Lactational transfer of PCBs and chlorinated pesticides in pups of southern elephant seals (Mirounga leonina) from Antarctica

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1. Introduction

Lactational transfer of persistent contaminants is an important route for marine mammals neonates exposure to lipophilic organochlorine contaminants (Addison and Stobo, 1993; Weisbrod et al., 2000; Metcalfe et al., 2004). Since neonates are at a critical stage in the development of neurological, immune and physiological systems, ingestion of toxic contaminants may have irreversible toxicological impacts. In pup and juvenile seals exposed to lipophilic contaminants, several toxic effects have been reported, including reduced immunocompetence, disruption to thyroid status and vitamin A dynamics, and indirect post-weaning mortality (Reijnders, 1986; De Swart et al., 1992; Simms et al., 2000; Levin et al., 2005; Sermo et al., 2005). In marine mammals, 85–95% of the caloric content in maternal milk is associated with the fat concentration (Kirby and Ortiz, 1994). Contamination of marine mammal neonates through lactational transfer is dependent on the milk fat content, as well as prey selection, previous parity history and the duration of lactation by the dam (Addison and Brodie, 1977; Aguilar and Borrell, 1994).

Pinnipeds and other marine mammals differ from terrestrial ones because their high rates of lactational energy transfer to the young, primarily because of the elevated milk lipid content (Carlini et al., 2000). This also contributes to the transfer of lipophilic contaminants to the young. In cetaceans and pinnipeds, more than 90% of organochlorine contaminants present in neonates are transferred through milk, greatly exceeding gestational transfer before birth (Addison and Stobo, 1993; Borrell et al., 1995). The lactation period of elephant seals is of approximately 23 d. Among the phocids, with the only exception of monk seals, elephant seals are the ones displaying a longer lactation period. Indeed, the lactation of many phocids only lasts a few days. The relative long suckling period of elephant seal pups represents an important source for transfer of lipophilic contaminants through the milk.

The energetics of lactation for southern elephant seals (Mirounga leonina) have been widely studied (Riedman, 1990; Fedak et al., 1994; Carlini et al., 1997, 2000). According to these studies, females fast and can lose about 180–235 kg, i.e. about 35% of their body mass during the lactational period. On average, the fat...
content in *M. leonina* milk corresponds to 47%. Considering that lactating elephant seal females do not travel to the sea for feeding during lactation, all the lipids present in the milk come from the blubber mobilization that can liberate lipophilic contaminants into the serum and then into the milk. While suckling, pups can gain body weight at a rate of around 5–6.5 kg d⁻¹. At the end of the lactation period, the proportion of fat relative to the total body weight in pups is 34.5%, but it is reduced to 29.4% in the post-weaning period. For pups, this not only represents a loss of the energy gained during the maternal lactation period, but also a remobilization of contaminants.

Results from our previous studies showed that pup, juvenile and adult southern elephant seals (*M. leonina*) from a population breeding in Elephant Island (Antarctica) are contaminated with chlorinated compounds (Miranda-Filho et al., 2007). In the present study, we sampled the blubber and milk of dams and the blubber of their pups among this same seal population to evaluate the lactational transfer of PCBs and organochlorine contaminants. According to Reeves et al. (1992), three of the four largest populations of southern elephant seals are in decline (i.e. Heard Island, Kerguelen Island and Macquarie Island populations). The fourth population, living in South Georgia Island, is stable. Although there may be several factors contributing to the reduced number of elephant seals in populations under decline, the involvement of persistent contaminants cannot be ruled out. Actually, samples of blubber, placenta and milk can be used as biomarkers of spatial and temporal trends in contaminant burdens (Tanabe et al., 1994, 2003; Aguilar et al., 2002; Aguilar and Borrell, 2005; Borrell and Aguilar, 2007). Therefore, the data reported in the present study may directly contribute to ongoing studies of contaminant trends in the Antarctic region.

