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Chiral Polychlorinated Biphenyls (PCBs) in Bioaccumulation, Maternal Transfer, and Embryo Development of Chicken

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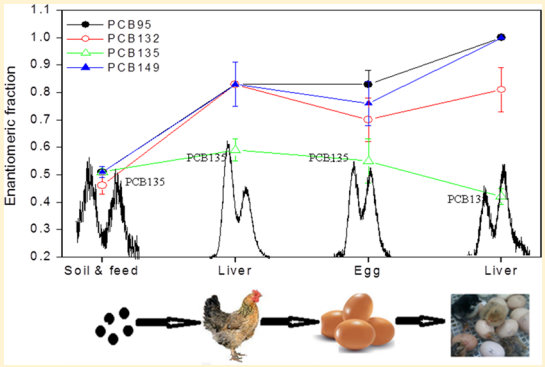
Supporting Information

ABSTRACT: Polychlorinated biphenyls (PCBs) and enantiomer fractions (EFs) of PCB enantiomers (PCBs 95, 132, 135, and 149) were investigated in soil and chicken feed, chicken (*Gallus domesticus*) tissues, eggs on 0, 7, and 14 days after the onset of incubation, and newborn chick tissues. The EF values of PCBs 95, 132, and 149 changed significantly from soil to chicken tissues, and the values in the liver exhibited the highest deviation from the racemic ratio, indicating enantiomer-selective metabolism in hens. Congeners, which are highly resistant to degradation, such as PCBs 138, 153, and 180, exhibited the highest maternal transfer potentials when muscle and liver were used to assess the maternal transfer. However, uniform transfer ratios were observed for most of the PCB congeners when visceral fat was used. The EFs of chiral PCBs in eggs either did not match with muscle or with liver or were similar to those in visceral fat. These results indicate that hens mainly mobilized visceral fat for egg formation and PCBs were deposited in eggs by associating with these lipid materials. Further enantiomeric enrichment of PCBs 95, 132, and 149 occurred in the newborn chick tissues. However, an opposite enantioselectivity for PCB 135 in newborn chicks was observed. These results indicate that the potential toxicity of PCB enantiomers to newborn chicks may be different from that of adults.



INTRODUCTION

Polychlorinated biphenyls (PCBs) are well-known environmental pollutants, which have been widely utilized in various industrial and commercial applications. Although PCB manufacturing has been banned since the 1970s, the occurrence of PCBs is still frequently reported worldwide in air, soil, sediment, and biota samples.[1−3] Because of the persistence and toxicity of PCBs, it is still essential to trace their environmental fate and potential adverse effects.[4] Chirality analysis is a useful tool for characterizing the biotransformation or degradation fate of PCBs because the enantiomer patterns can only be affected by biological rather than physical and chemical processes.[5] Because PCBs are released as racemic enantiomers in commercial products, enantiomer analysis in environment and biota could provide identical evidence for biological transport and distribution of PCBs.[6,7]

Among all of the 209 PCB congeners, 19 PCB congeners are axially chiral and routinely assessed to assess the elimination and biotransformation of PCBs. The distributions of enantiomer-specific PCBs in environmental and biota samples have been ubiquitously reported in previous studies.[5] The *in vivo* biotransformation and uptake from diet are both implied as important biological pathways in chiral PCB alternation.[7,8] Because of their relatively higher tropic levels in food webs, birds and mammals are considered to have higher metabolic enzymatic capacity for PCB detoxification.[6,9,10] Seven seabird species from Northwater Polynya have significantly nonracemic enantiomeric compositions of PCBs 91, 95, and 149 that are considerably different from the racemic residues in their arctic cod and zooplankton prey.[8] As a result, seabirds seem to have higher capabilities for enantiomer-selective biochemical weathering of PCBs than do aquatic organisms. Although the chiral PCBs are widely used in characterizing biochemical processes related to PCBs in aquatic environments, such as those in piscivorous birds, the enantiomeric biotransformation of PCB enantiomers in terrestrial avian species has not been fully understood. The eggs of top predatory birds in Spain were reported to have nonracemic enantiomer fractions (EFs) for most chiral PCBs.[11] However, the reasons for the nonracemic PCB chiral signatures in terrestrial avian species are still unclear, and further studies are required to gain insight into the enantiomer enrichment process in terrestrial avian species.[5]

Bird eggs can be easily collected, and they can directly reflect the pollutants transported from female adults. Therefore, eggs have long been used as a useful tool to monitor the

