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## Trophic transfer of hexabromocyclododecane in the terrestrial and aquatic food webs from an e-waste dismantling region in East China

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Trophic transfer of hexabromocyclododecane (HBCD) was investigated in both the terrestrial and aquatic food webs from an e-waste dismantling region in East China. The mean  $\Sigma_3$ HBCD concentrations in the terrestrial species varied from 0.91 (0.16–1.85) ng g<sup>-1</sup> lipid weight (lw) in dragonflies (*Pantala flavescens*) to 40.3 (22.1–51.1) ng g<sup>-1</sup> lw in rats (*Rattus norvegicus*). The isomeric profile indicated that  $\alpha$ -HBCD presented a decreasing trend along the trophic level (TL) (from 97.2% to 16.3% of  $\Sigma_3$ HBCDs), while  $\gamma$ -HBCD showed a reverse trend (from 2.8% to 73.6% of  $\Sigma_3$ HBCDs). The trophic magnification factor (TMF) derived from the slope of the regression line between TLs and ln-transferred  $\Sigma_3$ HBCDs was 0.10, suggesting a trophic dilution of HBCD in the terrestrial food web. By contrast, in the aquatic species,  $\Sigma_3$ HBCD concentrations varied from 5.02 (3.5–6.55) ng g<sup>-1</sup> lw in apple snails (*Ampullaria gigas spix*) to 45.9 (14.9–67.8) ng g<sup>-1</sup> lw in grass carps (*Ctenopharyngodon idellus*).  $\alpha$ -HBCD was the dominant isomer, followed by  $\gamma$ -HBCD in the majority of species. A positive linear relationship was observed in the plots of ln  $\Sigma_3$ HBCDs versus TLs ( $R^2 = 0.81$ ,  $p = 0.06$ ). The TMF for  $\Sigma_3$ HBCDs was 6.36, indicating a trophic magnification of HBCD in the aquatic food web. Although these results demonstrated the distinct trophic transfer of  $\Sigma_3$ HBCDs in different ecosystems, further research is needed to eliminate the uncertainty of the tendencies, due to the non-significant relationship and limited species.

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### Environmental impact

E-waste recycling areas have been singled out as a major hot spot for persistent organic pollutants (POPs) for many years. In the present study, various terrestrial and aquatic species were collected to investigate the concentration levels and trophic transfer of hexabromocyclododecane (HBCD) from the Taizhou e-waste recycling region in East China. The contamination levels demanded that strict measures should be taken to reduce environmental pollution and protect human health. The results about the trophic transfer of HBCD demonstrated the inconsistent tendencies: trophic dilution in terrestrial food webs and trophic magnification in the aquatic food webs. More research work should be conducted on the trophic transfer of this compound in the aquatic and terrestrial species, which may shed light on the different biomagnification potential in aquatic and terrestrial systems.

## Introduction

Hexabromocyclododecane (HBCD) is the most widely used cycloaliphatic additive brominated flame retardant (BFR) today, and has received considerable attention following restrictions on polybrominated biphenyls (PBBs) and polybrominated

diphenyl ethers (PBDEs).<sup>1,2</sup> It is mainly employed in polystyrene foams used as thermal insulation in construction, and upholstery textiles, such as residential and commercial furniture, draperies, and wall coverings.<sup>3</sup> Commercial HBCD products mainly contain three stereoisomers ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD), in which  $\gamma$ -HBCD occupies approximately 75–80% of total technical formulation, followed by  $\alpha$ -HBCD (10–13%) and  $\beta$ -HBCD (1–12%).<sup>4,5</sup> In 2001, the global market demand of HBCDs reached 16 700 tons, of which 9500 tons were consumed in Europe.<sup>4,5</sup> In 2007, the consumption of HBCDs in Europe increased to 11 000 tons.<sup>6</sup> Correspondingly, the product capacity in China was up to 7500 tons in the same year.<sup>7</sup>

As an additive BFR, HBCD could be released into the environment during production, use, disposal or recycling processes.<sup>8</sup> It was first detected in sediment and fish from a Swedish river in 1998.<sup>9</sup> After that, it has been found extensively

