BLUBBER MOBILIZATION OF PBDES IN NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*) DURING THE POST-WEANING FASTING DURATION – COMPARISON WITH PCBS

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Introduction

Northern elephant seals (NES) (*Mirounga angustirostris*) face a large risk for exposure to environmental toxic fat-soluble molecules such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), among others because of their high trophic-chain position. During lactation, high concentrations of pollutants are transferred from maternal blubber stores to the pup through the consumption of lipid-rich milk[1, 2]. Upon weaning, NES pups fast and mobilize primarily lipids from their large adipose tissue stores. This developmental fasting strategy also releases lipophilic contaminants into the pup's circulation. The presence of these circulating PCBs and PBDEs might generate potential toxic effects[3, 4].

Previous studies elucidated the dynamics of mobilization of PCBs from blubber to blood during NES's post-weaning fast. PCBs remained preferentially in the adipose tissue stores during the first part of the fast before being released in high concentrations at the end of the fasting period[1]. The phenomenon has never been investigated for PBDEs in weaned NES. Frouin et al.[5] showed that the PBDE contents in blubber and serum are strongly correlated in harbour, grey and harp seal pups. Furthermore, as a possible consequence of lactation, juvenile harbour seals and harbour porpoises have shown higher levels than adults[6]. However, little is known about the mobilization of PBDEs during energetic deprivation stages.

The objectives of this work were to investigate the levels and the profiles of PBDEs in serum and blubber (by differentiating inner blubber from outer blubber) of NES in order to understand the dynamics of mobilization of those pollutants and compare them to PCBs during post-weaning fast periods.

Materials and methods

Seal sampling. The study took place at Año Nuevo State Reserve, CA, USA (37°06'30''N, 122°20'10''W) during 2010 (January – April). All work was conducted under the National Marine Fisheries Service Marine Mammal Permit #87-1743-05. Twenty-two pups were longitudinally studied at 1-, 4-, 7- and 10-week post-weaning (later assigned early, middle, late and very late fast, respectively). Each animal was marked with dye and was followed by observing the colony each day. NES were initially immobilized with an intramuscular injection of Telazol at a rate of 1 mL per 100 kg of estimated body mass and sedation was maintained through subsequent intravenous injections of Ketamine (All drugs from Fort Dodge Animal Health, Fort Dodge, IA, USA) as needed. At each capture, blood was collected from the extradural vein into Vacutainer tubes (Becton-Dickinson, New Jersey, USA). Samples were centrifuged for 15 min at 4°C and serum was aliquoted into microtubes and stored at -20°C until analysis. At the first three captures, blubber samples were taken after subcutaneous injection of lidocaine for local anaesthesia. A blubber biopsy extending the full depth of the fat layer was taken in the lateral pelvis area using 6-mm biopsy punches (Acu-punch, Acuderm Help Medical, France) and stored in aluminium foil at -20°C until analysis.

Chemical analysis. Blubber biopsies were cut into three equal parts. Approximately, 0.130 mg of inner (closest to the muscle) and outer (closest to the skin) layers were analyzed separately as well as 1.5 mL of serum. In all

samples, 30 PCB congeners (IUPAC numbers: CB 28, 47, 49, 52, 74, 95, 99, 101, 105, 110, 118 , 128, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 199, 206 and 209) and 6 PBDEs (IUPAC numbers: BDE 28, 47, 99, 100, 153 and 154) were targeted. Additionally, 12 HO-PCBs (4-HO-CB107, 4-HO-CB120, 4'-HO-CB127, 4'-HO-CB130, 4-HO-CB138, 4-HO-CB146, 4-HO-CB162, 4-HO-CB163, 4'-HO-CB172, 4-HO-CB187, 4-HO-CB193, 4-HO-CB208) were analysed in the serum. Details of the preparation, extraction, clean-up, quality control and chromatography analysis are described elsewhere[6,7].

Statistics. Results were analysed using SAS Enterprise Guide 4.1. Linear mixed models were used to test differences in contaminant concentrations between each of the fasting periods. The level of statistical significance was set at $p \leq 0.05$. For a defined fasting period, results were analysed using an ANOVA on SAS 9.3 as well as Tukey's test ($\alpha = 0.05$).

