Maternal transfer of PCBs, PBDEs and their hydroxylated metabolites in grey seal (*Halichoerus grypus*) from the Isle of May, Scotland

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Introduction

Seals produce a lipid rich milk which is known to contain high levels of POPs, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)[1,2]. Suckling pups are thus exposed to high amounts of toxic chemicals while being at a critical developmental period of their life. Previous studies have shown that pup or juvenile seals exposed to POPs had reduced immunocompetence and impairment of thyroid and vitamin A homeostasis[3-6]. Several studies have already investigated the lactational transfer of PCBs, particularly in grey seals, and all have reported a preferential transfer of mostly lower chlorinated PCB congeners from mother to pup[7-9]. However, little is known about the transfer of PBDEs from mothers to pups in marine mammals. A recent study showed that the transfer of PBDEs during lactation in grey seals decreased with increasing Br substitution[10]. However, this work investigated levels and profiles in blubber of five mother-pup pairs sampled only once at an advanced stage of lactation. The objectives of this study were to investigate the levels and profiles of PCBs and PBDEs in maternal blubber, serum and milk as well as in pup serum collected at early and late lactation in a UK grey seal population in order to characterise the transfer of these molecules from mothers to their offspring. The transfer of PCB and PBDE hydroxylated metabolites (HO-PCBs and HO-PBDEs, respectively) and naturally-produced methoxylated PBDEs (MeO-PBDEs) was discussed as well.

Material and methods

Seal sampling. Twenty grey seal mother-pup pairs from the Isle of May (IOM), Scotland, were studied during the breeding season in November-December 2008. Animals were sampled at the beginning and at the end of the lactation period in order to collect maternal blubber, serum and milk as well as pup serum samples. All the samples were stored in a freezer at -20°C until analyses. Sampling techniques are described elsewhere[1,11].

Contaminant and vitamin A analyses. For blubber, approximately 150 mg of each end of the biopsy (outer and inner parts) were analysed separately. Details on the sample preparation, extraction, clean-up, quality control and chromatography analyses are described elsewhere[6,12,13]. In all samples, 35 PCB congeners (IUPAC numbers: CB 18, 28, 44, 47, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 146, 149, 151, 153, 156, 158, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206 and 209), 6 PBDEs (IUPAC numbers: BDE 28, 47, 99, 100, 153 and 154) and 2 naturally-produced methoxylated PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) were targeted. Additionally, 20 HO-PCBs (3HO-CB 118, 138, 153, 180, 4HO-CB 109, 120, 127, 130, 146, 162, 163, 172, 177, 187, 193, 198, 199, 202, 208 and 4-diMeO-CB202) as well as 3 HO-PBDEs (6-HO-BDE47, 5-HO-BDE47 and 4-HO-BDE49) were investigated in milk and serum samples.

Statistics. Statistical analyses were conducted using SPSS 17 for Windows. Contaminant levels were log-transformed to normalise the data and to reduce inter-individual variability. Linear mixed models were used to test differences in contaminant concentrations between both lactation periods. Other important confounding factors, such as the individual and the body condition were also included in the statistical analyses. The individual was considered as a random explanatory variable, while the lactation period and body condition were determined as fixed variables. The level of statistical significance was set at $p \leq 0.05$.

Results and discussion

PCB profiles and changes throughout lactation. In all tissues and for both periods, CB-153 was the predominant PCB congener followed by CB-138 or CB-180. Together, these PCB congeners accounted for more than 55 % of the total PCBs. This pattern is in accordance with what is usually observed in UK grey seals[8]. Tri-CBs were not detected in maternal serum and milk and were only found in very low concentrations in some maternal blubber and pup serum samples. Therefore, these congeners will not be further discussed here. Concerning the tetra-CB group, CB-44, CB-47 and CB-49 were detected in less than 50 % of the samples in all tissues. On the other hand, CB-52 was found in more than 90 % of the samples in all tissues. CB-209, a deca-CB was detected in all blubber samples, while it was only sporadically found in the other tissues. In general, maternal blubber layers contained higher proportions of hepta-, octa-, nona- and deca-CBs compared to other tissues, while percentages of tetra-, penta- and hexa-CBs were higher in maternal serum, milk and pup serum (results not shown). As the highest chlorinated PCBs are also the most lipophilic, the greater presence of these compounds in blubber was expected.