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in various environmental matrices, such as air, soil, sediment, zooplankton, aquatic and terrestrial species, human blood and breast milk.<sup>4–6,8,10</sup> HBCD has the potential to disrupt thyroid axis *in vivo* and *in vitro* animal models, including mammals, fishes and birds.<sup>3,6</sup> Other adverse effects of HBCD were also observed, including reproductive failures, damage of the nervous system and oxidative stress.<sup>6</sup> HBCD has the similar properties and empirical observations of long-range transport, persistence and bioaccumulation with PBDEs and some other persistent organic pollutants (POPs). It had therefore been included in the POP list of the Stockholm Convention in May 2013.<sup>11</sup>

The lipophilicity of HBCD leads to its bioaccumulation in living organisms, and possible trophic biomagnification in food webs.<sup>4,12</sup> In the aquatic food webs, the trophic transfer of HBCD has been reported in many studies,<sup>3,13–18</sup> where the trophic magnification factors (TMFs) of total HBCDs were greater than one, suggesting a trophic magnification. However, information on HBCD in terrestrial food webs is scarce to our knowledge. Sun *et al.*<sup>19</sup> demonstrated trophic magnification of  $\alpha$ -HBCD in three terrestrial birds at the e-waste and rural sites, in the Pearl River Delta, South China. Biomagnification of  $\alpha$ - and  $\gamma$ -HBCD was reported from grain to spotted dove (*Streptopelia chinensis*),<sup>20</sup> while biodilution of  $\alpha$ -HBCD was observed in the rat-owl and sparrow-kestrel food chain.<sup>1</sup> Further systematic studies on the trophic transfer should be made due to these inconsistent results.

E-waste recycling areas have been singled out as major hot spots for POPs in China.<sup>21</sup> HBCD was also reported in recycling areas with a relatively higher level in matrices like soil,<sup>22</sup> home-produced eggs<sup>23</sup> and terrestrial passerine birds,<sup>19</sup> *etc.* But the bioaccumulation and biomagnification of HBCD in these places were limited. Previously, we have paid close attention to the Taizhou e-waste recycling region in East China for many years.<sup>2,24–27</sup> In the present study, the terrestrial and aquatic species were collected in this region to investigate the levels and isomer profiles of HBCD, and evaluate trophic transfer. This approach allows comparisons of the trophic magnification of HBCD in different ecosystems.

## Materials and methods

### Reagents and standards

Dichloromethane (DCM) and *n*-hexane were of pesticide residue grade; methanol and acetonitrile were of HPLC-grade, purchased from J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water was produced by a Milli-Q system (Millipore, USA) in the laboratory. Silica gel 60 (0.063–0.100 mm) was supplied by Merck (Darmstadt, Germany). Analytically pure concentrated sulfuric acid, sodium hydroxide and anhydrous sodium sulfate were obtained from domestic manufacturers (Sinopharm Chemical Reagent Co., Ltd). The preparation and activation of neutral, acid and basic silica gels were described in our previous studies.<sup>28,29</sup> Native HBCD mixtures ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -HBCD, >98% purity) were purchased from Accustandard Inc. (New Haven, CT, USA). Isotope standard solutions <sup>13</sup>C<sub>12</sub>- $\gamma$ -HBCD and d<sub>18</sub>- $\gamma$ -HBCD (>98% purity) were bought from Wellington Laboratories Inc. (Guelph, Ontario, Canada).

### Sampling area and sample collection

The sampling location was at a village (N28°33', E121°22') located in Luqiao District, Taizhou City, East China. Taizhou is one of the largest e-waste dismantling regions in China, with a recycling history of over 30 years.<sup>26</sup> Although strict environmental management policies were implemented by the local government in 2005, contamination of POPs was still reported.<sup>24</sup> In the present study, various terrestrial and aquatic species were collected between July 2012 and October 2013, as shown in Table 1. For the terrestrial species, butterflies (*Pieris rapae* Linne) and dragonflies (*Pantala flavescens*) were captured using butterfly nets. Frogs (*Rana plancyi*) and grasshoppers (*Oxya japonica*) were collected by hand. Rats (*Rattus norvegicus*) were netted by using a rat cage. Aquatic species, including grass carps (*Ctenopharyngodon idellus*), stone snails (*Bellarnya purificata*) and apple snails (*Ampullaria gigas* spix) were collected from an adjacent brook. Frogs are an amphibian, which lives both in water and on land. It was used in both the aquatic and terrestrial food webs. For frogs, grass carps and rats, muscle tissues were employed. The shells of stone snails and apple snails were removed. Grasshoppers, dragonflies and butterflies were pooled to obtain enough material for analysis. Then all of them were freeze-dried, ground into powder and sealed in clean zipper bags, and then stored at –20 °C until further treatment.

