. To both fractions isooctane was added as a keeper before reducing volumes to 30 µL. Before quantification octachloronaphtalene was added to calculate recovery rates for the internal standards. Together with the plasma samples, 10 blanks and five SRMs were analysed.

Chromatographic separation and quantification

An 8065 gas chromatograph (Fisons Instruments, Manchester, UK) was equipped with a 30 m ZB5-MS column (0.25 mm id and 0.25 µm film thickness; Zebron, Phenomenex, USA), a guard column (0.53 mm id, 2.5 m length deactivated, Agilent) and a restriction capillary (0.18 mm id, 1.5 m length deactivated, J&W). Helium (6.0 quality, Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1 mL/min. One µL of the sample extract was injected on-column with an AS800 automatic injection system (Fisons Instruments). The following temperature program was used: 70 °C (2 min), then 15 °C/min to 180°C and 5 °C/min to 280 °C (10 min isothermal). Analysis was carried out by low-resolution mass spectrometry (LRMS) using a MD 800 mass spectrometer (Fisons Instruments, San Jose, CA, USA) with ionization energy of 70 eV. The transfer line temperature was held at 280 °C and the ion-source temperature was set to 220 °C. The limit of detection (three times signal/noise ratio) for analyzed PCBs, HO-PCBs, DDTs and HPCs ranged from 0.001 to 0.055 ng/mL wet weight, LOD for PCP was 0.226 ng/mL wet weight. Statistical analysis were performed using the SAS software version 8.

Results and Discussion

The concentrations of PCBs, HO-PCBs and DDTs were summed up and along with 4-,HO-HpCS and PCP illustrated in figure 1 as geometrical mean values. It is displayed that sum-PCB levels dominate in the Kautokeino population with 5.878 ng/mL, followed by DDT with 1.888 ng/mL and HO-PCBs with 0.735 ng/mL. Hammerfest samples represent a similar pattern with about three times lower concentrations as Kautokeino; PCBs are found with 2.148 ng/mL, DDTs with 0.697 ng/mL and HO-PCBs with 0.213 ng/mL. PCP and 4-,HO-HpCS values represent single compound parameters. Both analyts show same distribution as the sum values: Kautokeino´s content are higher compared to Hammerfest´s: 4-,HO-HpCS has a geometrical mean of 0.093 with a spreading range from 0.035 (LOD) to 0.607 in Kautokeino and 0 in Hammerfest with a range from 0.035 (LOD) to 0.179 (see table 1). PCP could be detected at levels from 1.153 to 20.761 with a geometrical mean of 4.432 in Kautokeino and at levels from 0.692 to 30.934 in the inland location.

All 14 analysed PCBs could be verified in the samples. Among the PCBs showed the higher chlorinated di-*ortho* congeners, PCB 138, PCB 153 and PCB 180, most dominance. In all samples they could be detected in concentrations over the LOQ with about threefold higher levels in Hammerfest. The mono-*ortho* congeners PCB

Table 1: Arithmetical and geometrical mean with quantified concentration ranges of assayed analytes in ng/mL wet weight. KK Kautokeino; HF Hammerfest;

