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Research Paper

Significance of biotransformation and excretion on the enantioselective bioaccumulation of hexabromocyclododecane (HBCDD) in laying hens and developing chicken embryos

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ABSTRACT

Although (–)-α-hexabromocyclododecane (HBCDD) and (+)-γ-HBCDD are preferentially enriched in chickens, the key factors contributing to their selective bioaccumulation in hens and their potential biotransformation in developing chicken embryos remain unclear. Herein, *in vivo* and *in ovo* exposure experiments using hens and fertilized eggs were conducted to investigate the absorption, excretion, and biotransformation of HBCDDs in chickens. γ-HBCDD (76%) exhibited a higher absorption efficiency than α- (22%) and β- (69%) HBCDDs. However, α-HBCDD was dominant in hen tissues, although γ-HBCDD accounted for >75% in the spiked feed. Moreover, chicken embryos biotransformed approximately 9.5% and 11.7% of absorbed α- and γ-HBCDDs, respectively, implying that diastereomer-selective elimination causes the predominance of α-HBCDD in hens. The concentration and enantiomer fraction (EF) of α-HBCDD in laid eggs were significantly positively correlated, suggesting enantioselective elimination. The EFs of α- and γ-HBCDDs varied between feces from the exposure and depuration periods, indicating the preferred excretion of (+)-α- and (–)-γ-HBCDDs. Furthermore, the enantioselective biotransformation of (–)-γ-HBCDD was confirmed in developing chicken embryos. These results show that excretion and biotransformation contribute to the diastereomer- and enantiomer-selective bioaccumulation of HBCDDs in chickens; The results may improve our understanding of the environmental fate and ecological risks of HBCDDs in biota.

1. Introduction

Hexabromocyclododecane (HBCDD) is among the most widely produced additive brominated flame retardants. It is primarily used in polystyrene insulation foams, upholstery fabric, building materials, and electronic equipment (Covaci et al., 2006; Kuribara et al., 2019). Although 16 stereoisomers are present, technical HBCDD mixtures mainly consist of three racemic isomers: α- (~10%), β- (~10%), and γ-HBCDDs (~80%) (Cariou et al., 2020). HBCDDs have been abundantly detected in environmental matrices (Covaci et al., 2006; Fromme et al., 2014), wildlife (Mukai et al., 2020; Poma et al., 2014), and humans (Meijer et al., 2008; Ryan and Rawn, 2014); Furthermore, that have been reported to have reproductive and embryonic toxicity on avian species (Crump et al., 2010; Fernie et al., 2009, 2011). Due to their persistence, bioaccumulation, and adverse effects on organisms, HBCDDs have been listed as the Stockholm Convention persistent organic pollutants (POPs) in 2013 (Cariou et al., 2020), an exemption was allowed to expanded polystyrene and extruded polystyrene foam from 2016 to 2021 (Zhang et al., 2018). However, they still warrant attention due to their environmental concerns.

Although γ-HBCDD is the predominant isomer in technical HBCDD formulations and abiotic samples, α-HBCDD is predominant in most biota (Janák et al., 2005; Law et al., 2014). Laboratory feeding studies have shown the preferential accumulation of α-HBCDD in avians and

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fish following technical exposure to HBCDD mixtures. This could be due to the diastereomer-selective absorption, elimination, and bioisomerization of the three diastereomers (Du et al., 2012; Letcher et al., 2015; Luo et al., 2013; Zheng et al., 2017). Du et al. (2012) reported bioisomerization from γ- to α-HBCDD in zebrafish. Similar results have also been observed in American kestrels (Letcher et al., 2015) and hens (Fournier et al., 2012). However, inconsistent results have been obtained in mirror carp (Esslinger et al., 2010), as no evidence for isomerization was found following exposure to γ-HBCDD via diet. Moreover, Fournier et al. (2012) confirmed that only 0.025–0.17% of ingested γ-HBCDD was transformed to α-HBCDD in hens. These results indicate that bioisomerization may not be the dominant reason for the predominance of α-HBCDD in biota, and diastereomer-selective absorption, excretion, and biotransformation may have greater impacts. However, these processes have rarely been studied.

The enantiomeric enrichment of HBCDDs have been reported in birds due to stereospecific biological processes. The preferential accumulation of (+)-α-HBCDD has been observed in guillemots (*Uria aalge*) and sea eagles (*Haliaeetus albicilla*) (Janák et al., 2008), whereas the enrichment of (–)-α-HBCDD has been observed in herring gulls, peregrine falcons, glaucous gulls, and passerine birds (Janák et al., 2005, 2008; Sun et al., 2012; Vorkamp et al., 2012). Limited studies have indicated the preferential enrichment of (+)-γ-HBCDD in terrestrial passerine birds (Sun et al., 2012). Recently, Zheng et al. (2017) and Omer et al. (2017) found enantioselective enrichment of (–)-α-HBCDD in chickens. However, an *in vitro* study (Zheng et al., 2015b) using chicken liver microsome has revealed the absence of enantiomeric bioisomerization for α-, β-, or γ-HBCDD. The specific reasons for the alteration in enantiomer fraction (EF) from diet to avian tissues remain unclear. Although the metabolism of HBCDDs have been indicated in adult avians (Dominguez-Romero et al., 2016; Letcher et al., 2015), little is known regarding the biotransformation and distribution of HBCDDs in the early life stages of avians. A previous study (Zheng et al., 2017) suggested that the EFs of α-HBCDD differed between hen and hatchling chick tissues. As chiral isomers could interact with different enzymes or endogenous compounds (Lu and Wong, 2011), it is therefore important for HBCDD toxicological studies, as avian embryos are more sensitive to xenobiotic pollutants than adults.