2. Experimental

2.1. Collection of biological samples and morphometric data

Samples of blubber and milk were obtained from live dam/pup pairs of elephant seals as they congregated during the reproductive period. They were captured during the 1999/2000 austral summer, on Shippock Reef and Great Beach, Elephant Island (61°13'S–55°23'W; South Shetlands, Antarctic Peninsula). Pups (n = 7) of both sexes and adult females (n = 7) were anaesthetized as previously described (Miranda-Filho et al., 2007). Morphometric data and blubber samples (~3 g) were collected and stored as previously described (Miranda-Filho et al., 2007). Morphometric data included girth, total length, and weight (see Table S1 – Supplementary Material). Blubber samples were collected with a scalpel and contained the more external layers (outer blubber). Milk samples (~25 mL) were also collected from adult females using a glass syringe after intramuscular injection of oxytocin (50 mL; 50 units). It must be noted that adult females were sampled in a late period of the lactation cycle. Milk samples were stored at −4°C in 30 mL glass containers until analysis. After the field collections, the biological material (blubber and milk) was transferred to the Laboratory in Brazil, where the samples were maintained at −80°C. In the fall of 2001, samples were transferred to Trent University in Peterborough (ON, Canada), where they were stored at −4°C until contaminant analysis.

2.2. Sample preparation and analysis

Sub samples of blubber (0.2 g) and milk (0.5 g) were grounded with granulated anhydrous sodium sulphate, extracted, prepared and analyzed as previously described (Miranda-Filho et al., 2007). Samples were analyzed by high resolution gas chromatography with an electron capture (63Ni) detector (HRGC-ECD) using a Varian model 3500 gas chromatograph equipped with a 60 m DB-5 (J&W) fused silica column (0.25 μm ID, 0.25 μm film thickness) and splitless injection. Analytes measured were identified by retention time. PCB analyzed included IUPAC congeners numbers 18, 28, 31, 33, 44, 49, 70, 74, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 156, 158, 170, 183, 187, 191, 194, 195 and 201. The total PCBs content was calculated as the sum of the detected congeners concentrations. PCB congeners quantification was done by comparison with standards obtained from the National Research Council (Halifax, NS, Canada). Organochlorine measured included hexachlorobenzene (HCB), hexachlorobutadiene, α-HCH, β-HCH, γ-HCH, aldrin, dieldrin, endosulfan, endosulfan sulphate, heptachlor, heptachlor epoxide, endrin, endrin-ketone, mirex, methoxychlor and: (a) p,p'-DDT, p,p'-DDE, p,p'-DDD, p,p'-DDE, p,p'-DDE, the sum of these compounds (ΣDDT); (b) cis- and trans-nonachlor, cis- and trans-chlordane and the sum of these compounds (Σchlordane). Organochlorine compounds quantification was done by comparison with standards obtained from the Wildlife Toxicology and Surveys Branch of the Canadian Wildlife Service (Hull, QC, Canada).

The contaminant concentration in blubber and milk was expressed on a lipid-normalized basis (i.e., ng g⁻¹ lipid). The mean lipid contents in the blubber (dams and pups) and milk samples were 65.9%, 69.6% and 54.7%, respectively. The Limits of Detection (LODs) on a lipid-normalized basis were 0.1–0.3 ng g⁻¹ for the PCB congeners, 0.2–0.3 ng g⁻¹ for mirex, HCHs and chlordanes, and 0.3–0.5 ng g⁻¹ for dieldrin and DDT compounds.

Procedural blanks were prepared to provide quality assurance for GC-ECD analysis. They were carried out after every 7 blubber and milk samples. Cod liver oil reference material (SRM 1588) purchased from the US National Institute of Standards and Technology (NIST) was analyzed for concentrations of PCBs and organochlorines pesticides and concentrations of all analytes measured were within certified values. For PCBs, HCHs, DDTs and chlordane compounds, the mean recovery (±standard deviation) was 74.2 ± 5.4%, 112.2 ± 5.6%, 114.4 ± 3.4%, and 117.4 ± 17.5%, respectively. Despite external standard method was used results described in the present study were not adjusted considering the mean recovery percentages. More information on the performance of the method can be found in Miranda-Filho et al. (2007).

2.3. Data analysis

For all statistical comparisons, lipid-normalized data were used to adjust for differences in the lipid content of the blubber. Data were log-transformed and compared using analysis of variance (ANOVA) followed by the Tukey's test.

Transfer ratio (TR) for each analyte measured was calculated by dividing the compound concentration in the milk by the concentration in the dam blubber (transfer from dam into the milk) or the compound concentration in the pup blubber by the concentration in the dam blubber (transfer from dam into the pup). Thus higher TR values indicate higher transfer rates. Correlation analysis between the log*Kow* (octanol/water partition coefficient) and TR values for PCB congeners and OC pesticides was performed using the linear regression model.

In all cases, the significance level adopted was 95% (P < 0.05). Analyses were performed using the software Statistica® (StatSoft, Tulsa, OK, USA).

