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Hexabromocyclododecane in terrestrial passerine birds from e-waste, urban and rural locations in the Pearl River Delta, South China: Levels, biomagnification, diastereoisomer- and enantiomer-specific accumulation

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ABSTRACT

Diastereoisomers and enantiomers of hexabromocyclododecane (HBCD) were determined in muscle and stomach contents of three terrestrial passerine birds from e-waste, urban and rural locations in South China. The highest HBCD concentration was found at the urban site, followed by the e-waste site, suggesting that HBCD was linked to urbanization and industrialization, as well as e-waste recycling activities. Trophic magnification for α-HBCD was observed in the terrestrial food chain. Diastereisomeric pattern in birds showed the predominance of α-isomer, with a minor contribution of γ-isomer. The enantiomeric analysis revealed a preferential enrichment of $(-)$-α-HBCD and $(+)$-γ-HBCD. The similarity in enantiomeric fractions of HBCD isomers between muscle and stomach contents of birds suggested that dietary uptake may be responsible for the observed nonracemic distribution of HBCD isomers in the studied birds although *in vivo* enantioselective biotic processes cannot be ruled out.

1. Introduction

Hexabromocyclododecane (HBCD, $C_{12}H_{18}Br_6$) is an additive brominated flame retardant used primarily in extruded and expanded polystyrene for thermal insulation in the building industry, with secondary applications in upholstered furniture, automobile interior textiles, car cushions, packaging material and electrical household equipment (Law et al., 2005; Marvin et al., 2011). The commercial product of HBCD consists mainly of three diastereomers: α-, β-, and γ-HBCD, with the gamma-isomer predominating in the mixture (Alaee et al., 2003). In 2003, the world market demand for HBCD was estimated to be over 22 000 metric tons (de Wit et al., 2010). Due to the widespread use, HBCD has become the subject of scientific concern about its environmental fate and toxicity (Covaci et al., 2006). Although there are no restrictions on the production or use of HBCD, voluntary programs to control and reduce emissions of HBCD into the environment have been introduced by the flame retardant companies in Europe. At present, HBCD has been proposed for authorisation under the European Union REACH regulations as a Substance of Very High Concern due to its persistent, bioaccumulative, and toxic properties (BSEF, 2009) and included on the USEPA's lists of Chemicals of Concern (USEPA, 2010). It is also currently under assessment for the addition to the lists of compounds in the Stockholm Convention and in the UN-ECE Protocol on Persistent Organic Pollutants (POPRC, 2009).

HBCD has been demonstrated to bioaccumulate in various types of biota. In contrast to the γ-dominated technical mixtures, α-HBCD appears to predominate in the majority of biota samples, particularly in higher trophic level species (Morris et al., 2004; Janák et al., 2005; Law et al., 2008; Peck et al., 2008). In addition, nonracemic HBCD distributions have also been detected in fish, marine mammals, bird and human samples (Covaci et al., 2006; Zhang et al., 2009). Selectivity to different HBCD diastereoisomers and enantiomers may be attributed to *in vivo* biotransformations, uptake by prey, or a combination of these processes. It remains unknown which is the key factor in determining diastereoisomer- and enantiomer-specific accumulation in biota. Meanwhile, the biomagnifications of HBCD in marine and freshwater food webs have been reported in several studies (Tomy et al., 2004; Wu et al., 2010; Haukås et al., 2010). However, few studies have been

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conducted to investigate the bioaccumulation and biomagnification of HBCD in terrestrial organisms which are expected to show different contaminant patterns from aquatic organisms due to distinct exposure route between terrestrial and aquatic biota. Therefore, more detailed information on occurrence of HBCD in terrestrial organisms is needed.

The Pearl River Delta (PRD) has become one of the manufacturing bases for electronic/electrical products in the world. It has also been one of the largest dumping sites of electrical waste (e-waste) in the world and houses many e-waste recycling sites, such as Qingyuan and Guiyu. Therefore, the environment and wildlife in this region are likely to be contaminated with brominated flame retardants (such as HBCD, PBDEs, and DBDPE). More recently, the occurrence of HBCD has been reported in biotic and abiotic samples collected from e-waste recycling, industrial, and urban areas in the PRD (Yu et al., 2008; He et al., 2010; Wu et al., 2010; Gao et al., 2011). However, no data on diastereoisomers and enantiomers of HBCD exist for rural locations in this region.

