HALOGENATED FLAME RETARDANTS DURING EGG FORMATION AND CHICKEN EMBRYO DEVELOPMENT: MATERNAL TRANSFER, POSSIBLE BIOTRANSFORMATION, AND TISSUE DISTRIBUTION

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Introduction

Halogenated flame retardants (HFRs), such as polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), decabromobiphenyl ethane (DBDPE), 1, 2-bis (2, 4, 6-tribromophenoxy) ethane (BTBPE), and dechlorane plus (DP), had long been recognized as hazardous environmental chemicals. The adverse effect of HFRs included disturbing thyroid function, neurobehavioral development, and reproduction success.[1-3] Since female birds transfer contaminants accumulated in lipid-rich tissues to their eggs, bird eggs were commonly used to study the maternal transfer potential of HFRs in avian.[4-5] However, studies on the HFR deposition kinetics from oocytes to eggs during egg formation are lacking. There were also limited studies considering the possible biotransformation and tissue distribution of HFRs during the embryonic development for avian, mammals, and human.[6]

In the present study, hen ovaries, oocytes, hatching eggs (on day 0, 7, and 14), and tissues of chicken embryos (liver and muscle) were collected from an electronic waste (e-waste) recycling site in south China. The objective was to characterize the concentrations and distribution of HFRs (e.g., PBDEs, DP, BTBPE, DBDPE, and PBBs) during egg formation and embryonic development. Special emphasis was placed on the maternal transportation of contaminants, potential biotransformation, and tissue distribution of HFRs in developing chicken embryos.

Materials and methods

Chickens (n = 12; 1 male and 11 female) several weeks old were purchased from the market and raised in an enclosed yard of a farmer house surrounded by e-waste recycling workshops in the Qingyuan County, Guangdong Province in October 2011. Hen eggs (n = 79) were collected during March to April in 2012 and stored at ambient temperature. The chickens were sacrificed in May 2012, hen ovaries (n = 11) were collected, and oocytes (n = 22, diameter: 0–1.5 cm) were also found next to the ovaries in the enterocoelia from 8 hens. The eggs were transferred to the laboratory in April 2012. After cleaning, eggs were put into an incubator at 37°C with 70% humidity for 21 days. Ten eggs were randomly collected on day 0, 7, and 14 after the incubation. Nine chickens were successfully incubated and then sacrificed. The chickens were dissected and their liver and muscle tissues were excised. Nine separated eggs were divided into albumin and yolk for analysis. All the samples were weighed and stored at –20°C until further analysis..

Ovaries, oocytes, eggs, and embryo tissues were extracted and cleaned according to a previously published method, [7] with minor modifications. Briefly, after being spiked with surrogate standards (BDEs 77, 181, 205, and 13C-BDE 209), approximately 2 g of the lyophilized samples were extracted with 190 mL hexane/acetone (1/1, v/v) for 48 h. An aliquot of the extract was used to determine the lipid content; the rest of the extract was subjected to gel-permeation chromatography in a column packed with 40 g of SX-3 Bio-Beads (Bio-Rad Laboratories, Hercules, CA) and further cleaned on a multilayer silica gel column packed with both neutral and acidified silica. The extract was concentrated to near dryness under gentle nitrogen flow and reconstituted in 300 µL of iso-octane for analysis. Prior to instrumental analysis, the extracts were spiked with known amounts of the internal standards BDE 118, BDE 128, 4-F-BDE 67, 3-F-BDE 153, 4'-F-BDE 208, and 13C-PCB 208..

All the HFRs were analyzed by gas chromatograph–electron capture negative ionization–mass spectrometer operated in the selected ion monitoring mode. A DB-XLB capillary column (30 m × 250 µmi.d. × 0.25 µm film thickness; J&W Scientific, CA) was used to separate the tri- to hepta-BDEs, PBB 153, and DP with its metabolites. For octa- to deca-BDEs, PBB 209, DBDPE, and BTBPE, a DB-5HT capillary column (15 m × 250 µmi.d. × 0.10 µm film thickness; J&W Scientific, CA) was used. Details of the instrumental conditions were published elsewhere. [8]

Results and discussion

Levels of HFRs. PBDE concentrations did not show significant differences among eggs at day 0, 7, and 14 after the incubation. Thus, all egg samples were treated as a group. PBDE concentrations, calculated as the sum of 13 PBDE congeners (BDE47, 99, 100, 153, 154, 171, 180, 183, 197, 196, 207, 206, 209) were in the range of 12.1–12400, 127–34200, 106–15700, 580–6040, and 563–18800 ng/g lw in ovaries of hens, oocytes (unshaped yolk), eggs, muscle, and liver of chicken embryo at day 0 post-hatching, respectively. DP was found in all the samples, covering a wide range from 1.10 to 87500 ng/g lw. *Anti*-Cl_{11}-DP was observed in 79% of the samples in concentrations between not detectable (nd) and 30.7 ng/g lw. BTBPE (<1–2620 ng/g lw) was detected in all egg samples, 95% of occeyt, 82% of ovary, 67% of chick liver and 44% of muscle. In contrast, DBDPE (nd–10700 ng/g lw) was detected in all the chick's muscles and chicks' livers while in 91% of ovaries, 86% of oocytes, and 70% of eggs. PBB 153 and 209 were found in 91% and 85% of the samples and PBB 209 concentration was 1–4 orders of magnitudes higher than that of PBB 153, indicating the exposure to technical decabromodiphenyl that is constituted by 97% of PBB 209.

