Assessment of prenatal exposure to POPs during second trimester using mother-fetus pairs of finless porpoises (*Neophocaena asiaeorietalis*)

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Introduction

Marine mammals receive large burden of organic contaminants including persistent organic pollutants (POPs) because of their high trophic level, long life span, and low metabolic activity. During the life time of marine mammals (from neonates to adults), POPs are steadily accumulated with age resulting in higher concentrations of POPs in mature specimens. However, mature females showed relatively lower residue level of POPs. The reason for this difference is known as the contaminants transfer to fetus and reduction during gestation and lactation. Prenatal exposure to POPs is severe problem because of vulnerability and low metabolic capacity of fetus. Several studies have reported that prenatal exposure of polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDTs) can cause adverse effects to fetal development[1-3]. Therefore, understanding the extent and mechanism of maternal transfer of POPs to fetus during the gestational period is important. Finless porpoises (*Neophocaena asiaeorientalis*) are small cetacean species living near coastal regions (< 5 km). In our study, four mother-fetus pairs of finless porpoises were collected as by-caught during their breeding season to investigate maternal transfer of POPs in early pregnant status. Until now, several studies investigated maternal transfer of POPs using mother-fetus pairs of cetaceans and pinnipeds. However, to our knowledge, only a few studies assessed the early-life exposure (< 6 months) to POPs and influence of parity and gestation period to POP accumulation for mother-fetus pairs. In our study, we investigated the body distribution of POPs in mature female and their fetus, assessed the process of maternal transfer of POPs during gestation period (2nd trimester), and calculated the partitioning of POPs between blubber of mother-fetus pairs. Considering physiological similarity of marine mammals with human, our result can be utilized for understanding early-life prenatal transfer and exposure to POPs for humans.

Materials and methods

Sampling information

In our study, four pairs of mother-fetus finless porpoise (*Neophocaena asiaeorientalis*) were collected as a by-caught from Yellow Sea of Korea during August 2016. After biometric measurement including body length and body weight, samples were moved and dissected in Cetacean Research Institute (CRI). Detailed biological information is presented in Table 1. According to previous study, the fetal growth rate of finless porpoises is estimated as 7.64 cm/month assuming the 11 months as a gestation period[4]. Considering the growth rate, the gestation periods of our samples were calculated between 3.6 and 6.5 months. From all mother samples, fourteen organs were collected (n=67; blubber, muscle, melon, liver, stomach, kidney, brain, heart, lung, intestine, mammary gland, pancreas uterus and fetal sac) and ovary and cord were also collected from two specimens. For fetus, blubber, kidney, stomach, liver, ovary, heart, lung and brain were collected from all specimens as common organs (*n*=36).

Chemical and instrumental analysis

The concentrations of PCBs (25 congeners), OCPs (17 compounds), and PBDEs (22 congeners) were measured following method described elsewhere[5,6]. Briefly, 0.4−2.0 g of each organs were homogenized with anhydrous Na_2SO_4 and extracted using Soxhlet apparatus during 12 h with dechloromehtane (DCM):hexane. The extracts were concentrated to 11 mL, and 1 mL was taken for measuring lipid contents. After removing lipid with gel permeation chromatography (GPC), ^{13}C-labelled internal standards were spiked and cleaned-up with multi-layer silica gel column with 150 mL of 15% DCM in hexane. After clean-up, eluents were concentrated and dissolved in 100 μL of nonane for instrumental analysis. The analysis of PCBs and OCPs was performed by gas chromatography equipped with mass spectrometer (GC-MS) with positive electron ionization mode. For PBDEs, GC-MS was operated with negative chemical ionization mode. The limit of quantification (LOQ) ranged from 0.01 to 0.5 ng/g for PCBs, 0.1 to 3.0 for OCPs and 0.01 to 1.5 ng/g for PBDEs. Average recoveries for internal standards were 90 ± 20% for PCBs, 77 ± 14% for BDE 77 and 51 ± 30% for $^{13}C_{12}$-BDE 209.