In the present study, three terrestrial resident passerine birds, namely light-vented bulbul (*Pycnonotus sinensis*, LVB), long-tailed shrike (*Lanius schach*, LTS), and oriental magpie-robin (*Copsychus saularis*, OMR), were collected from three different types of locations (e-waste, urban and rural) in the PRD, South China. Concentrations of α-, β-, and γ-HBCD and their enantiomeric distributions in muscle and stomach contents of birds were determined. The three species are resident birds with relative small-scale territories and foraging areas, which can serve as bioindicators for monitoring local pollution of HBCD. The objectives of this study were: (1) to explore the occurrence and geographic distribution of HBCD in the PRD region; (2) to evaluate the bioaccumulation and biomagnification of HBCD in terrestrial bird species; (3) to examine the diastereoisomer- and enantiomer-specific accumulation and the potential role of diet in these bird species.

2. Materials and methods

2.1. Chemicals

Native α-, β-, γ-HBCD were purchased from AccuStandard, Inc. (New Haven, CT). $^{13}C_{12}$-labeled α-, β-, γ-HBCD and d_{18}-labeled α-, β-, γ-HBCD were obtained from Cambridge Isotope Laboratories (Andover, MA) and Wellington Laboratories (Guelph, ON), respectively. All of the solvents used for the extraction and cleanup were HPLC grade. Commercial HBCD products were purchased from the marketplace in China.

2.2. Sample collection

A total of 56 birds, including 19 LVBs, 10 LTSs, and 27 OMRs, were collected from three sampling sites between September 2009 and March 2011 in the PRD, South China (Table 1). The detail of avifaunal sampling method was given in Zhang et al. (2011). Information on feeding and migratory habits of the three bird species is given in Table S1. The urban site is located in Guangzhou, a highly industrialized and urbanized city of the PRD region. The e-waste site is situated in Qingyuan, approximately 70 km north of the urban site, where approximately 700 000 t e-wastes are disposed each year within an area of 330 km^2 using crude recycling techniques. The rural site is located in Dinghu Mountain at the west side of the PRD with intensive agricultural activities, which was chosen as a background reference of the PRD because the regional atmospheric pollutants carried by prevailing westward and southwestward winds are blocked by a cluster of mountains and hills in the west of the delta (Mai et al., 2003). The map of sampling sites is given in Fig. 1. Birds were immediately transported to laboratory and euthanized with N_2. Necessary permit for all research was obtained from Forestry Bureau of Guangdong Province. Pectoral muscle and stomach contents were excised from each bird and stored at −20 °C until chemical analysis. The stomach contents in semi-digested form, collected from birds in the same species and same site, were pooled into 1–3 composited samples depending on mass.

2.3. Sample preparation and instrumental analysis

The procedure used for sample extraction was described previously (He et al., 2010). Briefly, a homogenized sample of muscle tissue or composited stomach contents was mixed with anhydrous sodium sulfate, spiked with surrogate standards ($^{13}C_{12}$-α-, β-, and γ-HBCD) and then Soxhlet extracted with 200 mL acetone/n-hexane ($v/v = 1{:}1$) for 48 h. The lipid content was determined gravimetrically on an aliquot of the extract. The remainder of the extract used for chemical analysis was subjected to gel permeation chromatography for lipid removal. Eluate from 90 to 280 mL containing HBCD was collected and concentrated to 1 mL, further cleaned up on a column packed with 8 cm neutral silica and 8 cm acidified silica and eluted with 30 mL n-hexane/dichloromethane ($v/v = 1{:}1$). The eluate was concentrated to dryness under N_2 and reconstituted in 200 μL of methanol, spiked with internal standard (40 ng d_{18}-α-, β-, and γ-HBCD) for HBCD diastereoisomer and enantiomer determination by LC-MS/MS.