The relative biomagnification factors from the dam’s milk to the blubber of the respective pup (lipid normalized) were calculated using the equation for “relative ratios” described by Boon et al. (1994): \( R_{\text{REL}}(\text{analyte}) = R_{\text{analyte, Consumer}}/R_{\text{CB-153}} \). Therefore, concentrations of the different analytes measured were expressed as a proportion of PCB-153, which is the most persistent and thus...
generally the most abundant congener found in biological tissues, as observed in the present study (Table 1). The relative biomagnification factor (BF) from prey/food source to the consumer was then calculated using milk as the food source (diet) and pup’s blubber as the consumer. Thus a BF value > 1 indicates biomagnification from milk to pup blubber (Beckmen et al., 1999).

3. Results and discussion

The analytical data obtained show that a range of PCB congeners (Table 1) and OC compounds (Table 2) were detected in blubber and milk samples collected from dam/pup pairs of southern elephant seals at the Elephant Island (Antarctica). As earlier reported for the same population of seals (Miranda-Filho et al., 2007), concentrations of most of the analytes measured followed the pattern: dam blubber > milk.

Concentrations of PCBs and DDT and its metabolites in elephant seal tissues were typically 1–3 orders of magnitude lower than those reported in blubber of pinnipeds from the northern hemisphere. In this case, concentrations of PCBs are typically similar to those of DDT compounds, reflecting the importance of industrial sources of PCBs in developed countries from the northern hemisphere. In southern elephant seal tissues, the relative concentrations of the analytes measured followed the pattern: \( \Sigma DDT > \text{mirex} > \Sigma PCB > \text{Echlorodane} > \text{HCB} > \text{heptachlor epoxide} > \text{dieldrin} > \text{methoxychlor} > \Sigma \text{CH} > \text{other organochlorines}. \) In blubber and liver samples of the California sea lion (Zalophus californianus) and the northern elephant seal (Mirounga angustirostris), which are pinnipeds from the northern hemisphere, the relative concentrations of persistent organochlorines followed the pattern: \( \Sigma DDT > \Sigma PCB > \text{Echlorodane} > \Sigma \text{CH} > \text{HCB} \) (Kajiwara et al., 2001). The importance of mirex, heptachlor epoxide, dieldrin and methoxychlor as contaminants in the southern elephant seals from Antarctica relative to the contaminant patterns for pinnipeds from the northern hemisphere may reflect contamination from pesticides that are currently used or were historically used for agricultural purposes in the southern hemisphere. On the other hand, a very high DDE/\( \Sigma DDT \) ratio in the blubber of dams (0.89; Table 2) and pups (0.91; Table 2) is an indicative of long-term, extremely distant pollution.

The lipid-normalized concentrations of most of the analytes measured were significantly lower in samples of maternal milk and pup blubber than in those of maternal blubber (Tables 1 and 2). The relative lipid-normalized concentrations for almost all analytes measured followed the pattern: dam blubber > pup blubber > milk. The differences are particularly apparent for some of the highly chlorinated PCB congeners (Table 1, see also Fig. S1 – Supplementary Material), DDT isomers (Table 2, see also Fig. S2 – Supplementary Material), cyclodiene pesticides and mirex (Table 2, see also Fig. S3 – Supplementary Material), and HCB (Table 2, see also Fig. S4 – Supplementary Material). The mean levels of PCB to octachlorobiphenyls) were consistently detected in the samples, and the concentrations of these PCBs were relatively low (Table 1).
congeners and mirex in milk and pup blubber were generally less than 50% of the concentrations in maternal blubber. However, the mean concentrations of the other OC pesticides detected in milk and pup blubber were generally only slightly lower than those in maternal blubber (Table 2). This pattern of lower lipid-normalized concentrations of contaminants in milk relative to the dam is consistent with the literature on the levels of lipophilic contaminants in the milk of phocid seals (Bacon et al., 1992; Kleivane et al., 1997; Beckmen et al., 1999; Wolkers et al., 2002; Debier et al., 2003).