Results and discussion

PBDEs profiles throughout fasting period. Weaned NES are marginally contaminated by PBDEs (Table 1 and Table 2). Total concentrations did not exceed 73 ng/g lipids in blubber and 400 pg/mL wet weight in serum for the most contaminated animal. In all compartments, BDE-47 was predominant, representing over 80% of total amount in blubber and over 95% in serum, followed by BDE-99 and BDE-100 congeners. BDE-28 and BDE-153 were not detected in any compartment, whereas BDE-154 was measured in very low concentrations in blubber and only in some serum samples. Indeed, this profile is representative of main commercial mixtures of PBDEs and bioaccumulative congeners[8].

Weaned NES seemed to be consistently less contaminated than Pacific harbour seals and California sea lions (2000 ± 2000 ng ∑PBDE /g lipids and 51,000 ± 75,000 ng ∑PBDE /g lipids, respectively)[9, 10]. BDE-47 levels increased significantly in the serum ($p < 0.01$) during the fast as well as in the inner blubber layer ($p < 0.05$). BDE-47 concentrations in the outer blubber layer remained constant between early and middle fast ($p > 0.05$) and increased at late fast ($p < 0.05$). At early fast, the concentrations of total PBDEs in inner blubber did not significantly differ from those in outer blubber. However, at the end of the fast, the concentrations became significantly higher in inner blubber layer (Table 3). This suggests a more prominent mobilization of lipids close to the muscle than to the skin and a less efficient mobilization of PBDEs from blubber as compared to fatty acids.

Table 1. Mean ± standard deviation (ng/g lipids) of PBDE congeners and PCB congener groups measured in blubber of weaned NES.

	Outer blubber			**Inner blubber**		
	Early fast	*Middle fast*	*Late fast*	*Early fast*	*Middle fast*	*Late fast*
Lipid content (%)	74 ± 12 [a]	84 ± 2 [b]	85 ± 3 [b]	85 ± 4 [a]	87 ± 3 [a]	85 ± 4 [a]
BDE 28	N.D	N.D	N.D	N.D	N.D	N.D
BDE 47	8 ± 6 [a]	9 ± 5 [a]	10 ± 6 [b]	9 ± 5 [a]	11 ± 8 [b]	16 ± 11 [c]
BDE 99	1 ± 1 [a]	N.D	1 ± 0 [a]	N.D	1 ± 0 [a]	1 ± 1 [b]
BDE 100	1 ± 1 [a]	1 ± 1 [b]	1 ± 1 [c]	1 ± 1 [a]	1.0 ± 1 [b]	2 ± 2 [c]
BDE 153	N.D	N.D	N.D	N.D	N.D	N.D
BDE 154	1 ± 1 [a]	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]	1 ± 0 [a]
Σ PBDEs	**11 ± 7 [a]**	**10 ± 6 [a]**	**13 ± 8 [b]**	**10 ± 6 [a]**	**13 ± 10 [b]**	**19 ± 13 [c]**
Σ tri-CBs	4 ± 4 [a]	3 ± 3 [a]	4 ± 3 [a]	3 ± 3 [a]	1 ± 3 [b]	2 ± 3 [a]
Σ tetra-CBs	19 ± 6 [a]	23 ± 8 [a]	25 ± 6 [a]	21 ± 5 [a]	27 ± 8 [b]	34 ± 9 [c]
Σ penta-CBs	112 ± 41 [a]	154 ± 82 [a]	192 ± 58 [a]	115 ± 34 [a]	193 ± 107 [b]	274 ± 87 [c]
Σ hexa-CBs	218 ± 64 [a]	302 ± 159 [a]	380 ±109 [a]	226 ±59 [a]	382 ± 212 [b]	557 ± 180 [c]
Σ hepta-CBs	64 ± 22 [a]	93 ± 56 [a]	126 ± 39 [a]	68 ± 21 [a]	126 ± 78 [b]	184 ± 65 [c]
Σ octa-CBs	3 ± 1 [a]	4 ± 2 [a]	5 ± 2 [a]	3 ± 1 [a]	6 ± 4 [b]	10 ± 4 [c]
Σ PCBs	**419 ± 131 [a]**	**580 ± 304 [a]**	**732 ± 212 [a]**	**436 ± 118 [a]**	**735 ± 406 [b]**	**1060 ± 341 [c]**

For a defined tissue, values within a row followed by different letters are significantly different ($p \leq 0.05$).
N.D = Not detected

Table 2. Mean ± standard deviation (pg/mL) of PBDE congeners, PCB congener groups and HO-PCB congener groups measured in serum of weaned NES.