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contamination burden in adult birds and to explore the maternal transfer processes.[12−14] A lipid normalized egg-to-maternal tissue concentration ratio can be used to assess the maternal transfer potential of chemicals in oviparous organisms, including fish, reptiles, and birds.[15] In previous studies, inconsistent results for maternal transfer of organohalogenated contaminants were reported. In blue tits, Van den Steen et al.[14] found that maternal transfer seemed to be selective for the more bioaccumulative and persistent compounds. However, Verreault et al.[13] reported that highly chlorinated PCB congeners are preferentially retained in maternal plasma of glaucous gulls rather than eggs. Tanabe et al.[16] found that the ratios of the PCB homologue group concentrations in subcutaneous fat of Adelie penguins and eggs did not vary. The different results are likely attributed to the species-specific variations and/or assessment methods of maternal transfer in these studies because whole body,[14] plasma,[13] and subcutaneous fat[16] were, respectively, used in these studies. In the ovogenesis process, endogenous lipid stores are mobilized and extensively modified and reprocessed by the liver and then transported by the bloodstream to the growing ovum via lipoproteins.[17,18] It is essential to know the maternal transfer mechanism of PCBs and other environmental contaminants, which is important not only for the standardization of maternal transfer investigations but also for evaluating the adverse effect of these contaminants on reproduction and further embryo development of birds.

Nonracemic distributions of some chiral organic pollutants have been found in eggs of raptors and seabirds.[8,11] The enrichment or depletion of one enantiomer over the other enantiomer in eggs may be due to the direct transfer of nonracemic proportions of contaminants from the mother to the egg.[6] Alternatively, it has also been hypothesized that nonracemic EFs of chiral contaminants in eggs may be the result of stereoselective microbial degradation in eggs.[19] The enantiomer signatures in eggs may possess different toxicological properties for embryos due to potential enantiomer-specific differences in chiral PCB toxicity.[20] For example, (+)-PCB 139 is a more potent inducer of ethoxyresorufin-*O*-deethylase activity than (−)-PCB 139 in chick embryo hepatocyte cultures.[20] Further investigations are necessary to understand the mechanism of changes in chiral contaminants during maternal transfer and the sensitive early life stages.

The aims of the present study are as follows: (i) to explore the PCB chiral signature alternations in bioaccumulation by quantifying the enantiomer pattern of chiral PCBs in dietary sources (chicken feed and soil) and chicken tissues, (ii) to reveal the PCB maternal transfer features by investigating the alternation in PCB congener patterns and chiral signature from chicken tissues to eggs, and (iii) to elucidate the PCB enantiomer patterns in newborn chicks so as to provide suggestions and implications for toxicological assessment in newborn avian species.

MATERIALS AND METHODS

Sample Collection. Chickens (*Gallus domesticus*, n = 11) 2 weeks old were purchased from the market and raised in a closed yard of a farmer house surrounded by e-waste recycling workshops in the Qingyuan County, Guangdong Province (23°32′ N, 113°03′ E) in October 2011. Hen eggs (n = 70) were collected from March to April 2012 and stored at ambient temperature. The chickens were sacrificed in May 2012 and individual ovum (unshaped yolk, n = 22; diameter, 0−1.5 cm) were also found next to the ovaries in the enterocoelia from eight hens. Eleven kinds of tissues (n = 11, liver, muscle, heart, lung, visceral fat, brain, stomach, intestine, ovary/testis, and serum and n = 10 for kidney) were collected. Among these, only seven tissues (liver, muscle, heart, lung, visceral fat, stomach, and intestine) were selected for discussion because EF values of chiral PCBs cannot be obtained for the other four tissues.

After cleaning, eggs were put into an incubator at 37 °C with 70% humidity for 21 days. All samples were weighed and stored at −20 °C until further analysis. Ten eggs were randomly collected on days 0, 7, and 14, after the onset of incubation. Nine chicks were successfully incubated and then sacrificed. The chicks were dissected, and their liver and muscle tissues were excised. Soil samples (n = 10) in the yard were collected during chicken breeding. Nine chicken feed samples (mixture of rice, wheat, and other grain) for chicken were also collected. All the samples were weighed and stored at −20 °C until further analysis.