The statistical treatments were performed on groups of congeners according to their degree of chlorination. Due to their low concentrations and detection frequency in the samples, nona and deca-CBs (CB-206 and 209) were combined in one group for further statistical analyses. The sum of all PCBs (from tetra to deca) remained stable in maternal outer blubber from early to late lactation, but increased significantly in inner blubber at late lactation. This increase was noticed in the different PCB groups, except for Σ octa-CBs ($p_{4\text{-Cl}} = 0.003$, $p_{5\text{-Cl}} = 0.001$, $p_{6\text{-Cl}} = 0.002$, $p_{7\text{-Cl}} = 0.006$, $p_{8\text{-Cl}} = 0.116$ and $p_{9\text{-}10\text{-Cl}} = 0.001$). At early lactation, PCBs might be less readily mobilised than lipids as suggested in other studies[1]. This phenomenon might be due, at least in part, to the higher affinity of PCBs and in particular for the higher chlorinated ones for blubber triacylglycerols than for more polar serum lipids (e.g. phospholipids)[1,2,8].

Table 1. Mean ± standard deviation of PCB congener groups, PBDE congeners, Σ HO-PCBs and Σ MeO-PBDEs measured in maternal blubber and serum of grey seals.

	Outer blubber (ng/g ww*)		Inner blubber (ng/g ww*)		Maternal serum (pg/ml)	
	Early lactation	*Late lactation*	*Early lactation*	*Late lactation*	*Early lactation*	*Late lactation*
Lipid content (%)	86.7 ± 3.8[a]	77.5 ± 8.8[b]	84.6 ± 5.0[a]	66.9 ± 12.9[b]	0.8 ± 0.1[a]	0.6 ± 0.1[b]
Σ tri-CBs	1 ± 0[a]	5 ± 8[a]	1 ± 0[a]	2 ± 4[b]	N.D	N.D
Σ tetra-CBs	14 ± 6[a]	16 ± 8[a]	12 ± 3[a]	17 ± 8[b]	114 ± 45[a]	106 ± 36[a]
Σ penta-CBs	126 ± 53[a]	139 ± 61[a]	98 ± 35[a]	160 ± 66[b]	541 ± 198[a]	585 ± 265[a]
Σ hexa-CBs	1232 ± 766[a]	1489 ± 829[a]	720 ± 378[a]	1377 ± 821[b]	2914 ± 1327[a]	4368 ± 2781[a]
Σ hepta-CBs	796 ± 599[a]	1014 ± 658[a]	379 ± 324[a]	767 ± 578[b]	945 ± 490[a]	1860 ± 1370[a]
Σ octa-CBs	196 ± 166[a]	253 ± 180[a]	74 ± 85[a]	157 ± 143[a]	96.4 ± 86.5[a]	241 ± 230[a]
Σ nona-deca-CBs	67 ± 60[a]	88 ± 66[a]	24 ± 34[a]	57 ± 60[b]	15 ± 22[a]	46 ± 62[a]
Σ PCBs	**2432 ± 1588[a]**	**3004 ± 1716[a]**	**1308 ± 817[a]**	**2538 ± 1645[b]**	**4626 ± 2073[a]**	**7205 ± 4630[a]**
BDE 28	0.4 ± 0.2[a]	0.4 ± 0.2[a]	0.4 ± 0.2[a]	0.5 ± 0.2[b]	N.D	N.D
BDE 47	45.4 ± 25.9[a]	49.4 ± 28.9[a]	34.2 ± 21.5[a]	53.9 ± 29.8[b]	88.9 ± 55.1[a]	107.1 ± 73.0[a]
BDE 99	5.0 ± 2.8[a]	6.5 ± 4.9[a]	3.9 ± 3.8[a]	7.4 ± 5.3[b]	5.6 ± 3.3[a]	9.9 ± 9.0[a]
BDE 100	10.9 ± 8.4[a]	13.0 ± 8.9[a]	6.7 ± 5.3[a]	12.9 ± 9.7[b]	8.7 ± 5.3[a]	17.5 ± 12.8[a]
BDE 153	10.4 ± 9.2[a]	13.5 ± 9.8[a]	4.5 ± 6.3[a]	7.6 ± 6.6[b]	3.2 ± 2.2[a]	7.1 ± 5.5[a]
BDE 154	7.4 ± 9.6[a]	9.2 ± 10.4[a]	3.9 ± 4.7[a]	7.0 ± 7.9[b]	2.6 ± 2.3[a]	7.0 ± 6.4[a]
Σ PBDEs	**79.9 ± 52.3[a]**	**92.4 ± 57.8[a]**	**54.0 ± 38.3[a]**	**89.7± 56.2[b]**	**112.7 ± 67.7[a]**	**152.5 ± 99.5[a]**
Σ HO-PCBs	N.A	N.A	N.A	N.A	558 ± 271[a]	654 ± 329[a]
Σ MeO-PBDEs	2.5 ± 1.0[a]	2.4 ± 1.1[a]	2.3 ± 1.1[a]	3.5 ± 1.7[a]	3.6 ± 1.8[a]	3.5 ± 1.9[a]