To address the existing knowledge gaps on diastereomer- and enantiomer-selective accumulation of HBCDDs in avians, here comparative studies were conducted on laying hens and developing chicken embryos. Hens were fed with technical HBCDD mixtures, and their laid eggs, feces, and tissues were examined. Fertilized chicken eggs were injected with known amounts of α- and γ-HBCDDs to evaluate their potential metabolisms, via chemical mass balance. The primary objectives of this study were to investigate the factors determining the diastereomer- and enantiomer-selective bioaccumulation of HBCDDs in hens and neonatal chicks, to improve our understanding of the environmental fate and ecological risk of chiral chemicals in avians.

2. Materials and methods

2.1. Standards and reagents

Standard solutions of α-, β-, and γ-HBCDDs, and HBCDD mixture were purchased from AccuStandard (New Haven, CT, USA). Standards of ^{13}C-α-, β-, and γ-HBCDDs and d_{18}-α-, β-, and γ-HBCDDs were obtained from Cambridge Isotope Laboratories (Andover, MA) and Wellington Laboratories (Guelph, Ontario, Canada), respectively. Pesticide-grade methanol, *n*-hexane and dichloromethane were acquired from CNW Technologies GmbH (Dusseldorf, Germany). Guaranteed reagent-grade dimethyl sulfoxide (DMSO), concentrated sulfuric acid, florisil, silica gel, and anhydrous sodium sulfate were supplied by Guangzhou Chemical Reagent Factory (Guangzhou, China).

2.2. In Ovo exposure

Chicken eggs (*Gallus domesticus*, n = 20) were collected from a local hatchery and were washed with 75% v/v aqueous ethanol solution. Three eggs were sampled to determine background levels of the target chemicals, which proved to be below the detection limit. According to the egg injection protocol validated in our previous study (Li et al., 2016b), DMSO was used as the vehicle, and the concentrations of α- and γ-HBCDDs in the DMSO were nominated as 15.0 and 2.0 ng/μL, respectively. Eggs were held upright and a hole was made on the round end (air cell) using a sterile needle. A syringe was pushed through the shell to reach the yolk, and a constant volume (20 μL/egg) of the DMSO solution was injected into the yolk. Four eggs (day-0 eggs) were randomly sampled to determine the exposure doses, and the other injected eggs were incubated for hatching. Seven chicks were successfully hatched on day 21. Neonatal chicks were euthanized with nitrogen, and tissues (liver, heart, stomach, and remaining yolk) were dissected excised and stored at −20 ℃ until further analysis. The study was approved by the Ethics Committee in the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences and all methods were performed in accordance with the relevant guidelines and regulations.

2.3. Exposure of hens

Commercial HBCDD mixture was dissolved in methanol and mixed with 1 kg of chicken feed under constant agitation to prepare the spiked feed. Four-month-old hens (n = 5) were purchased from a local farm, and were housed in cages separately. One gram of the spiked feed was given to each hen per day with control feed during the exposure period (30 days). Followed by 52 days of depuration period, in which only control feed was supplied. Spiked feed samples (n = 3) were collected at the beginning, middle and the end of the exposure period to determine the doses of HBCDD diastereoisomers. Hen feces samples were collected over three days in both exposure (n = 3) and depuration (n = 3) periods. Laid eggs (n = 20) were collected in depuration period (see Supporting Information, Table S1). Three hens were used as control group and were treated with control feed during the experiment. Target analytes were not detected in all the samples of the control group.

2.4. Sample preparation and analysis

Samples were extracted and purified as described in previous studies (Li et al., 2019; Zheng et al., 2017) with minor modification. Briefly, 2 g of lyophilized samples (or the entire sample if less than 2 g) were spiked with internal standards (^{13}C-α-, β-, and γ-HBCDDs), followed by Soxhlet extraction with 200 mL hexane/dichloromethane (1/1, v/v) for 48 h. an aliquot of the extract was used to determine lipid content by gravimetric method, and the remaining extract was treated with concentrated sulfuric acid for lipid removal. A multilayer gel column, packed with florisil, neutral silica, acid silica, and anhydrous sodium sulfate, was used for further purification, and eluted with 40 mL hexane and 40 mL dichloromethane. The combined eluate was concentrated to near dryness under a stream of nitrogen and reconstituted in 200 μL of methanol. Known amounts of recovery standards (d_{18}-α-, β-, and γ-HBCDDs) were spiked prior to instrumental analysis. For potential HBCDD metabolites, another 30 mL acetone: dichloromethane (1:1, v/v) eluate was collected and concentrated to 100 μL in methanol.

