Environment International 46 (2012) 6–15

Contents lists available at SciVerse ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation

Marie Vanden Berghe [a], Liesbeth Weijs [b,c], Sarah Habran [d], Krishna Das [d], Céline Bugli [e], Jean-François Rees [a], Paddy Pomeroy [f], Adrian Covaci [b,c], Cathy Debier [a,*]

[a] Institut des sciences de la vie, Université catholique de Louvain, Croix du Sud 2/L7.05.08, 1343 Louvain-la-Neuve, Belgium
[b] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[c] Ecophysiology, Biochemistry & Toxicology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[d] Laboratoire d'Océanologie, Université de Liège, Allée de la Chimie 3, 4000 Liège, Belgium
[e] Institut de statistique, Université catholique de Louvain, Voie du Roman Pays, 20, 1348 Louvain-la-Neuve, Belgium
[f] Sea Mammal Research Unit, University of St Andrews, St Andrews, Fife KY16 9AJ, Scotland, United Kingdom

ARTICLE INFO

Article history:
Received 9 December 2011
Accepted 26 April 2012
Available online 31 May 2012

Keywords:
Grey seal
Organochlorines
Maternal transfer
Blubber
Milk
Serum

ABSTRACT

Twenty grey seal (*Halichoerus grypus*) mother–pup pairs from the colony of the Isle of May (Scotland) were sampled at early and late lactation in order to study the transfer of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and their metabolites (HO-PCBs and HO-PBDEs) as well as organochlorine pesticides (OCPs), such as DDT and metabolites (DDXs) and hexachlorobenzene (HCB). The transfer of the naturally produced MeO-PBDEs was also investigated. Generally, concentrations (on a lipid weight basis) of the sum of PCBs, PBDEs and DDXs tended to be higher in all tissues at late lactation (for maternal outer blubber ΣPCBs = 3860 ± 2091 ng/g, ΣPBDEs = 120 ± 74 ng/g and ΣDDXs = 559 ± 207 ng/g; for maternal inner blubber ΣPCBs = 4229 ± 3274 ng/g, ΣPBDEs = 148 ± 118 ng/g and ΣDDXs = 704 ± 353 ng/g; for maternal serum ΣPCBs = 1271 ± 796 ng/g, ΣPBDEs = 27 ± 16 ng/g and ΣDDXs = 242 ± 125 ng/g; for milk ΣPCBs = 1190 ± 747 ng/g, ΣPBDEs = 55 ± 36 ng/g and ΣDDXs = 357 ± 160 ng/g; for pup serum ΣPCBs = 1451 ± 901 ng/g, ΣPBDEs = 48 ± 31 ng/g and ΣDDXs = 395 ± 201 ng/g). In all tissues, ΣMeO-PBDEs were found at very low levels or even undetected and their concentrations appeared to increase at late lactation only in maternal inner blubber (2.7 ± 1.3 to 5.3 ± 2.9 ng/g for early and late lactation, respectively) and milk (0.6 ± 0.3 to 1.1 ± 0.5 ng/g for early and late lactation, respectively). The transfer from inner blubber to maternal serum was selective and strongly depended on the log K_{ow} value of the compounds, with less lipophilic compounds being more efficiently released. Only a limited amount of HO-PCBs was transferred during lactation as 4-HO-CB-107 was the only metabolite detected in milk (29 to 40 pg/g lw). On the contrary, most of HO-PCB metabolites found in maternal serum were also detected in pup serum. These findings suggest not only a transplacental transfer of HO-PCBs from mothers to pups but also the possibility of endogenous biotransformation in suckling pups or accumulation of undetectable low amounts from milk.

1. Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) are well known anthropogenic and lipophilic compounds that bioaccumulate throughout the trophic chain. PCB and PBDE metabolites such as hydroxylated PCBs (HO-PCBs) and hydroxylated PBDEs (HO-PBDEs) may have comparable or even higher toxic effects than their parent compounds in mammals, especially on thyroid and vitamin A metabolism (Meerts et al., 2000, 2002). Although differences among species exist, it has been suggested that the formation of HO-PCBs results from the metabolic biotransformation of PCBs by cytochrome P450 enzymes (CYPs) in order to make them more water soluble and thus easier to eliminate. However, due to their high affinity for blood proteins (Letcher et al., 2000), specific metabolites were found to be retained in human (Dirtu et al., 2010) and marine mammal blood (Gabrielsen et al., 2011; Weijs et al., 2009c). By contrast, HO-PBDEs can be either biotransformation products of synthetic PBDEs mediated by CYPs or produced by marine organisms such as sponges or algae (Malmvärn et al., 2005; Vetter et al., 2002; Wiseman et al., 2011). They can also result from the demethylation of other naturally-