Sample Preparation and Analysis. Chicken tissues, ovum, and eggs were extracted and cleaned according to a previously published method.[21] Briefly, after being spiked with surrogate standards (PCBs 30, 65, and 204), 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 by gravimetric method; 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 PCBs 24, 82, and 198. PCB congeners were analyzed by an Agilent 6890 gas chromatograph equipped with a 5975B mass spectrometer (GC-MS) in electron impact ionization (EI) mode. A DB-5MS capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) was used to separate the PCB congeners. Six chiral PCB congeners (PCBs 84, 95, 132, 136, 149, and 183) were chosen for enantiomer analysis. Enantiomer signatures of chiral PCBs 95, 136, and 149 were determined on a Chirasil-Dex (25 m × 0.25 mm i.d., 0.25 μm film thickness) column. PCBs 84, 132, and 183 were separated on a BGB-172 (30 m × 0.25 mm i.d., 0.18 μm film thickness) capillary column. Of the six chiral PCB congeners, four chiral congeners (PCBs 95, 132, 136, and 149) were detectable. The enantiomer fractions (EFs) were calculated using the area of the (+)-enantiomer divided by areas of both enantiomers (−) and (+) for PCBs 95, 132, 135, 149. The (−)-enantiomer elutes first for PCBs 95, 132, and 149, and the (+)-enantiomer elutes first for PCB 135.[11,22] The details of other kinds of sample preparation and instrument analyses are given in the Supporting Information.

Quality Assurance and Quality Control. The methods for quality assurance (QA) and control (QC) were performed by regular analysis of procedural blanks, spiking blanks (a mixture of 7 PCB indicators spiked in solvent blanks), and blind triplicate samples. Procedure blanks contained traces of target chemicals, but the levels were less than 1% of the analyzed concentration in most samples. The recoveries in spiking blanks were 81 ± 9.6% for seven PCB indicators (PCBs 28, 52, 101, 118, 138, 153, and 180). The recoveries of surrogate standards were 98 ± 2.5, 95 ± 8.5, and 86 ± 1.6% for PCBs 30, 65, and 204, respectively. Target chemicals in the triplicate samples were consistent (RSD < 15%).

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Table 1. Enantiomer Fractions of Chiral PCBs (mean ± standard deviation) and ∑PCB Concentrations (median and range) in Dietary Sources (ng/g dry weight), Chicken Tissues (ng/g lipid weight), Eggs during Incubation (ng/g lipid weight), and Newborn Chick Tissues (ng/g lipid weight)

	EFs PCB 95	EFs PCB 132	EFs PCB 135	EFs PCB 149	∑PCBs[a]
Dietary sources					
chicken feed	0.50	0.62	0.47	0.51	8.8 (3.9−45)
soil	0.51 ± 0.01	0.46 ± 0.03	0.51 ± 0.04	0.51 ± 0.02	23300 (19000−25400)
Chicken tissues					
liver	0.83 ± 0.08	0.83 ± 0.07	0.59 ± 0.05	0.83 ± 0.08	648 (74−1410)
muscle	0.66 ± 0.06	0.60 ± 0.05	0.54 ± 0.07	0.72 ± 0.01	574 (318−5540)
heart	0.80 ± 0.08	0.66 ± 0.09	0.50 ± 0.06	0.73 ± 0.09	615 (33−2220)
lung	0.76 ± 0.08	0.66 ± 0.06	0.53 ± 0.04	0.74 ± 0.05	500 (44−884)
visceral fat	0.80 ± 0.09	0.67 ± 0.08	0.50 ± 0.05	0.71 ± 0.08	441 (29−1300)
stomach	0.74 ± 0.11	0.62 ± 0.08	0.49 ± 0.08	0.72 ± 0.06	375 (36−788)
intestine	0.79 ± 0.09	0.66 ± 0.08	0.52 ± 0.06	0.73 ± 0.07	480 (51−1110)
Eggs and newborn chick tissues					
ovum	0.83 ± 0.02	0.71 ± 0.05	0.50 ± 0.05	0.77 ± 0.02	439 (73−1400)
0 day	0.82 ± 0.06	0.68 ± 0.09	0.55 ± 0.12	0.75 ± 0.08	514 (65−1050)
7 day	0.82 ± 0.04	0.68 ± 0.10	0.55 ± 0.11	0.76 ± 0.06	660 (376−1150)
14 day	0.85 ± 0.04	0.72 ± 0.08	0.55 ± 0.11	0.76 ± 0.09	877 (644−1570)
chick liver	1	0.81 ± 0.08	0.42 ± 0.03	1	1580 (861−3840)
chick muscle	not detected	0.65 ± 0.05	0.42 ± 0.02	0.89 ± 0.10	1370 (20−4860)

[a] ∑PCBs: sum of PCBs 28, 52, 60, 66, 74, 85, 99, 101, 105, 110, 118, 130, 138, 139, 146, 149, 153, 164, 170, 180, 187, 194, and 196.