HBCDDs were analyzed on an Agilent 1200 series liquid chromatograph coupled with an Agilent 6410 triple quadrupole mass spectrometer with an electrospray interface in negative ionization mode. HBCDD diastereoisomers and enantiomers were separated using an XDB-C18 column (5 cm × 4.6 mm × 1.8 μm; Agilent) and a Phenomonex Nucleosil β-PM chiral column (20 cm × 4 mm × 5 μm; Macherey-Nagel, GmbH & Co., Germany), respectively. Details of the instrumental parameters for HBCDDs are given elsewhere (Zheng et al., 2017). Identification of HBCDD metabolites was performed according to a previously reported

method (Zheng et al., 2015b), which is provided in the Supporting Information.

2.5. Quality assurance and quality control

The methods for quality control were performed by regular analysis of procedural blanks, spiked blanks, spiked matrices (egg and muscle samples), and replicates. No HBCDDs were detected in procedural blanks. The recoveries of the internal standards were 82 ± 9–91 ± 13%. The recoveries of spiked α-, β-, and γ-HBCDDs were 86 ± 5 to 101 ± 11%. The relative standard deviations (RSDs) for the analytes were <15% (n = 3). The limits of quantification (LOQs) were set as signal-to-noise ratio of 10, and ranged from 0.02 to 0.09 ng/g lw for α-, β-, and γ-HBCDDs in all the samples. Chiral HBCDD signature was expressed as enantiomer fractions, which were defined as the ratio of (+) enantiomer and the sum of (+) and (–) enantiomers. The enantiomer fraction was corrected by d_{18}-labeled instrument standards according to the method described by Marvin et al. (Marvin et al., 2007).

2.6. Statistical analysis

Statistical analysis was performed with GraphPad Prism 8. The normality was checked by D'Agostino & Pearson test and the data was log transformed to display a normal distribution. Unpaired sample *t*-test was used to investigate the difference of EFs between feces and spiked feed. Paired samples *t*-test was used to examine the difference in α-HBCDD concentrations among hen tissues. One-way ANOVA was conducted to assess the difference in EFs between hen tissues and spiked feed, and between day-0 eggs and chick tissues, and to assess the difference in percentages of α-HBCDD among chick tissues. Pearson correlation analysis was performed to examine the association between egg concentrations and EF values of α-HBCDD. The criterion for significance was set at $p < 0.05$ throughout the study.

3. Results and discussion

3.1. Bioaccumulation of HBCDDs in hens

The mean concentrations of the α-, β-, and γ-HBCDDs in spiked chicken feed (n = 3) were 58.3 ± 6.17, 31.2 ± 3.17, and 275 ± 15.3 ng/g (dry weight), respectively (Table 1). The spiked feed comprised mean proportions of 15 ± 0.8%, 9 ± 0.3%, and 76 ± 1.0% of the α-, β-, and γ-HBCDD isomers, respectively (Fig. 1). These values are comparable to those of technical HBCDD mixtures (Letcher et al., 2015). The difference in HBCDD mass between the spiked feed and chicken feces from the exposure period was defined as the absorption in the gastrointestinal tract. The gastrointestinal absorption efficiencies of the α-, β-, and γ-HBCDDs were 22%, 69%, and 76%, respectively. The efficiency of α-HBCDD was considerably lower than those of β-, and γ-HBCDDs, suggesting that stereo-selective gastrointestinal absorption occurs in hens. Relatively low lipophilicity (5.07 and 5.47 for α- and γ-HBCDDs) (Wu et al., 2010) may contribute to this result. A previous study (Zheng et al., 2017) reported a lower accumulation ratio for γ-HBCDD (0–2.40) than for α-HBCDD (4.27–12.9), which could be attributed to the higher elimination rate for γ-HBCDD than for α-HBCDD (Letcher et al., 2015). Although α-HBCDD was detected in chicken tissues following exposure to γ-HBCDD, Fournier et al. (2012) found that only a small percentage of ingested γ-HBCDD was transformed to α-HBCDD in hens, suggesting that the bioisomerization of γ-HBCDD was not the main reason for the observed higher elimination rate. Thus, diastereomer-selective biotransformation is the more likely explanation for the lower accumulation ratio and faster elimination rate of γ-HBCDD in hens compared to α-HBCDD. The EFs of α-, β-, and γ-HBCDDs in feces from the exposure period exhibited no significant (unpaired sample *t*-test, $p > 0.05$) variation compared to those in the spiked feed (Fig. 2). This indicates the absence of enantioselective absorption.