* Corresponding author. Tel.: +32 10 47 37 32; fax: +32 10 47 37 28.
E-mail addresses: marie.vandenberghe@uclouvain.be (M. Vanden Berghe), liesbeth.weijs@ua.ac.be (L. Weijs), S.Habran@ulg.ac.be (S. Habran), krishna.das@ulg.ac.be (K. Das), celine.bugli@uclouvain.be (C. Bugli), jf.rees@uclouvain.be (J-F. Rees), pp6@st-andrews.ac.uk (P. Pomeroy), adrian.covaci@ua.ac.be (A. Covaci), cathy.debier@uclouvain.be (C. Debier).

0160-4120/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2012.04.011

produced PBDE analogs, the methoxylated polybrominated diphenyl ethers (MeO-PBDEs), as demonstrated in microsomal fractions of several species of vertebrates (Wan et al., 2009; Wiseman et al., 2011). MeO-PBDEs have already been detected in wildlife and marine mammals at concentrations sometimes greater than PBDEs (Teuten et al., 2005; Wan et al., 2009; Weijs et al., 2009a, 2010).

Because seals are top predators, have a long life span and are characterised by high lipid content (blubber), large concentrations of POPs are found in their tissues, especially in the blubber (Sørmo et al., 2003; Weijs et al., 2009b). When food intake is irregular or nonexistent during breeding, lactation and moulting in most species of seals, important amounts of POPs are mobilised from blubber into the bloodstream, exposing targeted organs to increased concentrations of toxicants (Debier et al., 2006; Vanden Berghe et al., 2010). During lactation, seals produce lipid-rich milk containing high levels of POPs (Debier et al., 2003b; Frouin et al., 2012; Sørmo et al., 2003; Vanden Berghe et al., 2010). Suckling newborns are thus exposed to high amounts of toxic chemicals, while being in a critical developmental period of their life. Previous studies have shown that pup or juvenile seals exposed to POPs, even to low concentrations, had reduced immune competence and impairment of thyroid hormone and vitamin A homeostasis (Hall et al., 2003; Mos et al., 2007; Simms et al., 2000; Sørmo, 2009). The lactational transfer of PCBs and OCPs in seals is more important at late lactation (Debier et al., 2003b). In addition, it appears to be selective, with a preferential transfer of mostly less lipophilic compounds from mother to pup, as shown for grey seals (*Halichoerus grypus*) (Debier et al., 2003a; Pomeroy et al., 1996; Sørmo et al., 2003). The lactational transfer of PBDEs in marine mammals has been much less investigated. Studies on grey seals and hooded seals (*Cystophora cristata*) also report a selective transfer of PBDEs during lactation (Ikonomou and Addison, 2008; Wolkers et al., 2006). However, those studies used a limited number of mother–pup pairs (5 to 6) and sampled only once during the lactation period. Therefore, the temporal trend in the transfer could not be established. To the best of our knowledge, only one recent study examined the changes in PBDE concentrations occurring in tissues of mothers and pups harp seal (*Phoca groenlandica*) sampled twice during the lactation (Frouin et al., 2012). However, as for the previous studies, only a limited number of individuals could be sampled (n = 6). In addition, the authors discussed their results for total PBDEs rather than for each single PBDE congener.

The presence and activities of CYPs in several species of seals (Nyman et al., 2001; Wolkers et al., 2002) suggest that they are theoretically able to produce HO-PCBs and HO-PBDEs. Indeed, HO-PCBs were detected in significant amounts in adult and pup seals (Gabrielsen et al., 2011; Løken et al., 2008). On the contrary, no or only low levels of HO-PBDEs were found in serum or plasma of harbour (*Phoca vitulina*) and ringed (*Phoca hispida*) seals (Routti et al., 2009; Weijs et al., 2009c), suggesting that formation or retention of these molecules is low in these animals. Studies on humans and rodents reported that HO-PCBs were transferred to offspring mainly through the placenta during gestation and to a lesser extent during lactation (Fangstrom et al., 2005; Guvenius et al., 2003; Meerts et al., 2002). However, information concerning the maternal transfer of HO-PCBs and HO-PBDEs in marine mammals is lacking. Gabrielsen et al. (2011) found positive correlations between serum HO-PCB levels and the age and body mass of hooded seal pups. The authors suggested thus a transfer of HO-PCBs via the milk or an endogenous biotransformation of PCBs in pups. Nevertheless, that study did not investigate changes occurring during lactation and the presence of HO-PCBs in milk.