Table 1. Biological information of four mother-infant pairs of finless porpoises analyzed in this study

	CRI6269		CRI6270		CRI6271		CRI6272	
	Mother	Fetus	Mother	Fetus	Mother	Fetus	Mother	Fetus
Body length (cm)	145[a]	44	130	28	140	50	153	38
Body weight (kg)	46	1.11	35	0.41	44	1.06	42[a]	0.88
Parity	> 3	−	> 7	−	> 1	−	> 10	−
Gestational period[a]	5.8 months		3.7 months		6.5 months		4.9 months	
lipid (%)	0.6−59	0.2−34	1.3−60	0.01−3.2	0.7-47	0.1−26	0.8−66	0.02−14

[a] Estimated value

Results and discussion:

Concentrations of POPs in mother-fetus pairs

The total concentrations of PCBs (∑PCBs), OCPs (∑OCPs), and PBDEs (∑PBDEs) in common organs from mothers (sum of blubber, kidney, stomach, muscle, liver, heart, mammary gland, lung, intestine, pancreas, uterus and brain) and fetus (sum of blubber, kidney, stomach, liver, gonad, intestine and brain) pairs are presented in Figures 1 and 2. Among analytes, CB 101, CB 153, CB 187, *p,p'*-DDE, *o,p'*-DDT, HCB, BDE 28, BDE 47, and BDE 71 were detected over 50% of all the organs analyzed in mothers and fetuses. This result indicates widespread distribution of these contaminants throughout all organs in mother-fetus pairs. The average concentrations of ∑DDTs (4900 ng/g lipid and 890 ng/g wet for mother; 3500 ng/g lipid and 150 ng/g wet for fetus) of four mothers and fetuses were the highest, followed by ∑PCBs (1500 ng/g lipid and 240 ng/g wet for mother; 950 ng/g lipid and 45 ng/g wet for fetus), HCB, ∑PBDEs, and ∑CHLs, indicating wide contamination of these contaminants despite of continuous regulation. The total POP concentrations (wet weight basis) of mothers showed 1.0 (∑PBDEs) to 59 (∑DDTs) times higher than those measured in paired fetus. Considering lipid contents, concentrations of HCB and ∑PBDEs in two fetuses were higher than their mothers. The detection and comparable concentrations of POPs found in earlier fetuses (3−6 months) suggest prenatal exposure to POPs occurred from relatively early stage of gestation.

Body distribution of POPs in mother-fetus pairs

Among organs, POPs are mainly accumulated in blubber because of their highly lipophilic properties. To investigate the body distribution of POPs in mothers and fetuses, relative contribution of POPs in each organ samples was calculated. For comparison, we used sum concentrations of CB 101, CB 153, CB 187, *p,p'*-DDE, *o,p'*-DDT, HCB, BDE 28, BDE 47, and BDE 71, which were frequently detected (> 50%). In mothers, approximately 62% of POPs are accumulated to blubber, followed by mammary gland (14%) and mesenteric lymph node (6%), while other organs showed lower contribution (< 5%). In fetuses, three specimens showed similar proportions with the highest contribution of blubber (> 90%), indicating blubber layer as a role of POPs reservoir. Other specimen (CRI 6270), which has the smallest body size, showed relatively lower contribution of blubber (56%). The gestational period of CRI 6270 was estimated to be 3.7 months, which is regarded as earlier stage to build a blubber layer. In CRI 6270, other organs showed higher contribution than other fetuses including brain (10%), liver (10%), gonad (7.5%), and kidney (6.3%). This result indicates that the POPs in younger fetuses are randomly distributed throughout the organs. Considering the vulnerability of fetuses, early exposure to POPs could be associated to adverse health effects.

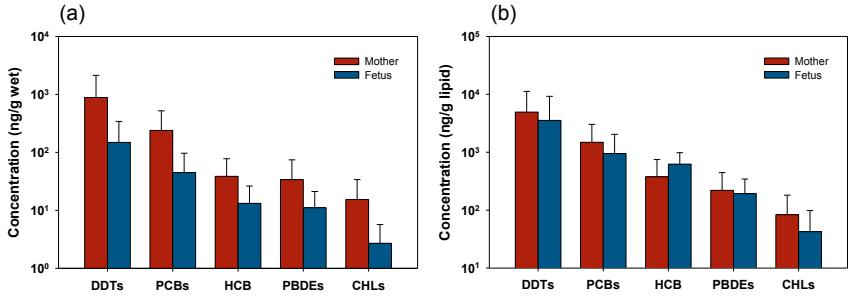

Figure 1. Average concentrations (± standard deviation) of total DDTs, PCBs, HCB, PBDEs, and CHLs in common organs from four mother-fetus pairs. (a) wet weight basis; (b) lipid weight basis.

