RELATIONSHIPS BETWEEN CHLORINATED AND BROMINATED ORGANIC POLLUTANTS IN HAIR, SOFT TISSUES AND BLOOD IN EAST GREENLAND POLAR BEARS

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Abstract

In this pilot study, we report for the first time on persistent organic pollutants (POPs) measured in polar bear (*Ursus maritimus*) hair, in an effort to validate hair as a non-invasive matrix representative of concentrations and profiles in internal organs and blood plasma. Among the findings, only five PCB congeners (CB 99, 138, 153, 170 and 180), one PBDE congener (BDE 47), oxychlordane, *trans*-nonachlor and *β*-HCH could be quantified in hair samples, which was likely a function of low sample weights (30-140 mg). Statistical analysis showed that the PCB profile in hair was similar to that of internal tissues, although a gender difference was found for concentrations in hair relative to concentrations in internal tissues. Females (n = 6) were found to display strongly negative correlations ($-0.78 \leq r \geq -0.55$, $0.065 \leq p \geq 0.26$ for sum PCBs) while males (n=5) showed weaker positive correlations ($0.21 \leq r \geq 0.84$, $0.077 \leq p \geq 0.74$ for sum PCBs). Even though most correlations were strong they were not significant ($p>0.05$), but this was due to small sample sizes. Further research is thus necessary to draw more definitive conclusions.

Introduction

Polar bears (*Ursus maritimus*) are top predators in the Arctic marine food web and are thus exposed to high levels of persistent organic pollutants (POPs) relative to other Arctic species[1]. In particular, polar bears from East Greenland have been documented to accumulate high levels of POPs[2,3]. Monitoring of POPs in polar bears has mostly been conducted on adipose tissue and to a lesser extent on full blood or plasma. Recently, studies have been performed on the internal tissue distribution of POPs[4,5]. However, to our knowledge, there are as yet no studies having investigated the use of hair in assessing the concentrations of POPs in internal tissues of polar bears. Keratinous tissues such as hair and feathers in other species have recently been proven to be useful as non-destructive and non-invasive biomonitors for POPs[6-8]. Hair can easily be sampled without harming individuals (e.g. during tagging operations) and access to historic hair samples may shed light on populations and periods from which other tissue samples can not be obtained. Moreover hair can simply be stored in envelopes or plastic bags and can be transported over large distances with Inter CITES Institutional permits and thus minimizing logistical difficulties. Conversely, POP concentrations are rather low in hair, because of its low lipid content[6] which makes the analysis of POPs in hair a challenging task. The present pilot study investigates POP concentrations in hair of polar bears at the same time assessing the usefulness of hair as a non-destructive biomonitoring matrix. POPs in hair should not only be quantifiable, but also related to concentrations in internal tissues. Internal tissue POP concentrations have been reported earlier in the present polar bear specimens allowing correlation analyses[4,5]. Furthermore, gender related differences were also explored.

Materials and Methods

Hair samples were obtained from 15 individuals (8 females, 7 males) that have been used in previous studies on POP tissue distribution in polar bears[4,5]. Samples from these polar bears were collected by local subsistence

hunters educated for that purpose in the Ittoqqortoormiit/Scoresby Sound area in central East Greenland (69°00'-74°00'N) during 1999-2001.

The hairs were washed with distilled water, dried at room temperature and cut in small pieces. An amount between 13 and 140 mg hair was weighed and incubated overnight at 40°C with HCl (4N) and a mixture of hexane/dichloromethane (4:1, *v:v*). After liquid extraction, clean-up was performed on acidified silica[9]. In all samples, 39 polychlorinated biphenyl (PCB) congeners (CB 18, 28, 31, 47, 49, 52, 74, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 158, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 205, 206 and 209), 8 polybrominated diphenyl ethers (PBDE) congeners (BDE 28, 47, 49, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p,p'*-DDT and *o,p'*-DDT) and metabolites (*p,p'*-DDE *p,p'*-DDD, *o,p'*-DDE and *o,p'*-DDD), hexachlorocyclohexanes (HCHs; α-, β- and γ-HCH), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)) and hexachlorobenzene (HCB) were analysed. For PBDEs, analysis was done using a GC/MS equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm), operated in electron capture negative ionisation (ECNI) mode. PCBs and DDTs were analysed using a GC/MS equipped with a HT-8 capillary column (25 m × 0.22 mm × 0.25 μm), operated in electron ionisation (EI) mode.