The present study investigated the lactational transfer of several POPs in grey seals. Grey seal females fast during a short lactation period (16 to 21 days), while producing milk rich in lipids (up to 60%) (Iverson et al., 1993; Pomeroy et al., 1996). During that time, females can lose between 25 and 50% of their initial body mass (Iverson et al., 1993; Pomeroy et al., 1999). In contrast, due to the important daily ingestion of milk, suckling pups rapidly gain mass (between 0.8 and 2.8 kg/day) (Iverson et al., 1993; Pomeroy et al., 1999). Maternal blubber, maternal serum, milk and pup serum from 20 mother–pup pairs were collected at early and late lactation in order to examine the changes in levels and profiles of PCBs, PBDEs, their related metabolites (HO-PCBs and HO-PBDEs), OCPs and MeO-PBDEs between early and late lactation. To the best of our knowledge, this is the first study to investigate the levels and profiles of PBDEs, HO-PCBs, HO-PBDEs and MeO-PBDEs in tissues of grey seal mother–pup pairs sampled at different times of the lactation period.

2. Material and methods

2.1. 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. On their arrival on the Isle, females were monitored and dates of birth were recorded by daily observations of the breeding areas. Animals were sampled at early (days 2–5) and at late (days 14–17) lactation in order to collect maternal blubber, serum and milk as well as pup serum samples. Pup blubber samples could not be collected due to permit limitation. However, Frouin et al. (2011) reported a strong relationship between ΣPBDE levels in blubber and serum of harbour, harp and grey seal pups. The investigators concluded that serum was a reliable indicator for ΣPBDE contaminations in seal pups. In addition, another study from the same authors (Frouin et al., 2012) recently measured several POPs in harp seal pup blubber and serum and the concentrations (expressed per unit of lipid weight) reported in both tissues fell within the same ranges. Data concerning pup age as well as body weights of mothers and pups at each capture are presented in Table S1 (supplementary information). Sampling techniques are described elsewhere (Debier et al., 2002, 2003b). Animals were handled and weighed under UK Home Office licence as described in Pomeroy et al. (1996). All the samples were stored at −20 °C until analyses.

2.2. Analysis

In all samples, 35 PCB congeners (IUPAC numbers: CB-18, -28, -44, -47, -49, -52, -87, -95, -99, -101, -105, -110, -118, -128, -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), 6 DDXs (*o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT), HCB and 2 MeO-PBDEs (2′-MeO-BDE-68 and 6-MeO-BDE-47) were targeted. Additionally, twenty HO-PCB congeners were investigated in serum and milk: 3-HO-CB (118, 138, 153, 180), 4-HO-CB (109, 120, 127, 130, 146, 162, 163, 172, 177, 187, 193, 198, 199, 202, 208) and 4-diHO-CB202. Three HO-PBDEs were targeted in all serum and milk samples as well (6-HO-BDE47, 5-HO-BDE47 and 4-HO-BDE49).

The method used for the extraction and clean-up of blubber samples is described in details in Covaci et al. (2008). In brief, approximately 150 mg of each end of the blubber biopsy (outer and inner parts) was homogenised with anhydrous Na_2SO_4 and internal standards (CB 143 and BDE 77) were added. The samples were extracted by hot Soxhlet with hexane/acetone (3/1; v/v) and the remaining extract was cleaned up on acid silica. After elution of the analytes with 20 ml hexane and 15 ml dichloromethane (DCM), the cleaned extract was evaporated and dissolved in 150 μl iso-octane.

Serum samples were analysed as already described in Weijs et al. (2009c) with small adaptations in order to include the analysis of MeO-PBDEs. Briefly, serum (about 1.5 ml) was spiked with internal standards (CB 143 and BDE 77 for compounds eluting in the 1st fraction, e.g. neutrals; 4′-HO-CB 159 and 3-MeO-BDE28 for compounds eluting