The profile of PCB congeners in the blubber and milk samples of southern elephant seals was similar to those reported for other marine mammals (Boon et al., 1994; Kannan et al., 1994; Thôme et al., 2002), being dominated by hexachlorobiphenyl congeners (i.e., 153, 138 and 128), heptachlorobiphenyl congeners (170, 180 and 187) and the pentachlorobiphenyl congener 118. In harp seals (Phoca groenlandica) from the Gulf of St. Lawrence in Canada, PCB congeners 153, 180, 87, 99 and 138 represented about 55% of the total PCBs measured in the milk and pup blubber (Wolkers et al., 2002). In Antarctic fur seals (Arctocephalus gazella), PCB congeners 99, 118, 153, 138, 180 and 187 were detected in milk samples (Bacon et al., 1992). In the present study with southern elephant seal (M. leonina), PCB congeners 118, 153, 138, and 180 represented approximately 78% of total PCBs detected in the milk, and about 54% of total PCBs detected in the blubber of pups and dams.

PCB congeners with higher degrees of chlorination (i.e. >4 chlorines) were detected in the milk and blubber samples of M. leonina. As the more highly chlorinated PCBs are the most lipophilic, with log Kow values > 6.5 (Mackay et al., 2000), one expect that these congeners would be present at the highest concentrations in the blubber reserves. During the fasting period, dam remobilizes these reserves into the circulatory system, which are late transferred to the milk (Sørmo et al., 2003). Results from the present study confirm previous observations that PCB congeners detected in marine mammals are predominantly those with higher degrees of chlorination, relative to profiles in terrestrial ones that includes less chlorinated PCB congeners (Aguilar and Borrell, 1988, 1994; Debier et al., 2003). In addition, pinnipeds have a higher capacity for hepatic metabolism than cetaceans, resulting in a greater degree of metabolic biotransformation of the less chlorinated PCB congeners (Tanabe et al., 1988). PCB congeners without chlorination in adjacent meta and para positions (i.e. congeners 44, 49, 52, 101, 110, 149 and 151) were not detected in the samples from M. leonina, which is consistent with the PCB patterns described for other marine mammals (Boon et al., 1994). These congeners are eliminated because of the relatively rapid rates of biotransformation mediated by cytochrome P450 (CYP450) enzymes (Borlakoglou et al., 1988). The rapid metabolism of these congeners is a consequence of the induction of metabolic enzymes from the CYP1A1 subfamily (Van den Brink et al., 2000).

As shown in Tables 1 and 2 (see also Figs. S1 and S2 – Supplementary Material), transfer of PCB congeners from dams to pups was proportionally less (mean TR = 0.54) than that of DDT compounds (mean TR = 0.62). DDT metabolites (i.e. p,p’-DDE, p,p’-DDD, o,p’-DDD, o,p’-DDE) showed higher transfer rates than the DDT isomers. In comparison to the highly chlorinated PCBs, the DDT compounds have lower molecular weights and are less lipophilic, with log Kow values < 6.5 (Mackay et al., 2000). Consequently, a higher lactational transfer of DDT compounds relative to PCBs might be expected in marine mammals (Addison and Brodie, 1987; Aguilar and Borrell, 1994). In ringed seals (Phoca hispida) from the Russian Arctic, the degree of lactational transfer of chlorinated contaminants was estimated as 38% for DDTs, 25% for PCBs and 30% for chlordanes (Nakata et al., 1998).

Compared to the levels of contaminants observed in the blubber of M. leonina dams, there were relatively high concentrations of chlordane compounds (Table 2, see also Fig. S3 – Supplementary Material), as well as hexachlorobutadiene, dieldrin, HCH, endrin-ketone, HCB and methoxychlor (Table 2, see also Fig. S4 – Supplementary Material) in the maternal milk and pup blubber. In fact, the higher transfer rates of OC pesticides from the dam blubber to the pup blubber, excluding DDT isomers and their metabolites, were observed for hexachlorobutadiene (1.1), heptachlor epoxide (0.85), dieldrin (0.85), α-HCH (0.72), endrin-ketone (0.71), trans-nonachlor (0.70), HCB (0.65) and methoxychlor (0.64). It is important to note that the mean transfer rate for all OC pesticides analyzed was 0.66 (Table 2). In polar bears (Ursus maritimus), it was also detected similar levels of contamination for chlordanes, HCHs and HCB in pups and milk samples (Bernhoft et al., 1997). In harp seal (P. groenlandica) and hooded seal (Cystophora cristata) from the Arctic region, it was observed that HCHs and HCB are efficiently transferred through maternal milk into the pups (Espelund et al., 1997). HCB was considered the most highly transferred organochlorine from females of the odontocete cetacean, Phocoenoides dalli (Kajiwara et al., 2002). In harp seals samples from the Gulf of St. Lawrence, high concentrations of HCB and γ-HCH were detected in blubber of seal pups, relative to the concentrations found in the dams. Also, trans-nonachlor was the predominant compound amongst the chlordane analytes measured in blubber of dams, blubber of pups, and in the milk, corresponding to 44%, 32% and 40% of the total chlordanes, respectively (Wolkers et al., 2002). In the present study, trans-nonachlor was also the predominant chlordane compound in M. leonina, showing a relatively high transfer rate (0.70). However, its concentration corresponded to 66.3%, 77.9% and 49.5% of the total chlordanes detected in blubber of dams, blubber of pups and milk, respectively.