Analyses of POPs in internal tissues were performed in the Letcher Labs at the National Wildlife Research Centre in Ottawa, Canada. Details on the procedures of analysis and data of the internal tissue POPs concentrations can be found in Gebbink et al.[4]. These results have been used in the present study in order to link hair concentrations to internal tissue concentrations of the polar bears and to investigate differences in accumulation profiles.

All statistical analyses were performed using STATISTICA 7.0 (StatSoft Inc. 1984-2004). Samples with levels below the LOQ were assigned a value of p x LOQ, with 'p' the proportion of measurements with levels above the LOQ. Compounds with over 50% of the measurements below the LOQ were excluded from statistical analysis. Data were log transformed as had been done for the tissues[4,5]. The log-transformed data met the assumptions of normality and consequently parametric tests were performed. Pearson correlations were performed to investigate the relationships between concentrations of POPs in hair and tissues of polar bears. Furthermore, t-tests were performed to investigate the differences in concentrations in hair between genders. All hair samples were washed with distilled water, but four hair samples were excluded from statistical analyses, because they were contaminated with either fat (from adipose tissue) or blood that could not be effectively removed by the washing. The concentrations in both clean and external contaminated hair samples are given in Table 1.

Results and Discussion

Levels

Only five PCB congeners (CB 99, 138, 153, 170 and 180), one PBDE congener (BDE 47), OxC, TN and *β*-HCH could be quantified in the clean hair samples of polar bears (Table 1). This may also be related to the low amount of hair that was available (13-140 mg). An amount of minimum 200 mg is normally required for reliable measurement of organic pollutants in hair or feathers, yet depending on the degree of pollution. *p,p'*- DDE was not quantifiable in the clean hair samples, which is probably due to metabolism of this compound in polar bears[4,5,10]. Furthermore, POP concentrations were not significantly different between males and females ($p > 0.1$ in all cases).

In the external contaminated hair samples, seven PCB congeners (CB 99, 118, 138, 153, 170, 180 and 187), two PBDE congeners (BDE 47 and BDE 99), *p,p'*-DDE, *p,p'*-DDT, OxC, TN, HCB, *α*-HCH, *β*-HCH and *γ*-HCH could be quantified. This is probably due to the contamination with adipose tissue. As can be observed from Table 1, concentrations in this contaminated hair were also much higher than in the clean hair samples. Washing of hair samples with an organic solvent may possibly remove such external contamination.

Table 1. Mean concentrations ± SE (ng/g hair dry weight (d.w.)) of organic pollutants in clean and adipose/blood contaminated hairs of East Greenland polar bears. Contaminated hair samples include samples that contained skin and adipose tissue, which polluted the hair and may not be removed by washing.

ng/g hair	Clean hair samples (n=11)	External contaminated hair (n=4)
Sum PCBs*	117 ± 29	1660 ± 437
BDE 47	1.02 ± 0.14	5.8 ± 0.77
OxC	8.36 ± 2.25	188 ± 72
TN	3.12 ± 0.82	42 ± 10
β-HCH	3.29 ± 0.62	28 ± 3.5

* Sum PCBs includes: CB 99, 138, 153, 170 and 180.

In comparison, the corresponding levels in adipose, blood, brain and liver are given in Table 2 (adapted from Gebbink et al.[3,4]). Concentrations in hair were much lower than concentrations in adipose tissue or liver, but were in the same range as concentrations found in the brain and even higher than concentrations found in blood.