For a defined tissue, values within a row followed by different letters are significantly different ($p \leq 0.05$).
N.D = Not detected
N.A = Not targeted or analysed
* wet weight

In female serum, Σ tetra- and penta-CB levels were relatively stable, while Σ hexa to deca-CB levels tended to slightly increase at late lactation, although not significantly (Table 1). In milk, Σ of all PCB congeners tended to

increase at late lactation but this increase was not significant for the highest chlorinated PCBs (Σ octa to deca-CBs) (p4-Cl = 0.007, p5-Cl = 0.010, p6-Cl = 0.009, p7-Cl = 0.009, p8-Cl = 0.159 and p9-10-Cl = 0.138) (Table 2). In serum of suckling pups, total concentrations of all PCB groups tended to increase at late lactation but this increase was significant only for the lowest chlorinated groups, tetra and penta-CBs ($p_{4\text{-Cl}} = 0.010$, $p_{5\text{-Cl}} = 0.017$) (Table 2). These trends observed in female serum, milk and pup serum corroborates previous studies[7-9] concluding to a better transfer of lower chlorinated PCBs into the milk and therefore into the suckling pup.

Table 2. Mean ± standard deviation of PCB congener groups, PBDE congeners, Σ HO-PCBs and Σ MeO-PBDEs measured in milk and pup serum of grey seals.

	Milk (ng/g ww*)		Pup serum (pg/ml)	
	Early lactation	*Late lactation*	*Early lactation*	*Late lactation*
Lipid content (%)	44.9 ± 6.0^a	57.5 ± 5.2^b	0.9 ± 0.2^a	1.2 ± 0.2^b
Σ tri-CBs	N.D	N.D	22 ± 8^a	56 ± 26^b
Σ tetra-CBs	8 ± 3^a	11 ± 4^b	216 ± 117^a	421 ± 210^b
Σ penta-CBs	37 ± 14^a	67 ± 30^b	1083 ± 614^a	2309 ± 1378^a
Σ hexa-CBs	186 ± 76^a	411 ± 265^b	5510 ± 3193^a	11626 ± 8558^a
Σ hepta-CBs	59 ± 24^a	158 ± 115^b	1886 ± 1112^a	4376 ± 3512^a
Σ octa-CBs	15 ± 6^a	23 ± 17^a	276 ± 188^a	710 ± 638^a
Σ nona-deca-CBs	2 ± 1^a	3 ± 3^a	49 ± 54^a	139 ± 140^a
Σ PCBs	**307 ± 115^a**	**674 ± 420^b**	**9042 ± 5108^a**	**19637 ± 13987^b**
BDE 28	0.1 ± 0.1^a	0.2 ± 0.1^b	2.8 ± 2.4^a	4.3 ± 3.6^a
BDE 47	10.5 ± 5.6^a	24.1 ± 15.0^b	261 ± 159^a	501 ± 366^a
BDE 99	0.6 ± 0.4^a	1.7 ± 1.7^b	14.1 ± 12.4^a	29.5 ± 30.4^a
BDE 100	1.1 ± 0.6^a	3.1 ± 2.4^b	26.7 ± 15.4^a	59.1 ± 44.6^a
BDE 153	0.3 ± 0.2^a	1.0 ± 0.8^b	13.6 ± 6.3^a	28.0 ± 22.1^a
BDE 154	0.3 ± 0.2^a	0.9 ± 1^b	8.4 ± 8.0^a	21.1 ± 27.2^a
Σ PBDEs	**13.0 ± 6.8^a**	**31.1 ± 20.2^b**	**325.7 ± 192.4^a**	**642.9 ± 468.5^a**
Σ HO-PCBs	$< 0.02^a$	0.02 ± 0.01^b	501 ± 420^a	1077 ± 484^a
Σ MeO-PBDEs	0.3 ± 0.1^a	0.6 ± 0.3^b	N.D	N.D