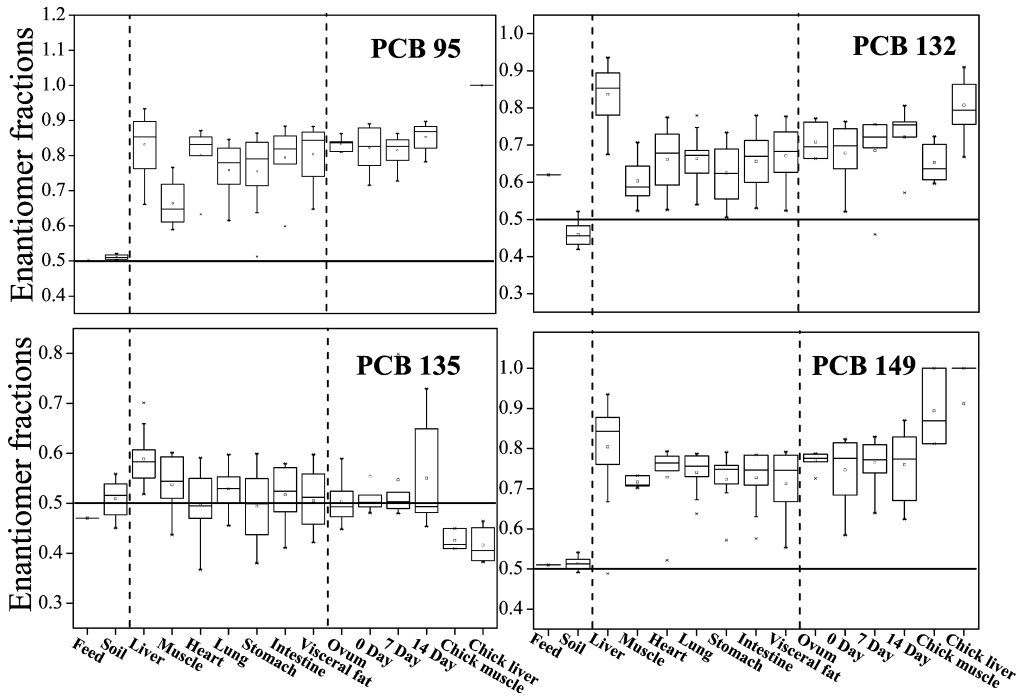

Figure 1. Enantiomer fractions of chiral PCBs (PCBs 95, 132, 135, and 149) in dietary sources (chicken feed and soil), mature hen tissues (liver, muscle, heart, lung, visceral fat, stomach, and intestine), ovum, eggs (0, 7, and 14 days after the onset of incubation), and newborn chick tissues (liver and muscle). The flat solid line represents EF = 0.5. The vertical dash lines separated dietary sources, chicken tissues, ovum, and eggs, and newborn chick tissues.

Instrumental QC was performed by regular injection of solvent blanks and standard solutions.

The limits of quantification (LOQs) were set as the mean value of target compounds detected in procedure blanks, plus three standard deviations. For the undetectable compounds in blanks, the LOQs were estimated as a signal-to-noise ratio of 10. The LOQs ranged from 0.13 to 2.2 ng/g lw (lipid weight) and <0.01 to 0.09 ng/g dw (dry weight) for PCBs. To investigate whether EFs changed during the sample treatment processes, matrix spikes with chiral PCB congeners (PCBs 91, 95, 84, 136, 135, and 149) were performed. The results showed no significant differences in EFs between before (0.496−0.504) and after (0.497−0.501) treatments, and the recoveries ranged from 94 ± 1.3% to 102 ± 1.4%.

