HOW IMPORTANT ARE THE HYDROXYLATED PCB METABOLITES (OH-PCB) IN HARBOUR SEALS (*PHOCA VITULINA*)?

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Introduction

Persistent organochlorine pollutants (OCs) such as the polychlorinated biphenyls (PCBs) and pesticides like DDT, hexachlorocyclohexane (HCH), hexachlorobenzene (HCB) and chlordanes, are lipophilic substances that can cross the lipophilic cell membranes of animal cells by diffusion. The fate of these substances in an organism varies considerably, and both degradation by metabolism and accumulation in lipid-rich tissues are possible outcomes. Several of these compounds have been shown to metabolize to various degrees, and some metabolites are also retained in the organism due to high lipid affinity, protein association or other mechanisms[1].

Numerous studies have shown that OCs may have endocrine disrupting effects. Several studies show that they may affect thyroid hormone function and homeostasis in experimental animals, wildlife species and humans[2,3,4]. Metabolism of parent compounds is very likely to alter their kinetics and toxicological properties. In recent years the awareness of the role of metabolites in the toxicological assessment of the parent compounds has increased considerably, and it is known that hydroxylated metabolites of PCBs (OH-PCBs) play a major role in inducing the negative effects that are seen on the thyroid hormone system in heavily exposed animals[5,6].

Harbour seals (*Phoca vitulina*) are members of the pinniped family of *Phocidae* (true seals) and can be found around temperate and Arctic marine coastlines of the Northern hemisphere. They prey mainly upon fish, and occasionally upon shrimp, molluscs and squid. The lifespan is up to 35 years, and usually females outlive males. Seals are almost at the top of marine food webs and because of their relatively long lifespan, the accumulation of OCs in these animals is of particular concern. It has been shown that in general there is a relationship between high trophic level and high metabolic capacity[7]. Hence, marine apex predators like the harbour seal are the ones that are most likely to have the highest concentrations of OH-PCBs. Studies conducted on polar bears have supported this hypothesis[8,9], but data on levels of OH-PCBs in the other marine mammals are scarce. Thus, the aim of the present study was to investigate the presence of OCs including OH-PCBs in liver and plasma of harbour seals.

Materials and Methods

In this study a selection of PCBs, pesticides and OH-PCBs were determined in plasma and liver of five adult male harbour seals. The seals were captured at Froan outside Trondheim on the Norwegian coast (Fig.1) in September 2003. The sampling was done in connection with the EU-project FIRE (Flame retardant Integrated Risk assessment for Endocrine disruption, contract No: QLT4-CT-2002-00596).

Fig. 1: Sampling location.

The Laboratory of Environmental Toxicology is accredited according to the requirements of NS-EN ISO/IEC 17025 (TEST 137). The multicomponent analytical method used for determination of OCs and metabolites is based on the method originally described by Brevik[10] and later modified by Bernhoft & Skaare[11] and Løken[12]. Standard procedures were used to ensure adequate quality assurance and control, and the precision, linearity, and sensitivity of the analyses were within the laboratory's accredited requirements.

The samples were weighed (~4 g plasma and ~3 g liver) in 80 ml sentrifuge tubes and added internal standards (PCB-29, -112 and -207, 4'-OH-[$^{13}C_{12}$]CB159 and 4-OH-[$^{13}C_{12}$]CB187), 4 ml 2% NaCl and 10 ml 1 M H_2SO_4. Extraction with cyclohexane and acetone was performed twice using an ultrasonic processor. Lipid contents were determined gravimetrically. The lipid-extracts were cleaned up with concentrated H_2SO_4. The organic supernatants were then extracted twice with 5 ml 1 M KOH in 50% ethanol. The organic phases from this extraction were analysed by GC-ECD to determine the following PCBs and pesticides; PCB-28, -31, -47, -52, -56, -66, -74, -87, -99, -101, -105, -110, -114, -118, -128, -136, -137, -138, -141, -149, -151, -153, -156, -157, -170, -180, -183, -187, -189, -194, -196, -199, -206 and -209 (IUPAC numbers), hexachlorobenzene (HCB), α-, β and γ-hexachlorocyclohexanes (HCHs), oxy-, *trans*-, and *cis*-chlordane, *trans*- and *cis*-nonachlor, *p,p*'-DDE, *o,p*'-DDD, *p,p*'-DDD, *o,p*'-DDT, *p,p*'-DDT and mirex. The GC-system used was an Agilent 6890 Series with autosampler and Agilent 7683 Series split/splitless injector operated in the pulsed splitless mode, coupled to two Agilent micro EC-detectors (μECD). The capillary columns in the system included a fused silica precolumn (1m x 0.25mm, Varian) which was followed by a splitter to two columns of different polarity (60 m x 0.25 mm, 0.25 μm film thickness). The carrier gas used was hydrogen (99.999%). GC Chemstation Rev. A.10.02 (Agilent) was used both for instrument control and data analysis.