Table 2. Mean concentrations ± SE (ng/g wet weight (w.w.)) of organic pollutants in tissues of polar bears from East Greenland. * Sum PCBs includes: CB 99, 138, 153, 170 & 180. "n.d." not detectable.

ng/g w.w.	Adipose	Blood	Brain	Liver
Sum PCBs*	4550 ± 620	29 ± 4.4	137 ± 24	2520 ± 430
BDE 47	42 ± 11	0.52 ± 0.07	1.98 ± 0.6	3120 ± 2650
OxC	752 ± 107	5.33 ± 0.7	16 ± 6.9	3980 ± 540
TN	201 ± 27	0.92 ± 0.23	3.3 ± 1.7	50 ± 15
β-HCH	145 ± 49	0.38 ± 0.19	n.d.	n.d.

Correlations between hair, tissues and blood

Correlations were performed on both pooled data (n=11) and separately for males (n=5) and females (n=6). A significant correlation for the pooled data was only found for *β*-HCH in hair and blood ($r = 0.67$, $p = 0.024$). When the correlations were performed for males and females separately, 2 correlations were significant in females and only 1 in males. However, 12 correlations had high correlation coefficients ($|r| < 0.74$) and were close to being significant ($p < 0.1$). This indicates that a higher sample size would certainly yield significant correlations. All correlations are given in Table 3 and Table 4.

Table 3. Pearson correlations (r) between concentrations of POPs in hair (ng/g) and tissues (ng/g ww) of polar bear females from East Greenland. (*) $p < 0.2$, * $p < 0.1$, ** $p < 0.05$; 'n.a.': compound was analysed in too few samples to investigate the correlations.

n = 6	Hair vs Adipose	Hair vs Blood	Hair vs Brain	Hair vs Liver
CB 99	-0.56	-0.36	-0.76*	-0.54
CB 153	-0.80*	-0.54	-0.59	-0.43
CB 138	-0.80*	-0.61	-0.66(*)	-0.78*
CB 180	-0.72(*)	-0.60	-0.74*	-0.96**
CB 170/190	-0.53	-0.35	-0.05	-0.91**
Sum 5 PCBs	-0.78*	-0.55	-0.77*	-0.78*
OxC	-0.43	-0.32	-0.77*	-0.66(*)
TN	-0.69(*)	-0.29	0.27	-0.11
β-HCH	0.58	n.a.	n.a.	n.a.
BDE 47	0.16	0.34	0.10	0.24

Table 4. Pearson correlations (r) between concentrations of POPs in hair (ng/g) and tissues (ng/g ww) of polar bear males from East Greenland. (*) p < 0.2, * p < 0.1, ** p < 0.05; 'n.a.': compound was analysed in too few samples to investigate the correlations.

n = 5	Hair vs Adipose	Hair vs Blood	Hair vs Brain	Hair vs Liver
CB 99	0.30	0.81*	0.49	0.46
CB 153	0.77(*)	0.21	0.65	0.51
CB 138	0.99**	0.46	0.35	0.57
CB 180	0.78(*)	0.28	0.54	0.59
CB 170/190	0.81*	0.18	0.37	0.59
Sum 5 PCBs	0.84*	0.21	0.55	0.55
OxC	0.44	-0.03	-0.21	0.23
TN	0.65	0.27	0.31	0.29
β-HCH	-0.15	n.a.	n.a.	n.a.
BDE 47	-0.035	0.13	0.70(*)	-0.54

It is remarkable that female polar bear hair concentrations are mostly negatively correlated to concentrations in tissues while this is not the case for males. As we found no significant differences of concentrations in hair, this result may be due to seasonal fluctuations of concentrations in female tissues[2,3]. This is also illustrated in Figure 2 showing that adipose tissue concentrations are increasing with age in males (r = 0,59, p < 0,1) and decreasing with age in females (r = 0,38, p > 0,1). This is probably due to transplacental transfer of POPs to the foetus and the consecutive transfer through lactation to the cubs. Furthermore a time gap between the sampling period, which was all year round in this case, and the period of hair growth (September), when the bears are fasting resulting in high blood concentrations, and start of milk excretion (December) may even enhance the effect of seasonal fluctuations in females. In summary, more research with larger sample size is needed to investigate the factors which influence the POP concentrations in polar bear hair and to assess the usefulness of polar bear hair for biomonitoring purposes.