Statistical Analysis. Statistical analyses were performed using the SPSS 16 software for Windows (SPSS). The level of significance was set at $p = 0.05$ throughout the study. PCB concentrations were log-transformed to ensure a normal

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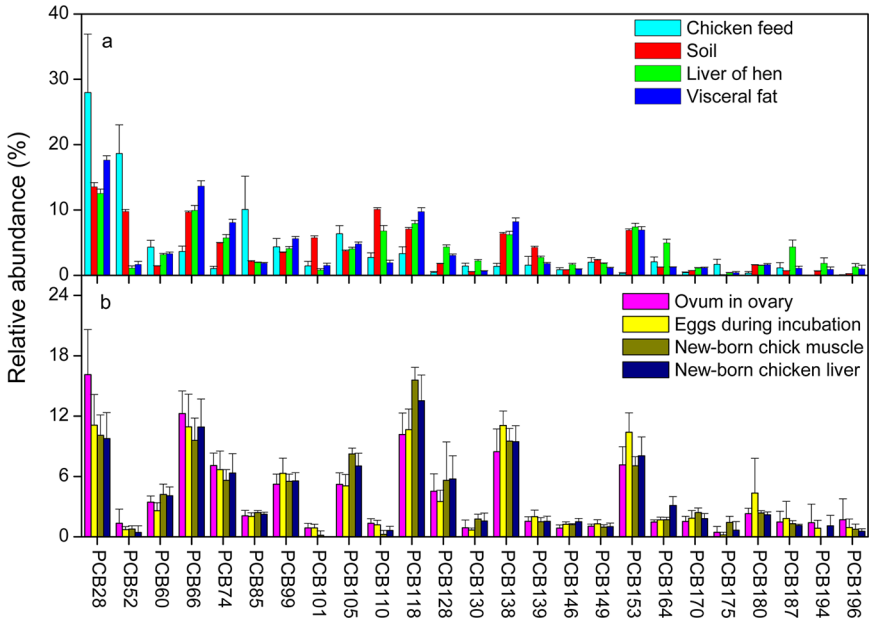

Figure 2. PCB congener contributions to total PCB concentrations were shown for individual groups of samples: dietary sources (chicken feed and soil) and chicken tissues (liver and visceral fat) in panel (a), ovum, eggs (0, 7, and 14 days after the onset of incubation), and newborn chick tissues (liver and muscle) in panel (b).

distribution. The normality test of data was conducted by nonparametric tests, i.e., one sample K−S test. The statistical differences in the PCB concentrations and EFs of chiral PCBs between different groups of samples were determined by one-way ANOVA with Tukey's Post Hoc test.

RESULTS AND DISCUSSION

Bioaccumulation and Tissue Distribution in Chicken. Total PCB levels in chicken feed and soil were 3.8−25, with a median of 8.8 ng/g dry weight (dw) and 19000−25000 with a median of 23000 ng/g dw. The PCB concentrations in chyme (collected from crop of chicken) were between 11 and 1100 ng/g dw, with a median of 40 ng/g dw. The EFs of PCBs 95, 132, 135, and 149 in soil samples were 0.51 ± 0.01, 0.46 ± 0.03, 0.51 ± 0.04, and 0.51 ± 0.02, respectively (Table 1, Figure 1; levels of chiral PCBs are shown in Table S1, Supporting Information). EF values for the above chiral PCBs were obtained for only one feed sample (0.50, 0.62, 0.47, and 0.51, respectively). Except for PCB 132, the EF values of chiral PCBs in feed were similar to those in the soil. As EF was only available in one feed sample and they are similar to EFs in soil except PCB 132, the EF values in soil were used in statistical analyses between soil and chicken tissues.

Median PCB concentrations were 104−1070 ng/g lipid weight (lw) in seven chicken tissues, and no significant differences were found in PCB levels among seven tissues (one-way ANOVA, $p > 0.05$). There were also no differences in PCB congener profiles among the seven tissues. However, compared with soil and feed samples, chicken tissues had much lower amounts of PCBs 52 and 101 (Figure 2, only liver and fat are shown for better readability; PCB congener profiles for all tissues are given in Figure S1, Supporting Information). This difference was derived from the PCB metabolism in chicken.[23,24] The gastrointestinal absorption and tissue distribution of PCBs in chicken is explained in another paper.[25]