	Arithmetical mean		Geometrical mean		Range	
Compound	KK	HF	KK	HF	KK	HF
PCBs						
PCB-99	0.153	0.072	0.116	0.058	0.020 - 0.653	< 0.019 - 0.261
PCB-101	0.035	0.030	0.012	0.026	< 0.021 - 1.084	< 0.021 - 0.078
PCB-105	0.090	0.059	0.065	0.052	< 0.028 - 0.507	< 0.028 - 0.166
PCB-118	0.299	0.151	0.230	0.121	0.035 - 1.113	< 0.024 - 0.582
PCB-128	0.065	0.016	0.049	0.000	< 0.023 - 0.265	< 0.023 - 0.088
PCB-138	1.131	0.426	0.989	0.331	0.106 - 3.835	0.071 - 1.500
PCB-149	0.016	0.009	0.000	0.000	< 0.022 - 0.104	< 0.022 - 0.144
PCB-153	2.175	0.794	1.677	0.620	0.172 - 7.473	0.125 - 2.576
PCB-156	0.039	0.015	0.034	0.014	< 0.021 - 0.106	< 0.021 - 0.057
PCB-170	0.658	0.246	0.539	0.211	0.049 - 2.367	< 0.055 - 0.705
PCB-180	1.639	0.559	1.385	0.439	0.093 - 5.321	0.089 - 1.773
PCB-183	0.139	0.046	0.122	0.038	0.012 - 0.405	< 0.017 - 0.152
PCB-187	0.504	0.147	0.395	0.123	0.037 - 1.630	0.024 - 0.477
PCB-194	0.162	0.039	0.147	0.036	< 0.025 - 0.576	< 0.025 - 0.158
HO-PCBs						
4-HO-PCB-97	0.000	0.000	0.000	0.000	n.d.	< 0.014 - 0.005
4-HO-PCB-107	0.127	0.037	0.119	0.025	< 0.016 - 0.35	< 0.016 - 0.145
3-HO-PCB-118	0.005	0.000	0.000	0.000	< 0.020 - 0.055	n.d.
4-HO-PCB-120	0.001	0.001	0.000	0.000	< 0.005 - 0.013	< 0.005 - 0.016
4-HO-PCB-127	0.007	0.001	0.000	0.000	< 0.034 - 0.163	< 0.034 - 0.028
4-HO-PCB-130	0.001	0.003	0.000	0.000	< 0.019 - 0.056	< 0.019 - 0.107
4-HO-PCB-134	0.000	0.000	0.000	0.000	n.d.	< 0.020 - 0.020
3-HO-PCB-138	0.063	0.020	0.063	0.020	< 0.026 - 0.219	< 0.026 - 0.099
4-HO-PCB-146	0.192	0.063	0.175	0.057	< 0.025 - 0.537	< 0.025 - 0.180
3-HO-PCB-153	0.078	0.023	0.073	0.024	< 0.027 - 0.213	< 0.027 - 0.094
3-HO-PCB-163	0.003	0.001	0.000	0.000	< 0.024 - 0.042	< 0.024 - 0.027
4-HO-PCB-172	0.05	0.010	0.043	0.000	< 0.013 - 0.326	< 0.013 - 0.070
4-HO-PCB-178	0.001	0.001	0.000	0.000	< 0.013 - 0.028	< 0.013 - 0.027
3-HO-PCB-180	0.005	0.001	0.000	0.000	< 0.011 - 0.051	< 0.011 - 0.032
4-HO-PCB-187	0.273	0.122	0.225	0.109	0.022 - 0.836	< 0.015 - 0.299
4-HO-PCB-199	0.000	0.004	0.000	0.000	n.d.	< 0.029 - 0.143
4-HO-PCB-202	0.002	0.001	0.000	0.000	< 0.038 - 0.033	< 0.038 - 0.050
DDTs						
o,p-DDE	0.000	0.000	0.000	0.000	n.d.	n.d.
p,p-DDE	2.731	1.034	1.888	0.697	< 0.044 - 9.528	0.192 - 5.275
p,p-DDT	0.028	0.005	0.000	0.000	< 0.043 - 0.547	< 0.043 - 0.219
Others						
4-HO-HpCS	0.119	0.030	0.093	0.000	< 0.035 - 0.607	< 0.035 - 0.179
PCP	5.702	4.514	4.432	2.821	1.153 - 20.761	0.692 - 30.934

105, PCB 118 and PCB 156 were not present in all samples, with an exception of PCB 118 in Kautokeino. This is shown a range from 0.035 to 3.835 and a geometrical mean of 0.230.