α-HBCDD was detected in hen liver, abdominal fat, intestine, and kidney, whereas γ-HBCDD was only detected in intestine and abdominal fat, and β-HBCDD was not detectable in hen tissues (Table 1). Higher amounts of α-HBCDD (contributing to >80%) were present in hen tissues, despite the quasi absence of this isomer in spiked feed. This is consistent with the previous report that α-HBCDD is the dominant HBCDD stereoisomer in biota (Law et al., 2014; Marvin et al., 2011). A previous laboratory study (Letcher et al., 2015) also observed more than 70% of α-HBCDD in the liver and fat tissues of American kestrels following dietary exposure to technical mixtures of HBCDDs. Although not significant (paired-samples *t*-test, $p > 0.05$), α-HBCDD levels were lower in liver tissue than in other tissues (Table 1), which is consistent with the findings of previous studies on avians (Dominguez-Romero et al., 2016; Zheng et al., 2017). One possible explanation is that the biotransformation of HBCDDs occurred in liver tissue, as the liver is an important organ for xenobiotic metabolism (Li et al., 2019). The EFs of α- and γ-HBCDDs in hen tissues differed significantly (one-way ANOVA, $P < 0.05$) from those in the spiked feed (Fig. 2), indicating the preferential enrichment of (–)-α-HBCDD and (+)-γ-HBCDD in hens. This is consistent with prior analysis of chickens collected from an e-waste

Table 1
Concentrations of HBCDD diastereoisomers and EF values of HBCDD enantiomers in spiked feed, feces, hen tissues, laid eggs, injected eggs, and chick tissues.

Samples	N	Lipid (%)	α-HBCDD	β-HBCDD	γ-HBCDD	EFs of α-HBCDD	EFs of β-HBCDD	EFs of γ-HBCDD
Spiked feed[a]	3	–	58.3 ± 6.17	31.2 ± 3.17	275 ± 15.3	0.481 ± 0.002	0.448 ± 0.004	0.524 ± 0.001
Feces (exposure)[a]	3	–	1.28 ± 0.111	0.274 ± 0.021	1.87 ± 0.229	0.496 ± 0.018	0.454 ± 0.012	0.503 ± 0.013
Feces (depuration)[a]	3	–	0.837 ± 0.279	nd[e]	0.915 ± 0.254	0.512 ± 0.011	–	0.476 ± 0.013
Hen tissues and laid eggs[b]								
Abdominal fat	5	90.3 ± 4.2	2.47 ± 0.97	nd	0.248 ± 0.103	0.404 ± 0.045	–	0.922 ± 0.047
Liver	5	25.8 ± 5.1	0.655 ± 0.21	nd	nd	0.375 ± 0.035	–	–
Intestine	5	44.8 ± 8.4	1.72 ± 0.63	nd	0.281 ± 0.10	0.361 ± 0.043	–	0.922 ± 0.023
Kidney	5	26.6 ± 5.0	1.25 ± 0.46	nd	nd	0.334 ± 0.011	–	–
Developing eggs[c] and chick tissues[d]								
Day-0 eggs	4	28.2 ± 3.1	250 ± 4.70	–	35.0 ± 2.43	0.496 ± 0.004	–	0.504 ± 0.009
Heart	7	12.4 ± 1.3	0.758 ± 0.095	–	nd	0.462 ± 0.035	–	–
Stomach	7	7.4 ± 1.2	1.94 ± 0.603	–	0.274 ± 0.102	0.465 ± 0.039	–	0.683 ± 0.038
Liver	7	40.2 ± 3.9	6.59 ± 1.71	–	0.199 ± 0.047	0.431 ± 0.048	–	0.828 ± 0.024
Yolk	7	37.6 ± 12	49.0 ± 8.66	–	3.84 ± 1.35	0.475 ± 0.023	–	0.685 ± 0.049
Carcass	7	21.4 ± 2.3	174 ± 16.4	–	27.1 ± 4.52	0.528 ± 0.017	–	0.686 ± 0.022
ΣChick	7	–	231 ± 11.5	–	31.4 ± 5.39	–	–	–

[a] ng/g wet weight.
[b] ng/g lipid weight.
[c] ng/egg.
[d] ng/tissue.
[e] not detected.