The EF values of PCBs 95, 132, and 149 changed significantly from dietary sources to chicken tissues (Table 1, Figure 1) (one-way ANOVA, $p < 0.05$). EFs of PCBs 95, 132, and 149 increased from nearly racemic in soil to 0.66−0.83, 0.60−0.83, and 0.71−0.83 in chicken tissues, respectively. PCB 135 showed no significant differences among soil and chicken tissues, except liver, where EF values were significantly higher than in soil. These results suggest a preference for (+)-enantiomer accumulation for all chiral PCBs in chicken. The nonracemic enantiomeric composition of chiral PCBs in organisms was reported to be caused by diet/prey or by *in vivo* biotransformation.[5] In the present study, the chiral PCBs could not be detected in the chicken gastrointestinal tract contents (chyme, intestinal contents, and feces), and it was not possible to investigate the chiral signatures during gastrointestinal absorption. In a study of gastrointestinal absorption of hexabromocyclododecane (HBCD) in fish, no enantioselective uptake was observed.[26] Considering the greatest enantiomeric enrichment in chicken liver for four chiral PCBs (Figure 1 and also discussion in the next), the EF change in the chicken should be attributed to *in vivo* biotransformation rather than gastrointestinal absorption. Nonracemic EFs of chiral PCBs (PCBs 91, 95, and 149) were observed in several seabird species, while racemic EFs were found in prey (zooplankton and fish), which suggested an avian biotransformation process.[7,8] These seabirds were believed to more efficiently detoxify PCBs than lower tropic level biota because of their higher CYP (cytochrome P450) enzyme activity levels.[8]

Among the seven tissues, liver had the highest EF values (for PCBs 132, 135, and 149) as compared to other tissues (one-way ANOVA, $p < 0.05$), and no significant differences were found in EFs among other tissues. For PCB 95, liver had the highest EF values (0.83) among all tissues (0.66−0.80). Moreover, liver also had significantly higher EF values (for PCB 135:0.59 ± 0.02) than soil (0.46 ± 0.03), although other chicken tissues did not have statistically different EF fractions with soil (Figure 1). Liver is well known to be the detoxification organ for PCBs, as the cytochrome P450 enzymes efficiently metabolize xenobiotic in the liver.[27−30] The rat CYP 2B1 enzyme degrades PCBs 45, 84, 91, 95, and 136 enantioselectively in *in vitro* assays.[28] The high

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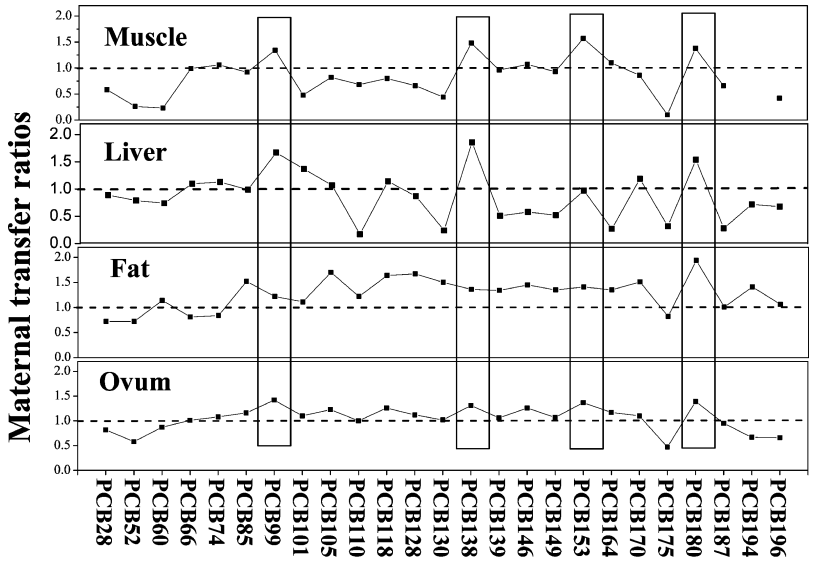

Figure 3. Maternal transfer ratios calculated by median PCB levels in eggs divided by those in different maternal tissues (muscle, liver, and visceral fat) and ovum.

activity of CYP enzymes involved in PCB metabolism might be the reason for the alternative chiral PCB signatures found in chicken liver.

Maternal Transfer. In order to fully elucidate the maternal transfer process from hen to eggs, we calculated transfer ratios using median concentrations (lipid normalized) of the chemicals in the eggs divided by those found in chicken tissues such as muscle, liver, fat, and ovum (Figure 3). The calculations were not conducted for serum because of the low detection frequencies (less than 50%) of almost half of the PCB congeners in serum. Muscle was chosen because it is the largest tissue in terms of mass and thereby could be an important endogenous store for xenobiotic. Previous studies have demonstrated some oviparous organisms (e.g., Atlantic salmon) use muscle lipid for egg formation.[31] The liver is directly linked with the maternal transfer of pollutants because the major constituents of the egg, i.e., yolk, are synthesized in the liver and transported to the oocyte for uptake. A full-size ovum is also called an egg yolk. In the present study, ovum was partly perfused with yolk materials in ovary (diameter less than 1.5 cm). Therefore, a comparison between ovum and egg was expected to provide information regarding *in ovo* metabolism, or microbial transformation, in eggs. Only egg samples from before the incubation process (0 day after the onset of incubation) were used because PCB levels in eggs increase during incubation due to the consumption of lipid, which will be discussed in the next section.