Association between POPs and parity/gestational period

In our study, parity and gestational period were recorded from each mother-fetus sample. To assess the influence of parity and gestational period for POP accumulation, the blubber concentration of mother-fetus pairs was used because blubber occupies higher contribution to total POP concentrations (Figure 2). Among mothers, CRI 6271 with the lowest number of pregnancy (n=1) showed the highest POP concentrations (Figure 2a). The concentrations of PCB and PBDE congeners were decreased with increasing parity, while the concentrations of OCPs were similar in CRI 6270 and CRI 6272. The decreasing slope ranged from -0.15 for BDE 28 to -116 for *p,p'*-DDE, suggesting *p,p'*-DDE is preferentially eliminated through delivery. For fetuses, we compared the POP concentrations with gestational period. The concentrations of POPs in CRI 6271 (6.5 months), which has the longest gestational period, showed the highest concentrations for all POP compounds except BDE 71. Overall, the concentration of POPs was increased with increasing gestational periods, suggesting time-dependent transfer of POPs from mothers to fetus during pregnant. The clear decreasing slopes were found in *p,p'*-DDE (slope: 75), *o,p'*-DDT (19), HCB (9.2), and BDE 47 (2.6) along with increasing gestational periods. The CBs 101 and 153 did not show any trend among CRI 6270, 6272 and 6271, while CB 187 was decreased. Through this result, it can be concluded that maternal transfer of POPs is determined by parity, gestational period, and physico-chemical properties of compounds, simultaneously.

Maternal transfer of POPs in mother-fetus pairs

To identity the relationship between physico-chemical properties of POPs and maternal transfer, the fetus-mother ratio (F-M ratio; blubber concentration) was compared with molecular weight and log K_{ow} value. Among four mother-fetus pairs, CRI 6270 and CRI 6271 were selected because they have the shortest and longest gestational periods, respectively (Figure 3). As molecular weight increases, both specimens showed decreasing F-M ratio until 400 Da and slightly increased. According to Figure 3b, CRI 6270 showed decreasing F-M ratio with increasing log K_{ow} while CRI 6270 did not show any trend. Through this result, it can be suggested that growing placenta may play a role as a barrier between mother and fetus with increasing gestational periods.

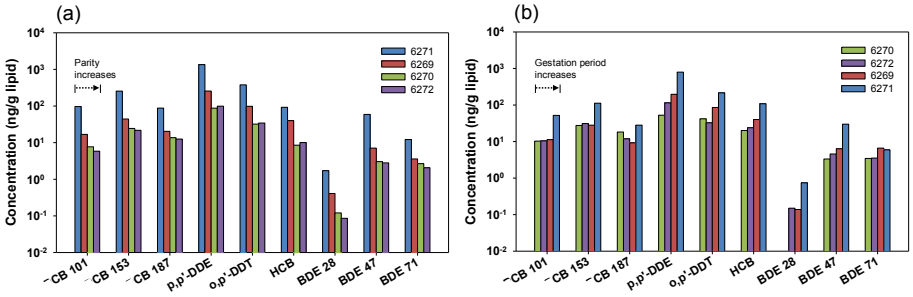

Figure 2. Concentration of predominant compounds of POPs in each specimen among (a) mothers and (b) fetuses

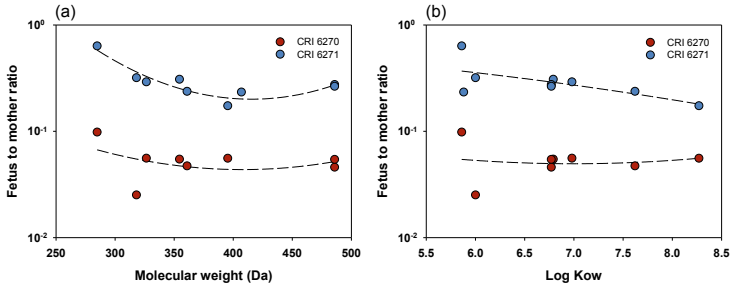

Figure 3. Regressions between fetus to mother ratios and (a) molecular weight and (b) log K_{ow} of POPs

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