For a defined tissue, values within a row followed by different letters are significantly different ($p \leq 0.05$).
N.D = Not detected;
N.A = Not targeted or analysed
* wet weight

PBDE profiles and changes throughout lactation. BDE-47 was the most dominant congener in all samples representing between 50 % and 80% of the total PBDEs depending on the tissue. The next congeners were BDE-99, BDE-100 or BDE-153 depending on the period and the tissue considered. These congeners are usually predominant in marine mammals worldwide[10-14]. In both maternal blubber layers and in milk, all the studied congeners were found in more than 95% of the samples. In maternal serum, BDE-28 was not found and BDE-153 and BDE-154 were found in less than 50% of the samples. In pup serum, BDE-28 was detected in less than 50 % of the samples, whereas BDE-153 and BDE -154 were found in more than 95% and 60% of the samples, respectively. Overall, maternal blubber layers contained higher percentages of BDE-100, BDE-153 and BDE-154 compared to other tissues, while the proportion of BDE-47 tended to be more important in female serum, milk and pup serum (results not shown), revealing an accumulation of higher PBDEs in maternal blubber reserves. Other measured congeners differed only slightly between tissues.

Levels of each PBDE congener increased significantly in maternal inner blubber at late lactation ($p_{BDE\text{-}28} = 0.021$, $p_{BDE\text{-}47} = 0.002$, $p_{BDE\text{-}99} = 0.004$, $p_{BDE\text{-}100} = 0.004$, $p_{BDE\text{-}153} = 0.009$ and $p_{BDE\text{-}154} = 0.006$), while they remained constant in outer blubber. The same hypotheses as suggested earlier for PCBs could be applied to the PBDE dynamics observed in inner blubber. In female serum, levels of each congener appeared to slightly increase at late lactation, although not significantly. In milk, levels of each congener were significantly higher at late lactation compared to early lactation ($p_{BDE\text{-}28} = 0.006$, $p_{BDE\text{-}47} = 0.009$, $p_{BDE\text{-}99} = 0.034$, $p_{BDE\text{-}100} = 0.011$, $p_{BDE\text{-}153} = 0.045$ and $p_{BDE\text{-}154} = 0.032$). Finally, in pup serum, although the concentrations of each congener tended to increase at late lactation, no

significant difference was found between both periods. Contrary to what is usually observed with PCBs, no clear trends towards a selective transfer were found for PBDE congeners in the present work. This is in contradiction with a previous study on grey seals (n=5) that concluded to a reduction in the efficiency of transfer of higher PBDEs from mother blubber to pup blubber[10]. However, as mentioned in the introduction section, that study did not focus on longitudinal samples of recaptured individuals.

Levels and profiles of PBDE and PCB metabolites. Because of the larger affinity of hydroxylated metabolites for blood compared to blubber, HO-PCBs and HO-PBDEs were not targeted in maternal blubber. No HO-PBDEs were found either in milk or serum samples. Similarly, 3HO-CB118 and 3HO-CB153 were not found in any investigated sample. 4HO-CB107 was found at the highest concentrations in all tissues followed by 4-HO-CB162. This profile of HO-PCBs agrees with the profile reported in serum of harbour seals[13]. 4HO-CB107 was the only hydroxylated metabolite found in milk at very low levels. This observation is in agreement with rodent studies which reported that only limited amounts of HO-PCBs are transferred to offspring through lactation[15]. Interestingly, in addition to 4HO-CB107 which was found in all pup serum samples, 2 other HO-PCBs were also detected: 4-HO-CB162 found in all pup serum samples and 4-HO-CB120 detected in 53% of the samples, mainly late lactation samples. It may be possible that these HO-PCBs are present in the milk but in concentrations below the limit of detection, allowing pups to accumulate these molecules through lactation. Another explanation would be that these HO-PCBs are transferred to the foetus during the gestation as reported for humans and rodents[16,17]. Finally, nursing pups might be able, to a small extent, to produce HO-PCBs through their own metabolism.

MeO-PBDEs. Two naturally-produced MeO-PBDEs, 2'-MeO-BDE 68 and 6-MeO-BDE 47, were targeted as well. Both compounds were detected in very low concentrations in maternal tissues and milk with a predominance of 6-MeO-BDE 47, but not in pup serum. This result agrees with a previous study reporting that 6-MeO-BDE 47 was the main congener found in marine mammals from the Northern hemisphere[14].

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