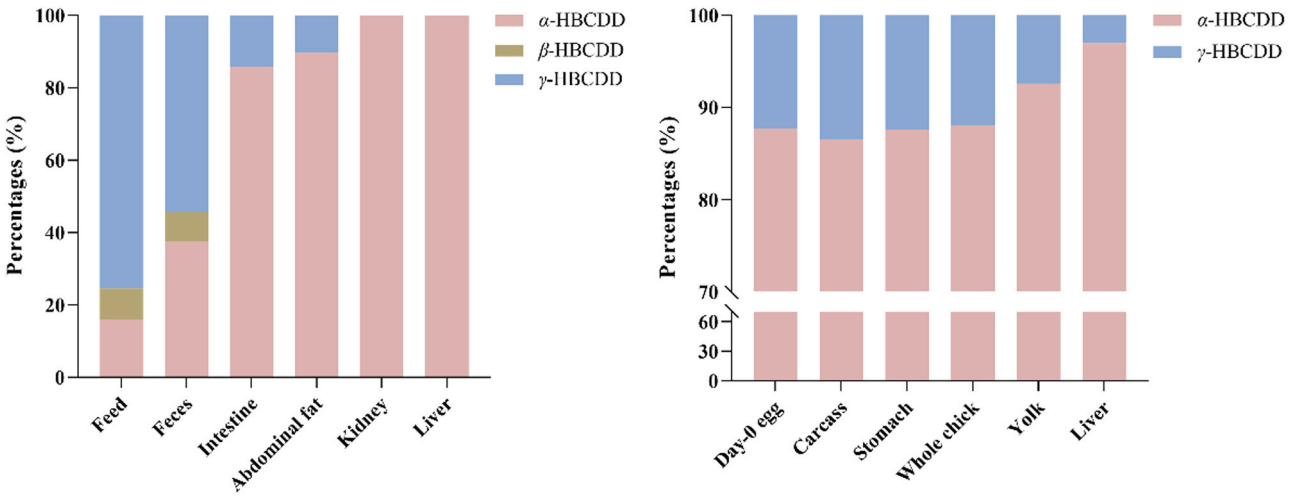

Fig. 1. Profiles of HBCDD diastereoisomers in spiked feed, feces from exposure period, hen tissues, day-0 eggs and neonatal chick tissues.

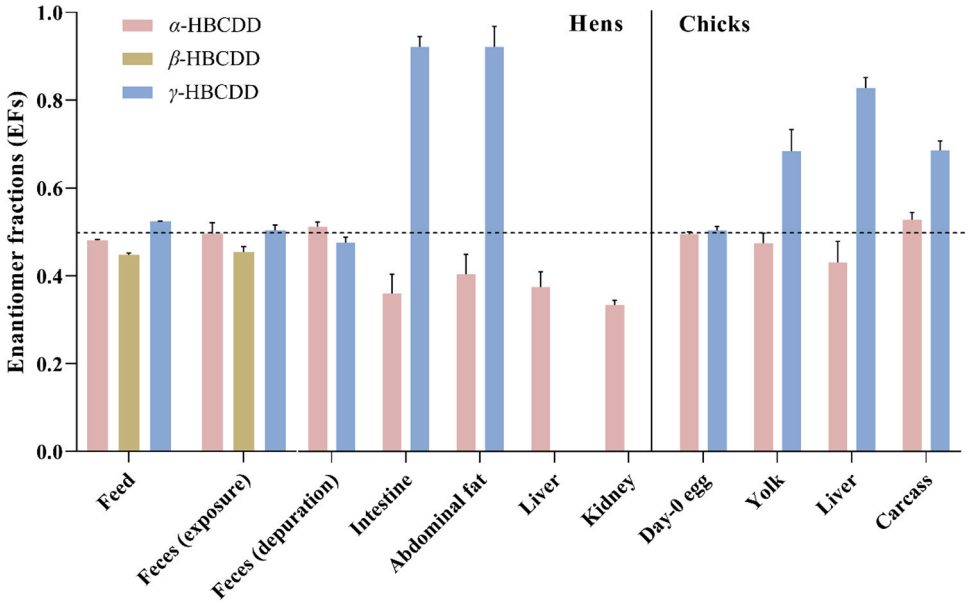

Fig. 2. Enantiomer fractions (EFs) of HBCDDs in spiked feed, feces, hen tissues, day-0 eggs and neonatal chick tissues. The dashed line represents racemic (EF = 0.5).

polluted area (Zheng et al., 2017). Significantly (unpaired sample *t*-test, $p < 0.05$) higher EFs of α-HBCDD and lower EFs of γ-HBCDD were found in feces from the depuration period, compared to feces from the exposure period (Table 1). This may have contributed to the observed enrichment of HBCDD enantiomers. This suggests that enantioselective excretion could be an important factor for preferential enrichment, although enantioselective biotransformation cannot be ruled out.

3.2. Bioaccumulation of HBCDDs in laid eggs

α-HBCDD was detected in all the laid eggs, with concentrations ranging from 0.349 to 3.04 ng/g lw; γ-HBCDD was detectable in 13 of the 20 laid eggs (Table 1). The concentrations of α-HBCDD in the laid eggs decreased over time during the depuration period (Table S1), which is consistent with the trend observed in a prior α-HBCDD exposure study (Dominguez-Romero et al., 2016). The EFs of α-HBCDD decreased whereas the EFs of γ-HBCDD increased in laid eggs. This occurred in accordance with the laying times (Fig. 3), which were the same as those in hen tissues. The greater deviation of γ-HBCDD observed in both hen tissues and laid eggs implies that γ-HBCDD could be more readily

