Transplacental transfer of persistent organic pollutants in La Plata dolphins (Pontoporia blainvillei; Cetartiodactyla, Pontoporiidae)

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HIGHLIGHTS
• POPs are transferred via placenta in La Plata dolphins.
• Dolphin blubber is the main tissue for accumulate POPs.
• POP transfer via placenta increases according to gestation period and fetal development of Pontoporia blainvillei.

GRAPHICAL ABSTRACT

Abstract
Persistent organic pollutants (POPs) accumulate in the fat tissue of living organisms and are found in relatively high concentrations in animals at the top of the food chain, such as dolphins. The ability of these compounds to interact with the endocrine system of marine mammals constitutes a risk for the reproduction and conservation of species. The La Plata dolphin, Pontoporia blainvillei, is exclusive to the southwestern Atlantic Ocean and is classified on the IUCN red list as a vulnerable species. Blubber, liver, kidney and muscle samples from four P. blainvillei mother-fetus pairs were analyzed to evaluate the transfer of POPs to fetal tissues through the placenta. The presence of POPs in fetal tissues indicates the maternal transfer of compounds. In the pregnant females, blubber was the tissue with POP highest concentration, followed by the liver, kidneys and muscles. In the fetuses, POP accumulation mainly occurred in the blubber followed by the muscles, liver and kidneys. Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs) were found in all tissues analyzed and had the highest concentrations among all compounds. The main PCB congeners in the fetal samples had five to seven chlorine atoms. The only polybrominated diphenyl ether (PBDE) in the fetal samples was 47 and was found only in blubber. The main DDT metabolite in the fetuses was p,p'-DDE. POP transfer via the placenta occurs in the first months of gestation and increases with fetal development, according to fetus/mother (F/M) ratio: HCB > DDT > PCB > PBDE > Mirex, which may follow the order of the octanol/water partition coefficient (Kow) values.

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1. Introduction

Persistent organic pollutants (POPs) accumulate and are biomagnified in the fat tissue of organisms due to their lipophilic characteristics with higher concentrations found in animals at the top of the food chain (Aguilar et al., 1999; Tanabe et al., 1994). POPs include organochlorine pesticides, such as DDTs, mirex, chlordane, hexachlorocyclohexanes (HCHs) and industrial products, such as PCBs and PBDEs (UNEP, 1995, 2009, 2011, USEPA, 2009). Such pollutants pose risks to marine mammal populations (Bila and Dezotti, 2007; Pestana et al., 2008).

Marine mammals bioaccumulate and biomagnify large amounts of POPs due to the fact they are top predators with high longevity and low biodegradation capacity (Borrel and Aguilar, 2007; Tanabe et al., 1988). Moreover, these mammals maintain a significant fat layer for energy and thermoregulation needs, making them particularly vulnerable to the lipophilic POPs (Weijjs et al., 2010a). A series of chemical substances present in the environment can disturb the endocrine system of aquatic and terrestrial organisms by interacting with reproductive hormone receptors, exerting a negative influence on the reproduction and thus the conservation of a given species (USEPA, 1997). Known as environmental endocrine disruptors, these compounds are defined as xenogenous agents that interfere with the production, release, transport, action, metabolism, link or elimination of natural hormones responsible for the maintenance, homeostasis, reproduction, development and/or behavior of organisms (USEPA, 2008). POP contents in dolphins vary with exogenous agents that interfere with the production, release, transport, action, metabolism, link or elimination of natural hormones responsible for the maintenance, homeostasis, reproduction, development and/or behavior of organisms (USEPA, 2008). POP contents in dolphins vary with exogenous agents that interfere with the production, release, transport, action, metabolism, link or elimination of natural hormones responsible for the maintenance, homeostasis, reproduction, development and/or behavior of organisms (USEPA, 2008).

2. Material and methods

2.1. Sampling

Between September 2013 and June 2015, four pregnant females (PA331, PA332, PA369, and PA397) with their respective fetuses (PA344, PA345, PA370 and PA401) (see Table 1 - considered as pairs A, B, C and D) were accidentally caught in fishing operations on the coast of the state of São Paulo (southeastern Brazil) (Fig. 1). Necropsies were conducted at the field research station of the Instituto Oceanográfico – Universidade de São Paulo. Blubber, muscle, kidney and liver samples were collected from fetuses and females. The samples were wrapped in aluminum foil and preserved at −20 °C until analysis at the Laboratório de Química Orgânica Marinha do Instituto Oceanográfico – Universidade de São Paulo [Marine Organic Chemistry Laboratory, Oceanographic Institute, University of São Paulo].