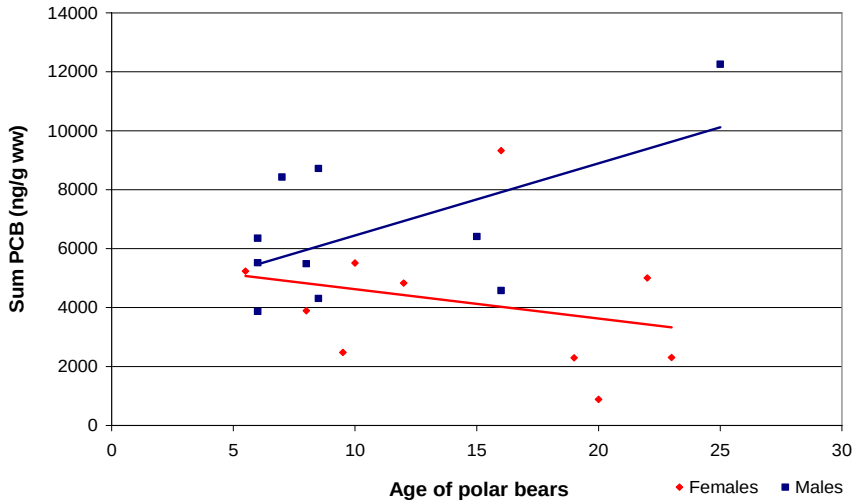

Figure 2. PCB concentrations versus age in adipose tissue of East Greenland male and female polar bears.

Profiles

The profile of PCBs was compared between hair and tissues (Figure 1). Only congeners that could be quantified in both hair and tissues are included. Overall, the PCB profile in hair is similar to the profile in the tissues, which suggests that polar bear hair may be reflecting the internal contamination. CB 153 and CB 180 are the most important congeners in all tissues. The profile in males and females is comparable, although for the brain the contribution of CB 153 is slightly higher and CB170/190 is slightly lower in females.

