Marine Pollution Bulletin xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Maternal transfer of organohalogenated compounds in sharks and stingrays

Liesbeth Weijs [a,b,c,*], Nathalie Briels [a,b,d], Douglas H. Adams [e], Gilles Lepoint [f], Krishna Das [f], Ronny Blust [a], Adrian Covaci [b]

[a] Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[b] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[c] National Research Centre for Environmental Toxicology (Entox), The University of Queensland, 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[d] Norwegian University of Science and Technology (NTNU), Department of Biology, Høgskoleringen 5, Realfagbygget, 7491 Trondheim, Norway
[e] Florida Fish & Wildlife Conservation Commission, Fish & Wildlife Research Institute, 1220 Prospect Ave., #285, Melbourne, FL 32901, USA
[f] Laboratory for Oceanology – MARE Center, University of Liège B6C, 4000 Liège, Belgium

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Bonnethead
Atlantic stingrays
Blacktip sharks
POPs
Maternal transfer
Embryo

ABSTRACT

Elasmobranchs can bioaccumulate considerable amounts of persistent organic pollutants (POPs) and utilize several reproductive strategies thereby influencing maternal transfer of contaminants. This study provides preliminary data on the POP transfer from pregnant females to offspring of three species (Atlantic stingrays, bonnethead, blacktip sharks) with different reproduction modes (aplacental, placental viviparity). Polychlorinated biphenyl (PCB) levels were generally higher than any other POPs. Stingrays and blacktip shark embryos contained the lowest POP concentrations while bonnetheads and the blacktip adult female had the highest concentrations. Results suggest that POPs are more readily transferred from the mother to the embryo compared to what is transferred to ova in stingrays. Statistically significant differences in levels of selected POPs were found between embryos from the left and right uterus within the same litter as well as between female and male embryos within the same litter for bonnetheads, but not for the blacktip sharks.

1. Introduction

Organohalogenated compounds can be passed between biota by several processes, including biomagnification, where pollutants in lower trophic level organisms are transferred to their higher trophic level predators. Biomagnification is a major pathway for several persistent organic pollutants (POPs) and is a frequent factor in biomonitoring studies (Kelly et al., 2004, 2008; Johnson-Restrepo et al., 2005; Burreau et al., 2006; Ikonomou and Addison, 2008). Additive to biomagnification, there is also the further potential of direct pollutant transfer from parent to offspring. This aspect of pollutant transfer has received comparatively less attention than biomagnification, although there have been a considerable number of studies regarding mammalian species (e.g. humans, marine mammals) dealing with body burdens of POPs in offspring (Soechitram et al., 2004; Greig et al., 2007; Vanden Berghe et al., 2012; Wang et al., 2012; Mori et al., 2014). Results for several contaminant classes have suggested a difference between prenatal maternal transfer (i.e. gestation) and postnatal maternal transfer (i.e. lactation) on the body burdens of POPs in mammalian offspring in terms of the types and amounts of contaminants (DeKoning and Karmaus, 2000; Ayotte et al., 2003; Guvenius et al., 2003).

Elasmobranch species have a wide variety of reproduction modes of which the influence on the maternal transfer during gestation remains largely unknown. Fortunately, recent attention has shifted to elucidating maternal transfer of POPs in several elasmobranchs. Lyons and Lowe (2013a,b) have investigated the mechanisms of maternal transfer of POPs in the common thresher shark (*Alopias vulpinus*), Lyons and Lowe (2013b) have focused on maternal offloading of POPs in round stingrays (*Urobatis halleri*), Mull et al. (2013) has found evidence of maternal offloading of POPs in young of the year white sharks (*Carcharodon carcharias*) and Olin et al. (2014) have attributed the high levels of PCBs (polybrominated biphenyls) found in juvenile bull sharks (*Carcharhinus leucas*) to maternal transfer.

* Corresponding author at: National Research Centre for Environmental Toxicology (Entox), The University of Queensland, 39 Kessels Road, Coopers Plains, QLD 4108, Australia. Tel.: +61 409 581 906.
E-mail addresses: liesbeth.weijs@uantwerpen.be, l.weijs@uq.edu.au (L. Weijs).

http://dx.doi.org/10.1016/j.marpolbul.2014.12.056
0025-326X/

Reproduction modes in elasmobranchs range from oviparity to viviparity with wide variation in terms of presence of placenta and nourishment to the embryo (i.e. yolk sac, oophagy, embryonic cannibalism, uterine milk) (Gilmore, 1993; Bone and Moore, 2008). The elasmobranch species investigated in the current study were Atlantic stingrays (*Dasyatis sabina*), bonnetheads (*Sphyrna tiburo*) and blacktip sharks (*Carcharhinus limbatus*). The reproduction mode in Atlantic stingrays is aplacental viviparity with histotrophy during which the embryos are initially nourished by yolk sac (first stages of gestation) and then uterine milk (histotroph; later stages of gestation) before parturition (Bone and Moore, 2008). Bonnetheads and blacktip sharks are both placental viviparous species, where embryos derive nutrients from the yolk sac in the first stages of gestation after which the depleted yolk sac becomes highly vascularized providing nutrients to the embryos directly from the maternal bloodstream (Wourms, 1977; Bone and Moore, 2008).