Maternal transfer potential of HFRs. A ratio calculated by dividing the median concentration of the chemicals in the egg by that in the oocyte was use to evaluate the maternal transfer potential of HFRs. Ratios for BDE153, 154, 183, 196, 197, BTBPE, PBB153 were larger than 1, indicating a more readily maternal transfer. Ratios for BDE47, 100, 99 were close 1. However, for BDE206, 207, 209, PBB209, DBDPE, syn-DP, and anti-DP, the ratios were all less than 1, indicating a selective retaining in mother. The maternal transfer potential was directly related to the octanol/water partition coefficient (log K_{OW}) of the chemicals (Figure 1). Chemicals with log K_{OW} ranging between 7 and 9 exhibited strong transfer efficiencies (egg-to-oocyte ratio > 1). This finding had never been obtained in previous studies. There was conflicting information about maternal transfer of organohalogen contaminants in birds. Tanabe et al [9] reported that chlorination extend of PCBs did not affect maternal transfer of PCB congeners in birds. Verreault et al. [5] showed that less-persistent compounds were favored for maternal transfer whereas the higher-halogenated compounds were less readily transferred. Van den Steen et al [4] found that maternal transfer in blue tits seemed to be selective for the more bioaccumulative and persistent compounds. The inconsistency between these studies could be attributed to differences in the methods used to determine maternal concentrations (serum, subcutaneous fat, or homogenized whole adult).

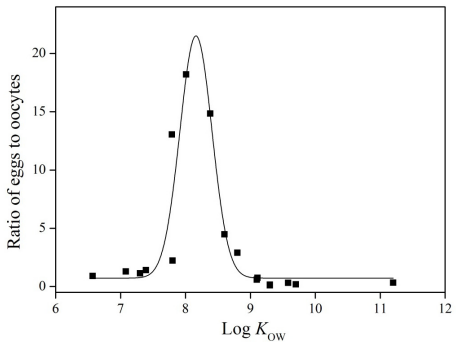

Figure 1, Correlation analysis between the median concentration ratio of eggs to oocytes and the octanol/water partition coefficients (log K_{OW}) of halogenated flame retardants.

The congener-specific maternal transfer resulted in a PBDE congener profile change between oocytes in hen and eggs. There are two distinctly different PBDE congener patterns in chicken in the present study. One was dominated by BDE 183, followed by BDE 197, 196, 209, 207, and 153. The other was dominated by BDE 209, followed by nona-, octa-, and hepta-BDE congeners. Lower brominated congeners such as BDE 47, 99, and 100 contributed less to the total PBDEs. The PBDE congener pattern suggested that deca-BDE and octa-BDE technical products were major sources of PBDEs in this region. The contributions of BDE183, 154, 153, 197, and 197 in eggs increased with a concomitant decrease in the contribution of BDE209 when compared to PBDE congener composition in oocytes due to the observed selective maternal transfer. The maternal transfer potential of syn-DP (ratio of egg to oocyte was 0.11) was lower than that of anti-DP (0.17). Consequently, f_{anti} values (anti-DP/(syn- + anti-DP)) in eggs (mean, 0.65) were significantly higher than those in oocytes (0.52) ($p<0.05$).

Possible biotransformation in chicken embryos. The concentrations of HFRs in the muscle and liver of chicken embryos were significant higher than those in eggs except for PBB209 in muscle whose concentration was below the detectable limit. This was likely caused by the lipid consumption during the last days of incubation.The lipid content in the muscle and liver (6.3% ± 1.7% and 13.1% ± 2.8%, respectively) was significant lower than that (14.3% ± 2.9%) in eggs ($p < 0.05$). Additionally, the weight of the eggs decreased due to the loss of water and energy consumption during the incubation, such that at day 14 after the incubation, the weight loss was 10%. These two factors resulted in contaminant accumulation in the tissues of chicken embryos.

The pollutant composition in the embryonated eggs differed from that in the tissues of chicken embryos (Figure 2). In the embryonated eggs, PBDEs were the dominating pollutants accounting for 69% of the total HFRs, followed by DP (16%), PBBs (12%), and the minor contributors BTBPE (1.9%) and DBDPE (1.2%). However, DBDPE became the predominant pollutant (53%) in the muscle of chicken embryos, followed by PBDEs (35%), DP (8.9%), BTBPE (3.3%), and PBBs (0.21%). Although PBDEs were still the dominating pollutants in the liver of chicken embryos, their percentage decreased from 69% in embryonated eggs to 49%, along with a concomitant increase in the relative amounts of DBDPE from 1.2% to 21%, respectively.