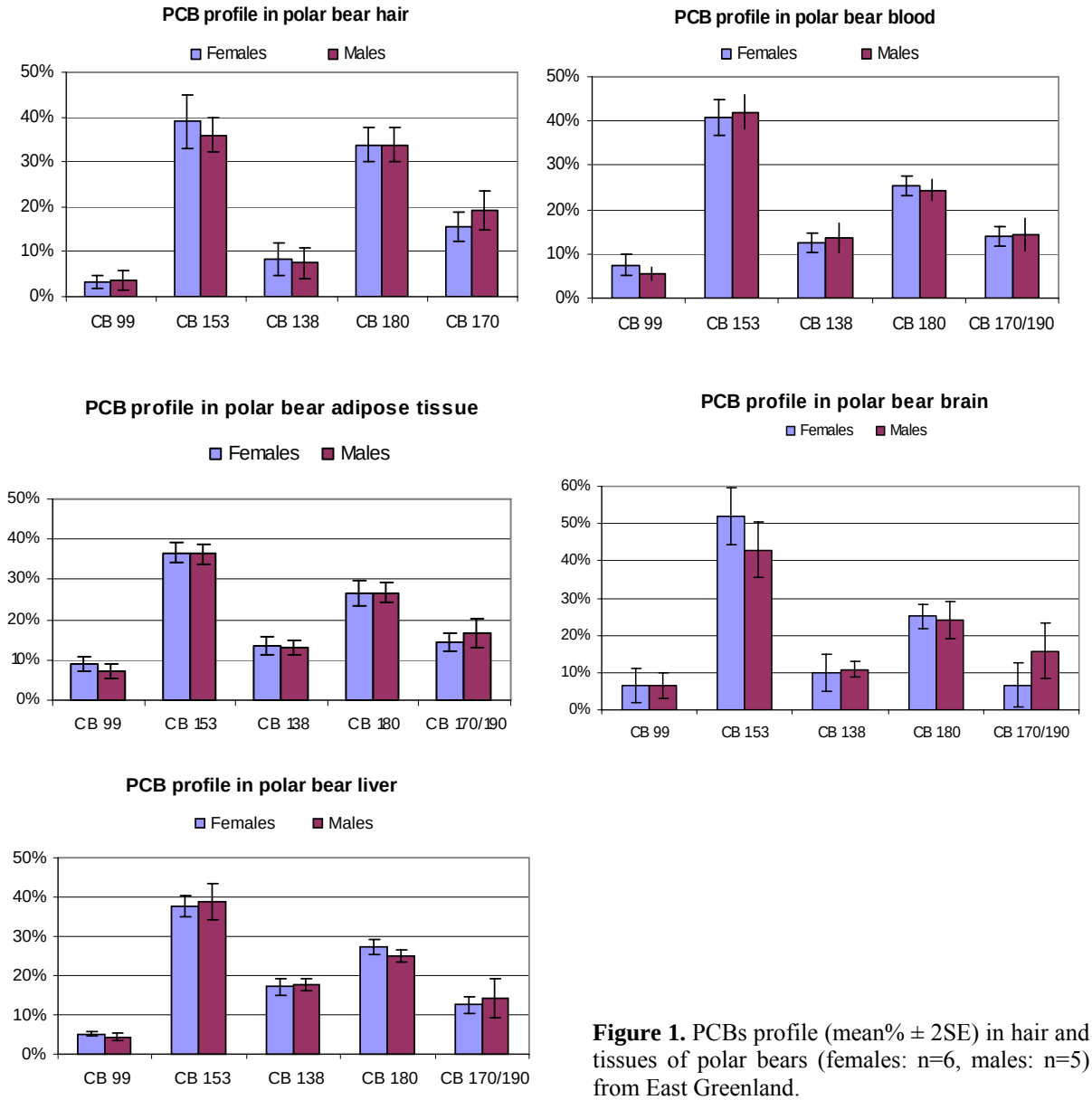

Figure 1. PCBs profile (mean% ± 2SE) in hair and tissues of polar bears (females: n=6, males: n=5) from East Greenland.

Conclusions

Only few POP compounds could be measured in hair of polar bears probably due to the low sample weight and the metabolic capacity of the polar bears for certain compounds (e.g. *p,p'*-DDE). Nevertheless, we found similar PCB profiles in hair compared to tissues, which suggest that hair is reflecting the internal congener contamination but not concentrations. However, there is a clear sex difference in the relation of concentrations in hair to concentrations in tissues: females show strong negative correlations, while males show weaker positive correlations. This is an interesting finding which might be related to seasonal fluctuations of POPs in tissues from females. The lack of significance in most correlations is more than likely due to small sample sizes, and therefore we recommend further studies on this issue with larger amounts of hair, and larger numbers of samples.

Acknowledgements
Dr. Veerle Jaspers and Dr. Adrian Covaci are postdoctoral fellows at the Funds for Scientific Research Flanders (FWO). Prof. Dr. Eens is funded by the University of Antwerp and FWO. Wouter Gebbink (Letcher Labs, NWRC, Ottawa) is acknowledged for carrying out the internal tissue and blood analyses for POPs. Danish Cooperation for Environment in the Arctic, the Commission for Scientific Research in Greenland, and the Natural Science and Engineering Research Council (NSERC) of Canada (to R.J. Letcher) are recognized for their financial support of the present study. Hanne Tuborg Sandell, Birger Sandell, Jonas Brønlund, and local hunters are acknowledged for conducting the field sampling.

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