Maternal transfer of POPs during gestation means that organisms are directly exposed to POPs, perhaps experiencing adverse effects, prior to birth. The influence of maternal transfer of POPs should be quantified and interpreted in species risk assessments. All species represented in this study have some similarities in terms of reproduction mode as they are all live bearers. However, there are important differences between the two reproduction modes in later stages of gestation as the stingray embryos rely on the protein and lipid-rich histotroph whereas blacktip sharks and bonnetheads rely on nutrients provided by the maternal bloodstream via pseudo placental connection. For lipophilic compounds, such as PCBs and PBDEs (polybrominated diphenyl ethers), the potential for maternal transfer can be greatly influenced by the type of tissue (Guvenius et al., 2003; Mori et al., 2014). Consequently, this may have a significant impact on the health and survival of embryos as a result of prenatal POP exposure as well as on the survival of newborns, juveniles and adults. Therefore, this study aims to provide further insight regarding the maternal transfer of selected POPs during gestation in three elasmobranch species that have two distinct reproduction modes.

2. Materials and methods

2.1. Samples

A total of 54 samples were analyzed for POPs and MeO-PBDEs (methoxylated polybrominated diphenyl ethers). These samples were from three different species (Atlantic stingray – $n = 7$; bonnethead – $n = 42$; blacktip shark – $n = 5$) and included liver, ova, ovary or the combination ova & ovary in case pre ovulated ova were too small to be analyzed separately (Table 1). In addition, dorsal muscle samples ($n = 13$) of one Atlantic stingray litter and one bonnethead litter were analyzed for stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to better understand their trophic position in relation to POPs. All sharks and rays were collected from estuarine waters of Indian River Lagoon (IRL) system on the Atlantic coast of Florida or from adjacent nearshore waters of the Atlantic Ocean from north of Cape Canaveral, Florida (latitude 28°40′N) south to Sebastian Inlet, Florida (latitude 27°50′N) during 2009 to 2012. For the POP analysis, 38 PCB (polychlorinated biphenyls) congeners, six PBDEs (polybrominated diphenyl ethers), six DDXs (dichlorodiphenyltrichloroethane and isomers and metabolites), HCB (hexachlorobenzene), five chlordanes (CHLs) and four MeO-PBDEs were targeted in all samples.

2.2. Sample preparation and POP analysis

The method used for the sample extraction and cleanup has been previously described (Weijs et al., 2015) and is briefly presented below. Approximately 0.2–0.3 g of ova, 0.6–0.7 g of ovary, 0.3 g of embryo liver and 0.2 g of adult (mother) liver was homogenized with Na_2SO_4, spiked with internal standards BDE 77 and CB 143 and extracted by hot Soxhlet extraction for 2 h with hexane/acetone (3/1; *v*/*v*). After lipid determination performed on an aliquot of the extract (typically 1/8th), the extract was cleaned on 8 g of acidified silica and analytes eluted with 20 ml hexane and 15 ml dichloromethane. The cleaned extract was evaporated to dryness and reconstituted in 150 µl iso-octane. Details of the analytical methods are given in Weijs et al. (2009) and are briefly given here. PBDEs, MeO-PBDEs and CHLs were measured by GC–ECNI/MS (gas chromatography–electron capture negative ion/mass spectrometry) on a 30 m × 0.25 mm × 0.25 µm DB-5 column by monitoring ions $m/z = 79$ and 81 (for PBDEs and MeO-PBDEs) and 2 specific ions for each CHL. PCBs and DDXs were measured by GC–EI/MS (gas chromatography–electron ionization/mass spectrometry) on a 25 m × 0.22 mm × 0.25 µm HT-8 column by monitoring 2 ions for each homologue group. This system was also used to confirm MeO-PBDEs.

2.3. Quality assurance/quality control (QA/QC)

Recoveries for individual PCB and PBDE congeners ranged between 75% and 104% (RSD < 12%). For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio *S*/*N* equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 10 ng/g lipid weight (lw) (Table S1). QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs, OCPs and PBDEs in whale blubber) was used to test the method accuracy. Obtained values did not deviate more than 10% from the certified values.

2.4. Litters and statistical analysis

Generally, levels of POPs were statistically tested and interpreted within each litter because the embryos were not in the same stage of development and because the mothers were not necessarily from the same age or area. Liver, ova and ovary samples of a pregnant female were also called a "litter" for consistency (Table 1). Results of embryos (per litter) were not pooled unless there were no statistically significant differences between female/male embryos or between embryos from the left or right uterus. Offspring/mother ratios were calculated and compared within the litter, within the species as well as among species. Overall, sample sizes were often too low to perform statistical analysis, but when possible, statistical tests were performed within each litter with non-parametric tests (Kruskal Wallis). For compounds that were detected in more than 50% of the samples, non-detects were replaced by a value of $f * LOQ$ (f is detection frequency and LOQ is limit of quantification). Statistical tests were performed using the SPSS software package (IBM SPSS Statistics Version 20). The level of statistical significance was $p = 0.05$. All concentrations are expressed in ng per g lipid weight (lw).

2.5. Stable isotopes

Measurements of $\delta^{13}C$ and $\delta^{15}N$ in muscle of one litter of Atlantic stingrays (1 mother and her 2 embryos) and one bonnethead litter (1 mother and her 9 embryos) were used to investigate the trophic position. Procedures for $\delta^{13}C$ and $\delta^{15}N$ follow Weijs et al. (2015). Briefly, after freeze drying (48 h), muscle samples were ground into a homogeneous powder of which 1.5 mg was used.

Please cite this article in press as: Weijs, L., et al. Maternal transfer of organohalogenated compounds in sharks and stingrays. Mar. Pollut. Bull. (2015), http://dx.doi.org/10.1016/j.marpolbul.2014.12.056