The alkaline phases were acidified with concentrated H_2SO_4, and re-extracted with 3 x 5 ml cyclohexane. These organic phases were then derivatized with acetic anhydride:pyridine (1:1) to yield the acetylated analogs to the OH-PCBs. The samples were analysed by GC-ECNI-MS to determine the following OH-PCBs; 4-OH-CB107, 4-OH-CB146, 4'-OH-CB106, 3'-OH-CB138, 4'-OH-CB130, 4'-OH-CB159, 4'-OH-CB172 and 4-OH-CB187[13]. The GC-MS system used consisted of an Agilent 6890 Series GC with autosampler, Agilent 7683 Series split/splitless injector operated in the pulsed splitless mode and Agilent 5673 quadropole mass spectrometer. The capillary column was a J&W Scientific DB-5 MS, with dimensions 0.25 mm i.d., 60 m length and 0.25 μm film thickness. The carrier gas used was hydrogen (99.999%), and the reagent gas was methane (99.995). MSD Chemstation Version D.01.00 (Agilent) was used both for instrument control and data analysis.

Calibration curves with 5 or more levels were used for quantification. Limits of quantification (LOQ) were set to 10 times the noise level, while limits of detection (LOD) were set to 3 times the noise level.

Table 1: Median and range of the concentrations (ng/g wet weight) of quantified OCs and extractable lipids in liver and plasma from male harbour seals (N = 5) captured at Froan, Norway. In calculating the median, levels that were between the LOD and the LOQ were included, and ½LOD were used for results under the LOD.

	Liver		Plasma	
	Median	**Range**	**Median**	**Range**
Lipids (%)	3.14	2.64–4.28	0.91	0.80–1.24
HCB	0.15	0.03–0.22	0.04	0.03–0.07
α-HCH	<0.14	<0.14	0.01	0.01–0.03
oxyKlordan	2.79	1.74–6.76	0.47	0.38–1.22
transNonaklor	1.39	1.25–3.65	0.43	0.01–1.00
pp-DDE	15.8	14.0–43.9	2.98	1.91–11.9
pp-DDD	3.59	3.11–5.76	0.13	0.04–0.14
pp-DDT	<1.30	<1.30	0.65	0.46–1.40
PCB-28	<0.17	<0.17	0.03	<0.01–0.04
PCB-52	0.62	<0.35–0.75	0.12	0.06–0.19
PCB-47	<0.35	<0.35–0.98	0.10	0.03–0.11
PCB-74	<0.25	<0.25–0.59	0.11	0.05–0.17
PCB-101	1.77	1.49–3.28	0.47	0.17–0.68
PCB-99	3.15	2.41–10.8	0.68	0.58–2.20
PCB-110	2.31	2.02–5.07	0.23	0.07–0.34
PCB-151	1.10	<0.40–2.30	0.10	0.03–0.17
PCB-149	1.98	1.41–2.94	0.28	0.12–0.40
PCB-118	0.60	<0.40–1.26	0.26	0.07–0.38
PCB-153	17.8	13.8–60.4	2.82	2.71–14.3
PCB-105	<0.40	<0.40	0.10	0.03–0.14
PCB-137	<0.22	<0.22–0.96	0.06	0.05–0.24
PCB-138	15.0	13.0–48.4	2.16	1.88–9.54
PCB-187	12.0	8.27–18.2	0.31	0.29–1.20
PCB-183	1.37	1.12–4.46	0.15	0.14–0.66
PCB-128	1.27	1.23–3.95	0.28	0.17–0.75
PCB-180	4.77	3.08–15.1	0.57	0.51–2.80
PCB-170	1.51	1.08–5.97	0.23	0.20–1.07
PCB-194	0.44	<0.35–1.16	0.08	0.07–0.33
ΣPCB_{20}	**59.7**	**53.3–181**	**9.27**	**7.30–35.4**
4-OH-CB107	0.30	0.20–0.42	1.56	1.08–2.83
4-OH-CB146	0.04	0.03–0.06	0.28	0.19–0.42
3'-OH-CB138	<0.10	<0.10	<0.07	<0.07–0.07
4'-OH-CB130	<0.09	<0.09	<0.06	<0.06–0.10
4'-OH-CB172	<0.02	<0.02	0.02	<0.02–0.03
4-OH-CB187	0.12	0.09–0.19	0.41	0.36–0.80
$\Sigma OH\text{-}PCB_6$	**0.49**	**0.40–0.79**	**2.37**	**1.83–4.25**

Results and Discussion

Levels of the highly lipophilic pesticides and native PCBs were considerably higher in the livers than in the plasma samples, while concentrations of the more polar OH-PCBs were highest in the plasma (Table 1). This is as expected as the lipophilic compounds are retained mainly because of lipid association, while the OH-PCBs are retained because of their affinity to specific proteins. OH-PCBs that have been detected in this study have the OH-group in *para* or *meta* position with adjacent chlorine atoms. This gives them structural similarities with the hormone thyroxine and thus an affinity for thyroxines transport proteins (mainly transthyretin, TTR), which has been shown to be the main reason for the retention of OH-PCBs in blood[1].