In the present study, mirex was detected in much higher concentrations in M. leonina dams than in pups and milk (Table 2, see also Fig. S3 – Supplementary Material). The relatively low rate of mirex lactational transfer may be due to its high molecular weight and high lipophilicity, as indicated by the high log Kow value of 6.8 (Mackay et al., 2000). In female harbor seals (Phoca vitulina) and grey seals (Halichoerus grypus), mirex was present at relatively high concentrations because of the low rates of lactational transfer observed (Bernt et al., 1999).

In a broad view, results from the present study clearly indicate that there are significant differences in the degree of lactational transfer of PCBs and OC pesticides from the dams of southern elephant seals to the pups. It is also clear from the discussion above that the transfer rate of the analytes measured is dependent on their lipophlicity. In fact, a significant inverse correlation was observed between the log Kow values and the transfer rate from dam into the milk for all PCBs analyzed and OC pesticides showing log Kow values > 4.7 (R² = 0.54; Fig 1A). Also, a very significant inverse correlation was observed between the log Kow values and the transfer rate from dam into the pup for all PCBs analyzed and OC pesticides showing log Kow values > 4.7 (R² = 0.70; Fig 1B). In both cases, the transfer rates of the OC pesticides showing log Kow values < 4.7 (endosulfan sulphate, HCHs, methoxychlor, and endrin-ketone) were not dependent on their lipophlicity and were in most cases around 0.6 (Fig 1). These findings clearly indicate that the different patterns of transference showed by organochlorine compounds with log Kow values < 4.7 and those with log Kow values > 4.7 are strictly associated with the partitioning of the contaminants into and out of lipid reserves and their transport across epithelial and endothelial membranes in the dam body during the lactational transfer.

When analyzing the potential for biomagnification as a result of the lactational transfer of PCB congeners and OC compounds from milk into the pups, most of the analytes measured had mean biomagnification factors close to 1.0, indicating that no significant
Biomagnification was observed (Tables 1 and 2). However, there were some exceptions, such as for PCB congener 201 (3.59) (Table 1), γ-HCH (2.11) (Table 2), and α,p'-DDE (6.09) (Table 2), where an important biomagnification process was observed. The mechanisms explaining why some lipophilic contaminants are biomagnified through trophic transfer in food chains are still being investigated (Mackay and Fraser, 2000; MacDonald et al., 2002). Therefore, it is difficult to speculate on why these contaminants show potential for biomagnification in the blubber of pup seals through the maternal milk. However, there are several steps that involve partitioning of contaminants into and out of lipid reserves, and their transport across epithelial and endothelial membranes during the lactational transfer. These toxicokinetic processes may provide opportunities for biomagnification of highly lipophilic compounds.

It was estimated that about 98% of the contaminant residues in grey seal pups sampled at weaning came from maternal lactational transfer (Addison and Stobo, 1993). Furthermore, significant amounts of PCBs and DDT compounds were detected in tissues of newly born grey seal pups (Jennsen et al., 1996). Data from the present study show that there is significant accumulation of organochlorine residues in southern elephant seal pups as a result of lactational transfer of these compounds from the mother. The mean transfer rate for PCBs and OC pesticides was 0.63. Exposure to these contaminants at early life stages may have toxic impacts. The impact of these contaminants on health and development of southern elephant seal pups is currently unknown, but it is known that these persistent contaminants may produce subtle biological effects, even at low concentrations (Beckmen et al., 2003; Sørmo et al., 2005).

Fig. 1. Relationship between the octanol/water partition coefficient (log $K_{ow}$) values and the transfer rate of organochlorine pesticides and PCB congeners from the dam blubber into the milk (A) or into the pup blubber (B) in southern elephant seals (Mirounga leonina) from Elephant Island (Antarctica). Values of log $K_{ow}$ were compiled from EPA (1998) and Hansen et al. (1999).