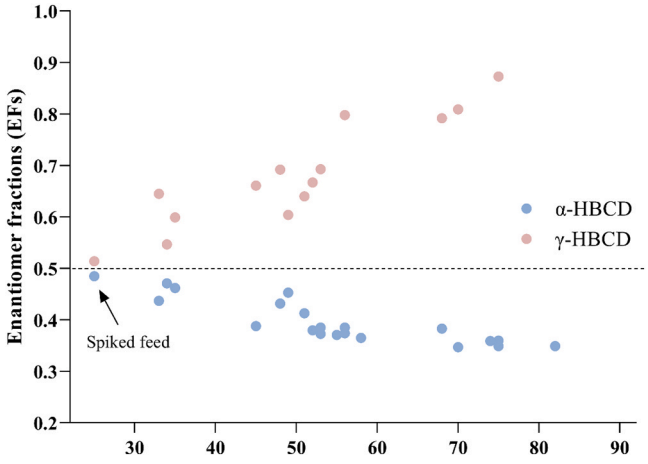

Fig. 3. Enantiomer fractions (EFs) of α- and γ-HBCDDs in laid eggs.

eliminated than α-HBCDD (Letcher et al., 2015). Furthermore, a significantly positive correlation (Pearson correlation analysis, $p < 0.05$, $R^2 = 0.5883$) was observed between egg concentrations and EF values of α-HBCDD in the depuration period, indicating that enantioselective excretion and/or biotransformation occurs in hens. Few studies have investigated the chiral signatures of HBCDDs in avians, suggesting the preferential enrichment of (–)-α-HBCDD in chickens (Omer et al., 2017; Zheng et al., 2017). The present study reveals that enantioselective elimination may account for the observed preferential bioaccumulation of HBCDD enantiomers in avians.

3.3. HBCDD diastereoisomers in developing chicken embryos

To further investigate the selective bioaccumulation of HBCDDs in chickens, α- and γ-HBCDD standards were exposed to developing chicken embryos with the same compositions as those observed in hen tissues (α-HBCDD accounting for ~90%). The difference in chemical amount between the day-0 egg and the remaining yolk was defined as the uptake amount during embryo development. The calculated uptake efficiencies of α- and γ-HBCDDs were 79.9% and 89.2%, respectively; these values are comparable to those obtained for chiral PCBs (~80%) in a previous egg injection study (Li et al., 2016b). γ-HBCDD exhibited relatively higher uptake efficiency than α-HBCDD, which is consistent with the abovementioned gastrointestinal absorption of HBCDDs in hens. In a laboratory feeding study on fish, Luo et al. (2013) observed a lower absorption efficiency for α-HBCDD than for β- and γ-HBCDDs. Similar results were also observed in juvenile rainbow trout (Law et al., 2006).

Compared with the day-0 eggs, the amounts of α- and γ-HBCDDs in neonatal chicks decreased by 7.6% and 10.4%, respectively (Table 1). As the analytes in the remaining yolk were not absorbed by chicken embryos, approximately 9.5% and 11.7% of the α- and γ-HBCDDs were metabolized during embryo development, respectively. Carbohydrate, protein, and lipid metabolism provide the energy needed for embryo development, and most of the lipid content of the yolk is assimilated into the embryonic tissues during this time (Noble and Cocchi, 1990). Xenobiotics could be metabolized during the complicated physiological and biochemical processes, which have been interpreted in previous studies (Li et al., 2016b, 2019). Few studies have examined the *in vivo* biotransformation of HBCDDs via chemical mass balance. A previous exposure experiment (Luo et al., 2013) indicated that more than 26% of initial HBCDDs were metabolized in fish after a 20-d depuration, which is comparable to the findings of our present study.

To further verify the biotransformation of HBCDDs, possible HBCDD metabolites were analyzed in developing chicken embryos. No possible metabolites were observed in the monitored ions in the background eggs, whereas pentabromocyclododecadiene (PBCDD) and tetrabromocyclododecadiene (TBCDD) were identified in neonatal chick liver (Fig. S1). This provides direct evidence for the metabolism of HBCDDs in developing chicken embryos. It is possibly that debromination is an important biotransformation pathway of HBCDDs in chicken. Previous *in vitro* studies using rat, trout, and chicken liver microsomes have reported the presence of debrominated HBCDD metabolites (Abdallah et al., 2014; Zheng et al., 2015b), which is consistent with the findings of our present study.

The composition of α-HBCDD in day-0 eggs was 87.7 ± 0.64%, which is comparable to that of neonatal chicks (88.1 ± 1.6%). However, significantly higher (one-way ANOVA, $p < 0.01$) percentages of α-HBCDD were found in the liver and remaining yolk among all of the chick tissues (Fig. 1). Variance in the lipophilicities and uptake efficiencies of the isomers may have contributed to the different profiles observed in the day-0 eggs and the remaining yolks. Liver showed the highest fraction of α-HBCDD (97.0 ± 0.7%) among all the tissues. Similar results have also been reported in hen liver tissue following oral exposure to γ-HBCDD (Fournier et al., 2012), and an increased proportion of α-HBCDD has also been observed in pipping chick liver tissue following exposure to technical HBCDD mixtures (Crump et al., 2010). Considering that γ-HBCDD has a higher metabolic rate than α-HBCDD, the present study demonstrates that stereo-selective biotransformation occurs in developing chicken embryos, though bioisomerization of γ-HBCDD to α-HBCDD cannot be ruled out.