All 17 HO-PCBs could be detected in the samples. HO-PCB 97, HO-PCB 134 and HO-PCB 199 were not present in the Kautokeino samples just as well as HO-PCB 118 in the Hammerfest samples. HO-PCB 187 shows the highest concentrations with a range from 0.022 to 0.837 together with a geometric mean of 0.225 in Kautokeino and a range from 0.015 (LOD) to 0.299 with a geometric mean of 0.109 in Hammerfest. Corresponding parent congeners, PCB 183 and PCB 187, show a positive correlation to the according metabolite HO-PCB 187. The levels of sum PCB and HO-PCBs were significantly ($p<0.01$) higher in Kautokeino, even after correcting for the age factor. The levels increased significantly with age as observed in previous studies.[3] For PCP there was no significant increase with age but there was a significant difference between the places.

Among the DDT group the metabolite *p,p´*-DDE was dominating with a geometrical mean of 1.888 and a range from 0.044 (LOD) to 9.528 in Kautokeino. In Hammerfest has *p,p´*-DDE been found in all samples with a geometrical mean of 0.697 and a range from 0.192 to 5.275. *p,p´*-DDT was verified in few samples, therefore geometrical means are 0 in both locations. Highest detected concentrations of *p,p´*-DDT are 0.547 and 0.219 in Kautokeino and Hammerfest, respectively.

A significant distinction in concentrations between the two locations could be shown for all analytes independent of age. In contrast to our expectations higher levels of POPs have been detected in the inland-population of Kautokeino. The reason for the levels of PCB and HO-PCBs being significantly higher in Kautokeino is not

known since we do not have detailed dietary information. It is surprising that the levels are higher in the inland community compared to the coastal population. However the coastal population had a lower mean age (Hammerfest: geom. mean 37 years; Kautokeino: geom. mean 54 years) and fish consumption on the coast is lower among the younger population.

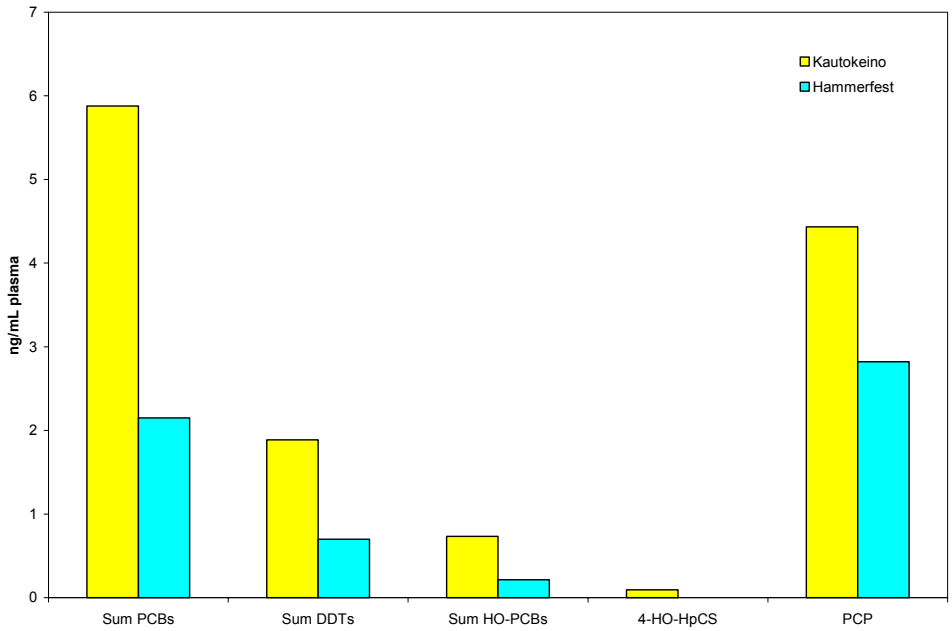

Figure 1: Geometrical mean of sum-levels of POPs in males from Kautokeino and Hammerfest (n=50 per location)

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