	Serum			
	Early fast	*Middle fast*	*Late fast*	*Very late fast*
Free fatty acids (mg/ml)	0.4 ± 0.1 [a]	0.6 ± 0.2 [b]	0.6 ± 0.2 [b]	0.8 ± 0.2 [c]
BDE 28	N.D	0 ± 1	N.D	N.D
BDE 47	66 ± 48 [a]	81 ± 53 [b]	93 ± 60 [c]	102 ± 33 [d]
BDE 99	2 ± 7 [a]	0 ± 1 [a]	0 ± 1 [b]	1 ± 3 [a, b]
BDE 100	3 ± 5 [a, c]	1 ±4 [b]	2 ± 7 [b]	2 ± 2 [a, c]
BDE 153	N.D	N.D	N.D	N.D
BDE 154	N.D	N.D	N.D	0 ± 1
Σ PBDEs	**72 ± 53 [a]**	**84 ± 61 [b]**	**96 ± 69 [c]**	**108 ± 38 [d]**
Σ tri-CBs	149 ± 109 [a]	179 ± 115 [b]	4 ± 10 [a]	11 ± 30 [c]
Σ tetra-CBs	510 ± 259 [a]	620 ± 280 [a]	274 ± 61 [b]	334 ± 134 [c]
Σ penta-CBs	1033 ± 232 [a]	1392 ± 368 [b]	1644 ± 391 [c]	1805 ± 677 [c]
Σ hexa-CBs	1641 ± 331 [a]	1979 ± 430 [b]	2601 ± 606 [c]	2763 ± 1025 [d]
Σ hepta-CBs	380 ± 139 [a]	362 ± 87 [a]	529 ± 158 [b]	572 ± 162 [c]
Σ octa-CBs	7 ±13 [a]	0± 0 [a]	4 ± 7 [a]	12 ± 8 [b]
Σ PCBs	**3723 ± 825 [a]**	**4533 ± 1179 [b]**	**5058 ± 1189 [c]**	**5850 ± 1416 [d]**
HO-penta-CBs	166 ± 68 [a]	183 ± 71 [b]	222 ± 91 [c]	365 ± 123 [d]
HO-hexa-CBs	94 ± 25 [a]	95 ± 27 [a]	101 ± 28 [a]	138 ± 23 [b]
HO-hepta-CBs	21 ± 4 [a]	20 ± 5 [a]	22 ± 5 [a]	25 ± 4 [b]
Σ HO-PCBs	**281 ± 9 [a]**	**299 ± 94 [b]**	**346 ± 112 [c]**	**529 ± 140 [d]**

Values within a row followed by different letters are significantly different ($p \leq 0.05$).
N.D = Not detected

Table 3. Results of ANOVA test comparing lipid normalized concentrations of total PBDEs and total PCBs between two compartments for one period of fasting. " *** " means a significant difference ($p < 0.05$); " / " means a no significant difference. O: outer blubber layer; I: inner blubber layer; S: serum.

	Early fast		*Middle fast*		*Late fast*	
Compartments	O - I	I -S	O -I	I - S	O - I	I - S
Σ PBDEs	/	***	/	***	***	***
Σ PCBs	/	/	/	***	***	***

PCBs profiles throughout fasting period. Total concentrations of PCBs found in both tissues were of the same order of magnitude as previously observed[1]. Despite this, weaned NES were marginally contaminated compared to other marine mammals, such as CA sea lions which had concentrations up 300 µg/g lipids[11]. Concentrations of PCBs in weaned NES were significantly raised in inner blubber layer and in serum during the fast while it remained constant in outer layer (Table 1 and Table 2). The PCB profiles of blubber and serum were mainly composed of tetra-, penta-, hexa- and hepta-CBs. Octa-CB congeners were quantified in only small concentrations in both tissues. CB-153 – which is the most persistent congener – was the major congener in all compartments, followed by CB-138 and CB-118.
The dynamics of PCBs throughout the fast were similar to those of PBDEs. Total PCBs were homogeneously distributed in the blubber at early fast. However, at late fast, the levels of PCBs became significantly higher in the inner blubber, suggesting a concentration of PCBs in the remaining amount of blubber with the progression of the fast. As already observed with PBDEs, a rise of PCBs in serum was detected throughout the fast (Table 2), suggesting that, even if PCBs are less efficiently released from blubber than lipids, their mobilization from this tissue remains significant.

PCBs metabolites levels throughout fasting period. Total hydroxylated metabolites assessed in serum increased significantly across the fasting duration. The dominance of the penta-, hexa- and hepta-HOCB is clearly established. HO-PCBs seemed to be accumulated in serum during the fasting confirming their hydrophilic behaviour.

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