Muscle tissue from fetus/mother pairs C and D and liver and kidney tissue from fetus/mother pair D were not available for analysis. The gestation period was estimated based on the body length of the fetuses (see Danilewicz et al., 2002). Detailed information on the biometric data of fetus/mother pairs is found in Table 1. Based on the carcass classification proposed by Geraci and Lounsby (1993), the individuals were grouped in the Code 2 category (fresh animals).

2.2. Chemical analysis

Samples of ~0.25 g of blubber, ~1.0 g of liver and kidney and ~2.5 g of muscle were ground with 15 g of anhydrous sodium sulfate and extracted in a Soxhlet apparatus for 8 h using approximately 80 mL of n-hexane and dichloromethane (1:1) (v:v). Before extraction, PCB 103 (2,2′,4,5′,6-pentachlorobiphenyl) and PCB 198 (2,2′,3,3′,4,4′,5,5′,6-octachlorobiphenyl) were added as surrogate standards. The extract was concentrated to 2 mL, from which a 0.2 mL aliquot was removed for lipid content gravimetric analysis (UNEP/FAO/IOC/IAEA, 1986). The remaining extract was submitted to treatment with concentrated sulfuric acid (96%), followed by elution in a 5% water-deactivated alumina chromatographic column with 20 mL of a mixture of 30% dichloromethane and 70% of n-hexane. Tetrachloro-m-xylene (TCMX) was added before injection in a gas chromatograph equipped with a 63Ni electron capture detector (GC-ECD) for the analysis of pesticides and a mass spectrometer detector (GC/MS) for the analysis of PCBs and PBDEs.

2.3. Instrumental parameters

2.3.1. GC-ECD

Agilent Technologies (model 6890 N) fused silica capillary column with 5% diphenylmethyldisiloxane (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used for separation. The carrier gas was H2 (0.7 mL min−1) and the makeup gas was N2. The compounds were identified based on retention time in comparison to certified external standards. The oven temperature used in the separation of compounds was set at 60 °C and increased to 150 °C (5 °C per min) held for 5 min, then up to 200 °C (1 °C per min) and finally reached 300 °C (8 °C per min), holding this temperature for 4.5 min (90 min run).

2.3.2. GC/MS

The column used was similar to that used in GC-ECD. MSD was operated in the electron impact ionization (EI) (70 eV) and select ion monitoring (SIM) mode. The carrier gas was He (1.1 mL min−1). In addition to the retention times, compounds were identified based on each mass/charge (m/z) ratio of quantitation ion. The oven temperature used in the separation of PCB congeners was set at 75 °C, held for 3 min, increased 15 °C per min to 150 °C, then up to 260 °C (2 °C per min) and finally reached 300 °C (20 °C per min), holding this temperature for 10 min until the end of the analysis in a 66 min run. For PBDEs, the heating ramp started at 70 °C, holding for 1 min, increased to 154 °C.
(12 °C per min), then up to 210 °C (2 °C per min) and finally 300 °C (3 °C per min) in a 66 min run.

POPs were quantified using analytical curves built with nine concentrations (1, 5, 10, 50, 80, 100, 150 and 200 pg μL⁻¹), with the coefficient ratio greater than or equal to 99.5% (t = 0.995) for each compound (Wade and Cantillo, 1994). Target compounds were purchased from Accustandard, USA: α-chlordane, γ-chlordane, oxychlordane, o,p′-DDD, p,p′-DDD, o,p′-DDE, p,p′-DDE, o,p′-DDT, p,p′-DDT, α-HCH, β-HCH, γ-HCH, δ-HCH, heptachlor, hexachlorobenzene, mirex, BDE 28, 47, 99, 100, 132, 138, 141, 149, 151, 153, 156, 157, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 199, 201, 203, 206 and 209.

2.4. Quality control

The blank, blank spike, sample, sample duplicate and sample spike for each tissue and standard reference material (SRM 1945) were processed for quality control (Wade and Cantillo, 1994). The recovery of surrogates was from 45% to 85% and the recovery of POP standards in the spiked samples was from 51% to 150%. The relative percentage difference between the duplicate samples was <25%. Concentrations reported in the SRM 1945 - NIST (Organics in Whale Blubber - National Institute of Standards and Technology) were in agreement with the acceptable limit of ±35%. Concentrations found in the blanks were subtracted from the respective samples. The detection limits (MDL) of all compounds were determined in accordance with the US Environmental Protection Agency (EPA) guidelines.