3.4. HBCDD enantiomers in developing chicken embryo

The α- and γ-HBCDDs were racemic in the day-0 eggs (Table 1). However, significantly different (one-way ANOVA, $p < 0.05$) EFs were found in neonatal chick tissues (Fig. 2), indicating the occurrence of enantioselective enrichment in developing chicken embryos. Deviations in the EFs of both α- and γ-HBCDDs were observed in the remaining yolk, although it was merely considered as cytoplasm containing nutritional reserves. It is therefore reasonable that a bidirectional material exchange could be expected between a chicken embryo and the yolk (Li et al., 2016b). As a main organ for metabolism, the liver exhibited the greatest degree of EF divergence (from 0.5) among all tissues. The enrichment of (+)-γ-HBCDD was observed in all the neonatal chick tissues, but was especially pronounced in liver tissue, elucidating the enantioselective biotransformation/isomerization of (–)-γ-HBCDD in developing chicken embryos. Nevertheless, inconsistent EF divergence of α-HBCDD was observed in the chick tissues. Carcass samples showed a preferential enrichment of (+)-α-HBCDD, whereas (–)-α-HBCDD was selectively enriched in liver tissue and in remaining yolk. Similar results were also reported by Zheng et al. (2017) who found that the EF of α-HBCDD was lower in liver tissue and higher in pectoral muscle tissue than in incubated eggs. This result indicates that enantioselectivity occurs in the transportation process among the tissues of neonatal chicks. A preferential absorption of (+)-α-HBCDD from the remaining yolk to the chick carcass may explain the selective enrichment of (+)-α-HBCDD in the carcass, and the relatively low EF in the yolk. In addition, one possible hypothesis for the preferential enrichment of (–)-α-HBCDD observed in liver is that the enantioselective biotransformation of (+)-α-HBCDD occurred in the chick liver. Differences in EFs for the α-HBCDD bioisomerized from β- or γ- isomers, compared with those from standards, have been shown in earthworms (Li et al., 2016a). This makes it possible that (–)-γ-HBCDD may be bioisomerized into (–)-α-HBCDD in chick liver; such a reaction has previously been reported under thermal stress (Esslinger et al., 2010). Chiral compounds can interact with different enzymes or endogenous chemicals (Lu and Wong, 2011; Rodman et al., 1991). The enantioselective biotransformation of PCB45 and PCB95 has been reported for rat CYP2B1 (Lu and Wong, 2011); however, the enantiomeric changes of HBCDDs were not catalyzed by cytochrome P450 (CYP) enzymes in a study on chicken liver microsomes (Zheng et al., 2015b). *In vivo* biotransformation includes both phase I and phase II enzymes, which can metabolize and/or bind with HBCDD enantiomers in developing chicken embryos. Previous studies (Li et al., 2016b; Zheng et al., 2015a) have elucidated that enantioselective biotransformation can contribute to the enrichment of (+)-atropisomer for PCB95, PCB132, and PCB149 in chickens. The present study, for the first time, revealed the enantioselective biotransformation of HBCDDs in developing chicken embryos. This finding implies that enantioselective biotransformation could be an important factor for the enantiomeric bioaccumulation of chiral chemicals in avians.

3.5. Tissue-specific distribution of HBCDDs in neonatal chicks

The ratio of tissue to total chick weight and the ratio of tissue analyte burden to total chick analyte burden were compared to evaluate the tissue-specific distribution of HBCDDs in neonatal chicks (Fig. 4A). α- and γ-HBCDDs were observed to be enriched in chick yolk. The remaining yolk contributed to 12% of the total chick weight, and α- and γ-HBCDDs accounted for 22% and 12% of the total analyte burden in the yolk, respectively. α-HBCDD tended to remain in the yolk, which could