HBCD diastereoisomers and enantiomers were analyzed by Agilent 1200 series liquid chromatography system coupled with an Agilent 6410 electrospray triple quadrupole mass spectrometer. HBCD diastereoisomers were separated through a XDB C_{18} reversed-phase column (4.6 × 50 mm, 1.8 μm, Agilent). For the separation of HBCD enantiomers, a Phenomonex Nucleosil β-PM chiral LC column (4.0 × 200 mm, 5 μm) containing permethylated β-cyclodextrion on silica (Macherey–Nagel, GmbH and Co, Düren, Germany) was used. The mass transitions of m/z 640.7→79, 652.7 → 79, 658.7 → 79 were monitored for native HBCD, $^{13}C_{12}$-HBCD and d_{18}-HBCD, respectively. More information on instrumental analysis was given in our previous study (He et al., 2010).

The enantiomeric composition is expressed as enantiomeric fraction (EF), which is normally calculated from the peak areas of the enantiomeric pairs by:

$$\mathrm{EF} = \frac{A_+}{A_+ + A_-} \tag{1}$$

Where A_+ and A_- are the peak areas of the corresponding (+) and (−) enantiomers.

In order to avoid the matrix effect, Marvin et al. (2007) introduced the corrected EF value calculated by the following formula:

$$\mathrm{EF}_{\text{corrected}} = \frac{\left(A_+/A_{+\mathrm{d}_{18}}\right) \times \mathrm{pg}\,A_{+\mathrm{d}_{18}}}{\left(A_+/A_{+\mathrm{d}_{18}}\right) \times \mathrm{pg}\,A_{+\mathrm{d}_{18}} + \left(A_-/A_{-\mathrm{d}_{18}}\right) \times \mathrm{pg}\,A_{-\mathrm{d}_{18}}} \tag{2}$$

$A_{+\mathrm{d}_{18}}$ and $A_{-\mathrm{d}_{18}}$ are the peak areas of eluting (+) and (−) enantiomers labeled by d_{18}, while pg A_+ and pg $A_{-\mathrm{d}_{18}}$ stand for the concentrations of d_{18}-HBCD.

Stable isotopes of carbon and nitrogen, expressed as $\delta^{13}C$ or $\delta^{15}N$, respectively, were analyzed by a Flash EA 112 series elemental analyzer interfaced with a Finnigan MAF ConFlo 111 isotope ratio mass spectrometer. Detail of analysis was given in Sun et al. (2012).

2.4. QA/QC

A procedural blank was performed in each batch of the samples. Only trace amounts of α-HBCD were detected in procedural blanks (less than 3% of the lowest

Table 1
Concentrations (ng/g lw) of HBCD in muscle of birds from e-waste, urban and rural sites in South China.

Locations	Species	N	$\delta^{13}C$ (‰)[a]	$\delta^{15}N$ (‰)	Lipid (%)	α-HBCD	β-HBCD	γ-HBCD	∑HBCD
E-waste	LVB	6	−25.5 ± 0.27	7.41 ± 0.69	2.6 (2.2–4.1)[b]	6.7 (nd[c]–16)	nd (nd–4.3)	nd (nd–9.8)	11 (nd–19)
	LTS	7	−23.2 ± 0.55	7.84 ± 0.67	4.1 (2.1–4.6)	5.1 (3.0–170)	nd	nd (nd–0.65)	5.1 (nd–170)
	OMR	12	−22.2 ± 0.44	8.10 ± 0.48	3.0 (1.8–5.1)	48 (11–830)	nd (nd–1.6)	1.5 (nd–26)	73 (11–850)
Urban	LVB	6	−26.1 ± 0.47	7.13 ± 0.14	2.8 (1.9–3.7)	13 (3.3–500)	nd	nd (nd–3.8)	15 (3.3–510)
	OMR	5	−21.3 ± 0.44	6.76 ± 0.62	2.6 (1.8–5.0)	290 (120–1700)	nd (nd–9.8)	nd (nd–86)	380 (120–1700)
Rural	LVB	7	−24.8 ± 0.26	5.65 ± 0.65	3.5 (2.1–4.8)	2.7 (nd–10)	nd	nd (nd–0.87)	2.8 (nd–10)
	LTS	3	−21.7 ± 0.50	9.12 ± 0.09	3.4 (2.2–3.6)	6.1 (nd–18)	nd	2.5 (nd–5.0)	8.6 (nd–23)
	OMR	10	−21.1 ± 0.56	9.22 ± 0.77	3.1 (2.2–4.2)	15 (nd–22)	nd	nd (nd–3.0)	16 (nd–22)

[a] Data from Sun et al. (2012).
[b] Median (range).
[c] Not detected.