Table 1

Biometric data (length in cm and weight in kg), individuals code, date and geographical coordinates, where pregnant La Plata dolphins (Pontoporia blainvillei) were incidentally caught in southeastern Brazil. The tissues available for analyses were also indicated. NA = not available.

<table>
<thead>
<tr>
<th>Data</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
<td>Female</td>
<td>Fetus</td>
<td>Female</td>
<td>Fetus</td>
</tr>
<tr>
<td>Notification date</td>
<td>PA331</td>
<td>PA344</td>
<td>PA322</td>
<td>PA345</td>
</tr>
<tr>
<td>Latitude</td>
<td>02/Sep/2013</td>
<td>02/Sep/2013</td>
<td>25° 01.726′</td>
<td>25° 01.726′</td>
</tr>
<tr>
<td>Longitude</td>
<td>24° 47.730′</td>
<td>24° 47.730′</td>
<td>47° 47.730′</td>
<td>47° 47.730′</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>138</td>
<td>59</td>
<td>135</td>
<td>43</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>29</td>
<td>2.6</td>
<td>26</td>
<td>0.92</td>
</tr>
<tr>
<td>Gestation period (months)</td>
<td>7-8</td>
<td>5-6</td>
<td>4-5</td>
<td>3-4</td>
</tr>
<tr>
<td>Tissues analyzed</td>
<td>Blubber</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Liver</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Kidney</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Muscle</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
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</table>

* Estimated based on body length of fetus (Danilewicz et al., 2002).
Protection Agency Protocol (US EPA, 1984) and were based on the standard deviation σ (Student’s t value with 95% confidence σ) of seven replicates of a spiked tissue sample containing target compounds at a concentration of 1 pg μl⁻¹. MDL ranged from 0.354 to 2.85 ng g⁻¹ for blubber, 0.103 to 1.56 ng g⁻¹ for liver and kidney and 0.035 to 0.626 ng g⁻¹ for muscle.

2.5. Data treatment

Concentrations were expressed as ng g⁻¹ on a wet and lipid weight (ww, lw) basis. DDTs represent the sum of o,p’-DDD, p,p’-DDD, o,p’-DDE, p,p’-DDE, o,p’-DDET and p,p’-DDET. HCHs represent the sum of α-HCH, β-HCH, γ-HCH and δ-HCH. Chlordanes represent the sum of α-chlordane, γ-chlordane, oxochlordane and heptachlor. PCBs correspond to the sum of fifty-one congeners and PBDEs correspond to the sum of seven congeners analyzed.

The fetus/mother (F/M) ratio was calculated to evaluate the transfer of pollutants from mothers to fetuses during gestation. This ratio regards the concentration of compounds present in the blubber of fetuses and their respective mothers. The F/M ratio was applied to all lipid classes detected above the MDL.

F/M Ratio = \( \frac{\sum \text{POP class fetus}}{\sum \text{POP class mother}} \)

Results were plotted using the ggplot2 package (Wickham, 2009) with Rstudio Team version 206 1.0.136 (Rstudio, 2016).

3. Results and discussion

3.1. Occurrence and distribution of POPs in tissues from mother-fetus pairs

The highest concentrations of POPs were found in the blubber of the fetuses and pregnant females. PCBs and DDTs were found in all tissues analyzed and at relatively higher concentrations in the females than fetus (Table 2). Most of the specimens analyzed exhibited HCH, chlordanes, PBDE and mirex at concentrations below the MDL for liver, kidney and muscle tissue.

PCB and DDT wet weight concentrations in the fetuses were higher in muscle in comparison to liver and kidney. In contrast, the liver exhibited higher concentrations than the two other tissues in adult females. The lipid content in fetuses was higher in muscle than the liver and kidney, whereas the opposite is true for adult females (Table 2), which may explain the difference in accumulation, as POPs have greater affinity for fat tissue (Aguilar et al., 1999; Tanabe et al., 1994). Moreover, these substances are transferred through the placenta to the fetuses while part of their tissues and organs are still under development (Weijts et al., 2010a, 2010b; Tilbury et al., 1999; Yang et al., 2007). The lipid contents were similar (2–6%) among liver, kidney and muscle, except for females PA331 and PA332 (0.75 and 0.60, respectively) and, therefore, the proportion of concentrations in lipid weight between mother and fetus were quite similar to wet weight data.

When introduced into the body, any substance is distributed through the blood flow reaching the organs. The blood flow rate that each tissue receives and the affinity to retain substances influence differences in POP concentrations. Thus, the more irrigated tissues, such as liver and kidney, concentrate more compounds than less irrigated tissues, such as blubber and muscle (Weijts et al., 2010b). However, POPs with greater affinity for lipids are present in lower amounts in the blood, as fat serves as a compartment to retain these lipophilic compounds (Weijts et al., 2010b).