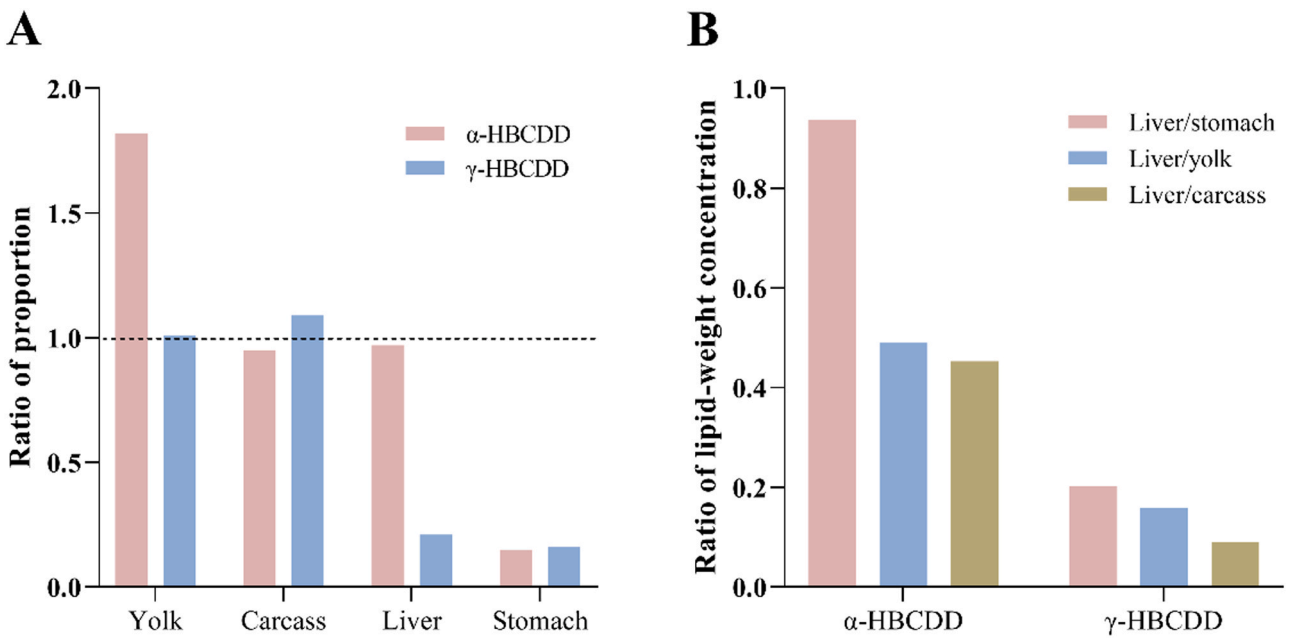

Fig. 4. (A) Proportions of α- and γ-HBCDDs is chick tissues relative to those in total chick compared to the percentages of the tissue weight to total chick weight. (B) Concentration (ng/g lipid weight) ratios of α- and γ-HBCDDs between chick liver and other tissues (stomach, yolk, and carcass).

be due to the lower absorption efficiency of α-HBCDD compared to that of γ-HBCDD. Stomach tissue exhibited defects for both HBCDDs; comparable percentages were found in carcass tissue, although γ-HBCDD exhibited a degree of enrichment. The different binding affinities of the target chemicals with endogenous lipids and proteins in embryos during tissue differentiation and organogenesis (Zheng et al., 2014) may have contributed to these results. Liver tissue showed defects for both isomers, especially for γ-HBCDD. This could be attributed to stereoselective biotransformation. Lipid-normalized concentrations also revealed insufficient accumulation of HBCDDs in chick liver tissue (Fig. 4B), thereby indicating possible impact of hepatic clearance on the tissue distribution of HBCDDs. A previous study (Li et al., 2016b) found that chick liver tends to enrich highly lipophilic chemicals, and a further study (Zheng et al., 2017) has suggested that chemicals with a log K_{OW} lower than 7.5 preferentially accumulate in pectoral muscle tissue, rather than liver tissue. HBCDD diastereomers have log K_{OW} values between 5.4 and 5.8 (Marvin et al., 2011), which could explain the preferential accumulation of α- and γ-HBCDDs in the chick carcass observed here.

4. Conclusions

In conclusion, diastereomer-preferred biotransformation for γ-HBCDD to α-HBCDD was confirmed in developing chicken embryo by chemical mass balance, as excretion was not expected to occur there. This indicates that the preferential accumulation of α-HBCDD in chicken may be attributed to diastereomer-specific elimination, as γ-HBCDD was more readily absorbed than α-HBCDD. However, the preferential enrichments of (–)-α- and (+)-γ-HBCDDs were observed in hens, and a significantly positive correlation was observed between the concentrations and EFs of α-HBCDD in laid eggs, indicating the occurrence of enantioselective excretion and/or biotransformation. The deviations of EFs in feces between the depuration and exposure periods suggest the preferred excretion of (+)-α- and (–)-γ-HBCDDs. Meanwhile, the enantioselective biotransformation of (–)-γ-HBCDDs was confirmed in developing chicken embryos. This study highlights the significance of excretion and biotransformation in the diastereomer- and enantiomer-selective bioaccumulation of HBCDDs in chicken. These processes are crucial in understanding the environmental fate and ecological risk of chiral chemicals in biota.

Supporting information

Additional experimental details. Table showing concentrations and enantiomer fractions of HBCDDs in laid eggs. Figure showing mass spectra of major ion clusters for possible HBCDD metabolites in chicks.

CRediT authorship contribution statement

Yun-Jiang Yu: Conceptualization, Supervision, Writing – original draft. **Zong-Rui Li:** Conceptualization, Methodology, Data curation, Writing – original draft. **Yu Zhu:** Data curation, Formal analysis, Investigation. **Liang-Zhong Li:** Methodology, Formal analysis, Investigation. **Luo-Hong Zhang:** Formal analysis, Investigation. **Ming-Deng Xiang:** Formal analysis, Investigation. **Eddy Y. Zeng:** Writing – review & editing, Supervision. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126749.

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