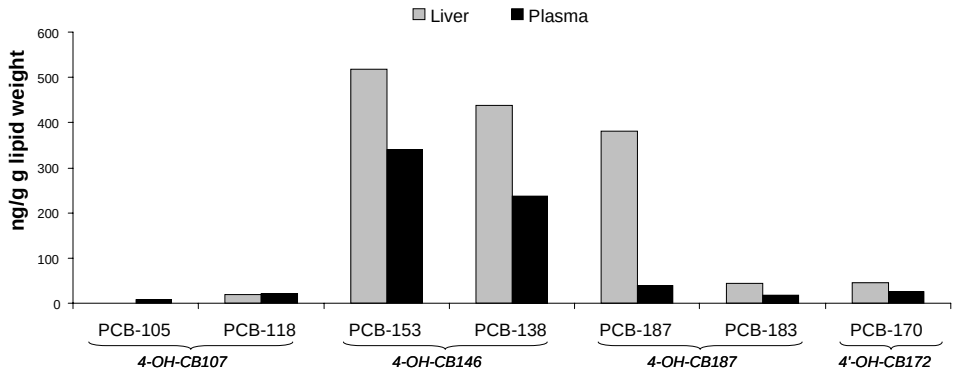

Fig. 2: Concentrations (ng/g lipid weight) of the relevant precursor PCBs in this study in both liver and plasma of five adult male harbour seals. The figure also shows which metabolites that are likely to be formed from the different PCB congeners[1].

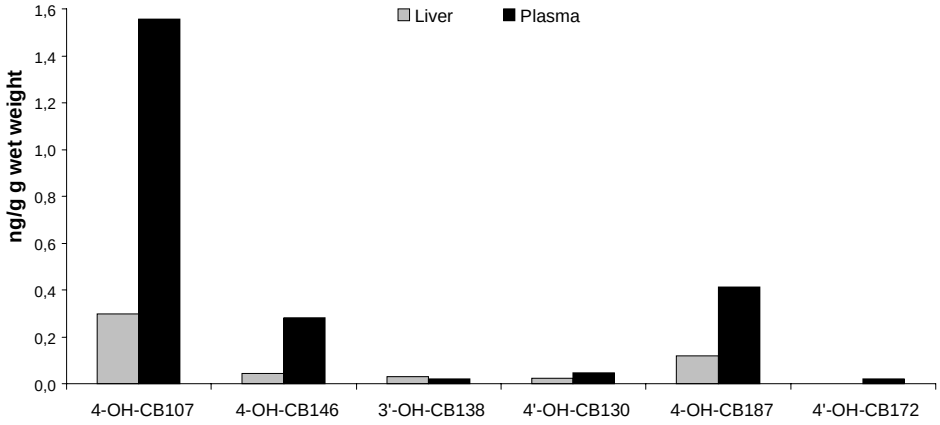

Fig. 3: Concentrations (ng/g wet weight) of the OH-PCBs determined in this study in both liver and plasma of five adult male harbour seals.

The two dominant PCB-congeners were PCB-153 and PCB-138 in both liver and plasma (Fig. 2), which is consistent with a previous study of harbour seal from the Norwegian coast[11]. From Fig. 2 it can be seen that when comparing the concentrations of some of the PCB congeners on a lipid weight basis, the differences between liver and plasma becomes rather insignificant. This is consistent with the fact that PCBs are retained in organisms mainly due to lipid association. In Fig. 2 it is also shown which of the included OH-PCB metabolites that are likely to be formed from the different PCB congeners[1].

The by far most dominant OH-PCB congener in both liver and plasma was 4-OH-CB107, with 4-OH-CB187 and 4-OH-CB146 as the second and third most abundant ones (Fig. 3). This is a different pattern than found in e.g. polar bears and humans[1]. The interspecies differences in relative ratios between the individual OH-PCB congeners might be due to species-specific biotransformation capacity with respect to PCB metabolism[14].

Selective metabolism of PCB-118 has been reported in harbour seals earlier[15], and this congener has been shown to be a precursor for 4-OH-CB107[1,16], which can explain why this is the most dominant congener. In addition one can not exclude the possibility for differences in type and affinity of ligand binding of TTR, even though this is a highly conserved protein present in all vertebrate species[1].

Among the pesticides determined in this study, the dominant compound in both liver and plasma were the DDT-metabolite *pp*-DDE, but the pattern differed slightly (Table 1). The major difference was the ratio between *pp*-DDE and *pp*-DDT, which was >12.2 in the liver and 4.6 in the plasma. Also the DDT-metabolite *pp*-DDD was considerably higher in liver than in plasma.

In summary, substantial amounts of specific OH-PCB metabolites (median sum of 6 OH-PCBs constituted approximately 26 % of median sum of 30 PCB congeners) are present in harbour seal plasma. Thus, it should be considered to include OH-metabolites in monitoring programs and risk assessment of persistent organic pollutants (POPs) when harbour seals are used as indicator species of POP pollution.

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