3.2. POPs accumulation profile

The main PCB congeners transferred from female to fetus in blubber, liver, kidney and muscle during gestation had five to seven chlorine atoms (i.e., penta, hexa and heptachlor, Fig. 2, Tables S1–4). These congeners are more resistant to degradation in organisms and therefore more persistent, which makes them particularly hazardous for marine mammals and other species that feed on them.

Table 2

<table>
<thead>
<tr>
<th>Lipids (%)</th>
<th>Blubber (n = 8)</th>
<th>Liver (n = 6)</th>
<th>Kidney (n = 6)</th>
<th>Muscle (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipids</td>
<td>PCBs</td>
<td>DDTs</td>
<td>PBDEs</td>
</tr>
<tr>
<td>PA331</td>
<td>Female A</td>
<td>78.4</td>
<td>163 (208)</td>
<td>102 (130)</td>
</tr>
<tr>
<td>PA334</td>
<td>Fetus A</td>
<td>60.5</td>
<td>64.5 (107)</td>
<td>83.4 (138)</td>
</tr>
<tr>
<td>PA332</td>
<td>Female B</td>
<td>87.9</td>
<td>291 (331)</td>
<td>136 (155)</td>
</tr>
<tr>
<td>PA345</td>
<td>Fetus B</td>
<td>24.4</td>
<td>89.0 (365)</td>
<td>72.9 (299)</td>
</tr>
<tr>
<td>PA369</td>
<td>Female C</td>
<td>86.9</td>
<td>723 (832)</td>
<td>408 (469)</td>
</tr>
<tr>
<td>PA370</td>
<td>Fetus C</td>
<td>29.1</td>
<td>124 (426)</td>
<td>95.2 (327)</td>
</tr>
<tr>
<td>PA397</td>
<td>Female D</td>
<td>81.3</td>
<td>1298 (1596)</td>
<td>516 (635)</td>
</tr>
<tr>
<td>PA401</td>
<td>Fetus D</td>
<td>2.37</td>
<td>2.83 (1021)</td>
<td>6.83 (288)</td>
</tr>
</tbody>
</table>

< represents values below MDL (method detection limit).
exhibit considerably greater bioaccumulation in comparison to other congeners (Leonel et al., 2010; Weijs et al., 2009; Yogui et al., 2003).

The dominant PCB congeners in the fetal and maternal blubber were 99, 101, 110, 118, 138, 149, 153, 180 and 187. The predominance of these congeners had also been reported in mother–fetus pairs of the beluga whale (Delphinapterus leucas) (Desforges et al., 2012) and the long-finned pilot whale ( Globicephala melas) (Weijs et al., 2013).

For other fetal tissues, the predominant congeners (99, 101, 110, 118, 138, 149 and 153) were generally similar to those found in the blubber, and the conjuger PCB 138 was predominant in all samples. Heavier compounds, such as 180 and 187, were also detected in muscles.

Borrell and Aguilar (2005) have shown that light PCB congeners (two to four chlorine atoms) are more easily transferred due to their low lipophilicity. However, only congeners 52 and 66 were found above the MDL in the fetuses analyzed while congeners with five to seven chlorine atoms were transferred in higher amounts (Fig. 2). The latter are more persistent in the organism and are therefore found in greater concentrations in marine mammals. In this study, that was observed independently of gestation period (Fig. 2).

Weijs et al. (2013) reported the predominance of these congeners in placental transfers in long-finned pilot whale fetuses, with lighter congeners transferred more during lactation.

Among pesticides, the main compound accumulated in pregnant females and fetuses was DDT, followed by mirex. The predominant DDT metabolite was p,p′-DDE. DDT passes through the placenta and its concentration in the umbilical cord blood varies in the same way than in the blood of exposed mothers (Goodman and Gilman, 2005). The mother–fetus pairs exhibited the predominance of the metabolite p,p′-DDE in all tissues analyzed. Several studies indicate that p,p′-DDE results from the metabolism of p,p′-DDT by organisms and the environment and is also more resistant to degradation than DDT (D’Amato et al., 2002).

Thus, p,p′-DDE is predominant in marine mammals due to biomagnification through the food chain (Alonso et al., 2015; Fillmann et al., 2007; Lailson-Brito et al., 2011; Leonel et al., 2010; Massé et al., 1986; Weijs et al., 2010a, 2010b; Yogui et al., 2003).