Liver and muscle exhibited similar transfer ratios for PCBs 99, 138, 153, and 180, and these were more easily deposited in eggs (Figure 3). For example, the maternal transfer ratios calculated based on muscle tissue ranged from 0.10 (PCB 175) to 1.57 (PCB 153). PCBs 52, 60, 101, 130, 175, and 196 have transfer ratios less than 0.5, while PCBs 138 (1.48), 153 (1.57), and 180 (1.38) exhibited the highest ratios among PCB congeners. As suggested in a previous study,[14] it seemed that the maternal transfer ratios were selective for the more bioaccumulative and persistent PCB congeners in muscle, namely, PCBs 138, 153, and 180. This could be attributed to the fact that chickens may invest a much larger percentage of lipid or energy in their eggs; therefore, more persistent and bioaccumulative congeners and compounds, along with the lipid storage in chicken, are more likely to be transferred to the eggs.[14] Almost all PCB congeners showed egg/ovum ratios around 1 (Figure 3), suggesting that the observed higher maternal transfer potentials for PCBs 138, 153, and 180 were not the result of *in ovo* metabolism or microbial transformation.

However, when visceral fat was used to calculate the maternal transfer potential for individual PCB congeners, a different scenario was observed. The calculated maternal transfer ratios were relatively uniform for most of congeners, especially for penta- to hepta-PCB congeners (Figure 3). This result was consistent with the report by Tanabe et al.,[16] in which the maternal transfer ratios of the PCB homologue concentrations in subcutaneous fat of Adelie penguin to its eggs did not vary.

The four chiral PCBs were detected in almost all eggs (Figure 1). The EF values of PCB 95 in eggs showed no significant differences from those in maternal tissues, except for in muscle, in which EF values were significantly lower than in eggs. Regarding PCBs 132 and 149, EFs in eggs were significantly different from those in liver but were not statistically different from those in other tissues. The EFs of PCB 135 in eggs showed no remarkable differences to those in maternal tissues. Muscle has been widely used to represent the maternal concentrations for investigating maternal transfer processes of oviparous organisms.[15] Liver has also demonstrated involvement in the modification of lipoproteins during maternal transfer.[32,33] However, the chiral signature of PCBs 95, 132, and 149 in eggs either did not match well with chicken muscle and did not match well with chicken liver. The high enzymatic activities in liver could be the reason for the difference between liver and egg. The difference in EF between muscle and egg suggested that the lipid from muscle is not the main source of yolk. In contrast, visceral fat exhibited EFs similar to those in eggs for all four chiral PCBs. Combined with the relative uniform ratio of egg/visceral fat, this suggests that lipid in fat tissue seems to be directly utilized to form eggs, and PCBs deposited to the eggs are associated with this lipid. Drouillard et al.[32] have demonstrated that yolk lipids did not resemble those from the food intake at the time of egg formation, as the isotopic ratios of PCB 153 in yolk lipids were identical to those in carcass lipids after introduction of diet. In previous studies, different mother tissues used in maternal transfer

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assessment could obviously alter the results of transfer ratios. The results in the present study suggest that PCB deposition in visceral fat could directly reflect the maternal transfer potential of PCBs in chicken.

Chicken Embryo Development and Tissue Differentiation. The PCB levels ranged from 65 to 1050, 376 to 1150, and 644 to 1570 ng/g lw in eggs 0, 7, and 14 days after the onset of incubation, respectively. Eggs collected 14 days after the onset of incubation had significantly higher PCB concentrations than eggs collected at 0 and 7 days (one-way ANOVA, $p < 0.05$). The PCB concentrations in chick liver and muscle were between 861−3840 and 20−4860 ng/g lw, which were both higher than those in eggs during incubations (one-way ANOVA, $p < 0.05$). This was likely caused by lipid consumption during the incubation. The lipid contents in the muscle and liver (6.3 ± 1.7 and 13.1 ± 2.8%, respectively) were significantly lower (14.3 ± 2.9%) than in eggs at 14 days after the onset of incubation (one-way ANOVA, $p < 0.05$).