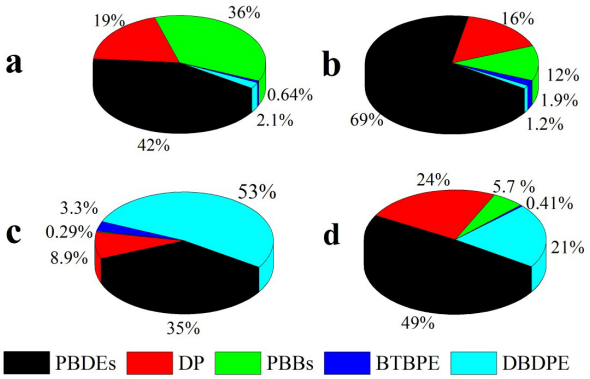

Figure 2, HFRs composition profiles in ovaries and oocytes (a), eggs (b), and muscle (c)/liver (d) of chicken embryo..

The dramatic change in pollutant pattern was most likely caused by the chemical-specific metabolism during the chicken embryo development. Debrominated and methoxylated metabolites of PBDEs in the mid-incubation and pipping bird embryos were detected in previous in ovo experiment.[6, 10] Winter et al. [6] showed that the amount of BDE 99 in in ovo BDE 99-treated zebra finch eggs at day 12 of incubation (around hatching) only represented approximately 20–30% of the nominal amount of BDE 99 injected, regardless of the injected dose. These results supported the hypothesis that PBDEs could be rapidly metabolized by the developing embryo. On the other hand, few studies have reported the potential metabolism of DBDPE in biota. Up to date, only one study reported the potential metabolism of DBDPE in rat but the metabolites has not been confirmed by synthetic standards.[11] The highest percentage for DBDPE in muscle of chicken embryo indicated that, as expected, this chemical highly resisted to the metabolism of the developing embryo. The f_{anti} values (0.68 ± 0.02 in the liver and 0.72 ± 0.03 in the muscle) in tissues of chicken embryo were higher than those in eggs (0.65 ± 0.07). The increased f_{anti} value in chicken embryo suggested that a stereoselective *syn*-DP metabolism occurred in the chicken embryo development.

Tissue distribution of HFRs in chicken embryos. The ratio of contaminant concentration in muscle over the sum of the concentration in muscle and liver was used to evaluate the tissue distribution of HFRs. BDE 47, 99, 100, 153, 154, 206, PBB 153, BTBPE, and DBDPE showed a preferential accumulation in muscle while BDE 196, 197, 207, 209, and PBB 209 showed a preference for the liver. The ratio of the concentration of the different chemicals was inversely related to their lipophilicity (log K_{OW}) with the exception of BDE 206 and DBDPE (Figure 3). It was difficult to explain why these 2 chemicals diverged from this trend. One possible reason for this behavior could be that the precision and the accuracy of the analytical method used in this study were weak for BDE 206, because its levels in all muscle samples and in 4 out of 9 liver samples were only 2 to 3 times higher than the blanks, leading to larger experimental errors. However, for DBDPE, this explanation was not valid because DBDPE was not detected in the blanks. The present study suggested that the lipid component in the liver had greater affinity for the highly lipophilic chemicals than the lipid component in the muscle, thus resulting in a selective accumulation of low-log K_{OW} chemicals in the muscle and high-log K_{OW} chemicals in the

liver during tissue differentiation and organogenesis. Some individual contaminants, such as DBDPE, might have specific affinity for certain components of the muscle, which could explain the preferential accumulation.

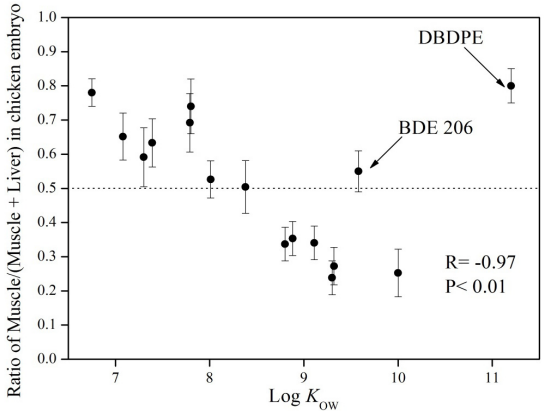

Figure 3, Correlation analysis between concentration ratio of muscle to sum of muscle to liver in chicken embryo and the octanol/water partition coefficients (log K_{OW}) of chemicals. Error bars represent ±1 standard error.

Acknowledgements

This work was supported by the ChineseAcademy of Sciences (No. KZCX2-EW-QN 105) and the National Nature Science Foundation of China (No. 21077105; 41273118; 41230639).

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