The main PBDE found in the pregnant females and the only one found in the fetuses was the congener 47. This congener, which has four bromine atoms in its molecule, is predominant in the environment, biota and humans (USEPA, 2008) and is more efficiently bioaccumulated. Desforges et al. (2012) and Weijs et al. (2013) also reported the predominance of this congener as well as of lighter PBDEs.

### 3.3. POPs transplacental transfer

Based on the total length of the fetuses and fetal growth rate (Danilewicz et al., 2002), the gestation period was estimated to approximately eight, six, five and four months for fetuses PA344, PA345, PA370 and PA401, respectively (Table 1). Total fetus length is a good indicator of the development stage (Weijs et al., 2013). Accordingly, the fetus/mother ratio (F/M) of the contaminants followed the order D (smallest fetus) < C < B < A (largest fetus) (see Table 1). The fetus PA370 had the greatest concentrations among fetuses and a smaller F/M ratio than the fetuses PA344 and PA345. Thus, this result confirms that the transfer of contaminants increases with the progression of the gestation period (Fig. 3). Although due to its short gestation period the high concentrations of fetus PA370 is a reflection of the high POP concentrations in its mother.

An F/M ratio > 1 indicates that the potential bioaccumulation of a given compound is higher in the fetus than in its mother, demonstrating greater ease of placental transfer. In the present study, no POP class had a F/M ratio > 1. HCB had the highest ratios (0.9, 0.6, 0.3), which were found in pairs A, B and C, respectively. Weijs et al. (2013) found a F/M ratio > 1 for HCB in G. melas, showing the greater potential for the bioaccumulation of this compound in fetuses compared to their mothers. As described in literature (Desforges et al., 2012; Alonso et al., 2015), there is a tendency for mothers to transfer less lipophilic and low log Kow (octanol/water partition coefficient, <6.5) halogenated compounds to fetuses. For example, HCB, which has six chlorines and a log Kow of 5.24. The compounds with the second highest ratios (0.8, 0.5, 0.2 and 0.1 for fetuses PA344, PA345, PA370 and PA401, respectively) were DDTs. The sum of DDTs was mainly composed of p,p′-DDE, which was found in greater concentrations in both the fetuses and adult females. The log Kow of this metabolite is 6.51, indicating a low to moderate tendency to be transferred from mother to fetus. The ratio F/M for PCBs (fetus PA344–0.4, PA345–0.3, PA370–0.2 and PA401–0.2), PBDEs (0.3, 0.3 and 0.1) and mirex (0.3, 0.2 and 0.06) for fetuses PA344, PA345 and PA370, respectively, were lower when compared to the ratios F/M of HCB and DDTs. Once more, the log Kow of those compounds may explain these results. Mirex, PCB–153 and PBDE–47 present a log Kow of 6.89, 6.89 and 6.84, respectively, showing higher tendency to accumulation in mothers than to transfer to the fetuses.

The risk of toxicity is higher during gestation and lactation than in any other stage of life (Borrell and Aguilar, 2005). For PCBs, Kannan et al. (2000) suggested a limit concentration of 17 μg g−1 lw observed in mammals surveyed in captivity (seals, European otters and mink) fed with field food items. For DDTs, the only threshold associated with reproductive failure and pathological alterations in cetaceans was 50 μg g−1 lw (Wagenman and Muir, 1984). No established toxicity thresholds were found in marine mammals for HCB and mirex. The absence of the blood–brain barrier in fetuses may lead to the accumulation of toxic chemical products in developing neural tissues in mammals (Tilbury et al., 1999). Therefore, POPs are particularly toxic during the

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**Fig. 2.** Distribution of PCB chlorination level in blubber of *Pontoporia blainvilliei* females and fetuses captured in fishing nets between 2013 and 2015 in southeastern Brazil.
initial stages of development suggesting that the levels for effects may be lower for embryos and youngsters than for adults (Borrell and Aguilar, 2005), representing a risk to the conservation of marine mammals.

4. Conclusion

The analyses of the blubber, liver, kidney and muscle samples from P. blainvili
to mothers-fetus pairs demonstrate POP transfers to fetuses and especially for PCBs and DDTs. A positive correlation was found between fetus gestation period and concentration of POPs, showing that transfer may increase throughout the gestation period. POPs observed in fetus/mother ratio was: HCB > DDT > PCB > PBDE > Mirex, which follows the order of log Kow values. The POP contents in La Plata dolphins represent a risk to the species conservation and underscore the need for special attention in future management plans.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.02.325.

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