PCB congener patterns did not significantly alter during the embryo development process, with PCBs 28, 66, 118, 138, and 153 being the most abundant congeners (Figure S2, Supporting Information). However, PCB 118 was the most abundant congener in the newborn chicks, with slightly decreased proportions of PCBs 138, 153, and 180 (Figure 2). Among 30 egg samples, we found that six samples exhibited a significantly different PCB congener profile than the remaining 24 egg samples. The abundances of highly chlorinated congeners (PCBs 153, 170, 180, 187, 194, and 196) were higher in the six samples than in the other 24 samples (Figure S3, Supporting Information). This was likely caused by the exposure to a highly chlorinated PCB source when hens laid these eggs. In the 24 eggs, there were no significant differences in PCB congener profiles between eggs and newborn chicks (Figure S3, Supporting Information). Thus, we highly suspected that the nine eggs where chicks had hatched exhibited similar congeners to those in the 24 egg samples. In this case, there were no significant differences in abundances of PCBs 118, 138, 153, and 180 between eggs and newborn chicks. PCB 101 was detected in all egg samples but was detectable in only one newborn chick muscle and one newborn chick liver sample, which suggested a further metabolism for PCB 101.

No significant changes in chiral PCB signatures were observed among eggs during incubation (0, 7, and 14 days after the onset of incubation) (Figure 1), suggesting no enantiomeric selective biotransformation during the first 14 days of incubation. All the four chiral PCBs were detectable in chick liver. However, only PCB 132 was detected in all chick muscle samples; PCBs 135 and 149 were detected in three of nine chick muscle samples, while PCB 95 was not detected.

Compared with eggs, the newborn chicks exhibited a further enantiomeric enrichment for PCBs 95 and 149, especially for chick liver, where only (+)-PCB 95 and (+)-PCB 149 (EFs = 1) were detected (Figure 1). PCBs 132 and 135 in chick liver exhibited greater enrichment for the (+)-enantiomer (0.81 ± 0.02) and (−)-enantiomer (0.42 ± 0.02), respectively, compared with eggs incubated for 14 days (0.72 ± 0.02 and 0.55 ± 0.02). Meanwhile, chick muscle had lower EFs of PCB 132 (0.65 ± 0.02) than eggs incubated for 14 days. Of the 21-day development period for the chick embryo, the last seven days are notable as an intense period of lipid metabolism and rapid embryonic growth. Nearly 80% of the entire lipid content of the yolk is mobilized and absorbed into the embryonic tissues over this time, while carbohydrate and protein metabolism predominate during the two preceding weeks.[33,34]

The alternation of enantiomer composition followed the same trend for PCBs 95, 132, and 149 between mature hens and newborn chicks. However, a reverse trend was observed for chiral PCB 135. A preference for (+)-PCB 135 was exhibited in livers of mature hens, while a preference for (−)-PCB 135 was observed in newborn chick livers (Figure 1). PCB enantiomers may interact differently with enzymes in chicken embryo hepatocytes. For example, (+)-PCB 197 had a greater capacity for inducing total CYPs than did (−)-PCB 197 in chick embryo hepatocytes, whereas the enantiomers of PCBs 88 and 139 had equal potencies as inducers of cytochrome P450.[20] The reasons newborn chicks exhibited enantioselectivity of PCB 135 opposite to that of mature hens were not clear. Little information was available on the different metabolism or other biological processes in mature and newborn creatures affecting chiral PCBs. The biotransformation or binding affinity of CYP enzymes with chiral PCBs should be investigated in both mature and newborn chickens in further studies. Additionally, the metabolism of PCBs during chicken embryo development indicated a highly toxicological risk caused by possible PCB metabolites in newborn chicks. Some of the PCB metabolites, such as OH-PCBs and $MeSO_2$-PCBs, are often considered to be more toxic than the parent molecule itself.[35−37] In addition, the chiral PCB 135 signatures changed differently in adult chicken and chick embryo development, suggesting different toxicological parameters of certain contaminant for adults and new lives, which deserved more attention.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed procedures on sample pretreatment and instrumental analysis; additional table on chiral PCB concentrations; and additional figures on PCB congener profiles in seven chicken tissues, eggs, and chick tissues. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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