### Sample cleanup and instrumental analysis

**Sample extraction and cleanup.** The extraction and cleanup procedure of HBCD for biota samples were described elsewhere.<sup>29</sup> In brief, 2 g of the powdered sample was mixed with 10 g of anhydrous sodium sulfate, and spiked with <sup>13</sup>C-labeled internal standards (<sup>13</sup>C<sub>12</sub>- $\gamma$ -HBCD). It was extracted using an accelerated solvent extractor (ASE, ASE300, Dionex, USA) with a mixture of *n*-hexane : DCM (1 : 1, v/v). The extract was concentrated to dryness and the lipid weight was determined by a gravimetric method. Then it was reconstituted in *n*-hexane and acidified silica gel (44%) was added to remove the lipid. After that, it was cleaned up through a multi-layer silica gel column. The resulting eluate was concentrated to complete dryness, redissolved in 200  $\mu$ L of methanol, and the injection standard (d<sub>18</sub>- $\gamma$ -HBCD) was spiked prior to instrumental analysis.

**Instrumental analysis.** Identification and quantitative determination of three HBCD isomers ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD) were performed using a Micromass Quattro Premier XE triple quadrupole MS spectrometer (Micromass, Manchester, UK) equipped with an Alliance 2695 LC system (Waters, Milford, MA.). The details about the instrument conditions were reported elsewhere.<sup>7</sup> HBCD isomers were separated on a Zorbax ODS reversed-phase column (150 mm  $\times$  3.0 mm i.d.  $\times$  5.0  $\mu$ m, Agilent, USA) with a mobile phase mixture of methanol/acetonitrile/water. Multiple reaction monitoring (MRM) was adopted in an atmospheric pressure chemical ionization (APCI) negative ion mode for mass spectrometric analysis. MRM transitions were [M – H]<sup>–</sup>  $\rightarrow$  Br<sup>–</sup> at *m/z* 640.6  $\rightarrow$  79.0 and 81.0 for native HBCD isomers, 652.6.6  $\rightarrow$  79.0 and 81.0 for <sup>13</sup>C<sub>12</sub>- $\gamma$ -HBCD and 657.6  $\rightarrow$  79.0 and 81.0 for d<sub>18</sub>- $\gamma$ -HBCD, respectively.

Table 1 Sample details and mean  $\Sigma_3$ HBCD concentrations (ng g<sup>-1</sup> lw) in the terrestrial and aquatic species

Species	Numbers	Lipid (%)	Trophic level	$\alpha$ -HBCD	$\beta$ -HBCD	$\gamma$ -HBCD	$\Sigma_3$ HBCDs
Apple snail ( <i>Ampullaria gigas</i> spix)	5 (pooled)	3.37 ± 0.56 <sup>a</sup>	2.81 ± 0.43	1.86 (nd–3.32) <sup>b</sup>	0.48 (nd–2.41)	2.68 (1.61–4.69)	5.02 (3.5–6.55)
Stone snail ( <i>Bellarmya purificata</i> )	3 (pooled)	4.80 ± 2.58	3.22 ± 0.18	13.8 (8.72–18.9)	nd <sup>c</sup>	nd	13.8 (8.72–18.9)
Frog ( <i>Rana plancyi</i> )	11	8.93 ± 2.18	3.26 ± 0.29	3.51 (nd–11.7)	0.55 (nd–1.81)	2.23 (nd–4.92)	6.28 (4.22–13.2)
Grass carp ( <i>Ctenopharyngodon idellus</i> )	5	16.9 ± 3.56	3.96 ± 0.12	41 (12–60.2)	0.55 (nd–2.73)	4.27 (2.56–6.9)	45.9 (14.9–67.8)
Rat ( <i>Rattus norvegicus</i> )	3	10.36 ± 2.7	2.13 ± 0.21	39.5 (20.9–47.8)	nd	0.84 (nd–1.3)	40.3 (22.1–51.1)
Grasshopper ( <i>Oxya japonica</i> )	5 (pooled)	10.63 ± 1.17	2.28 ± 0.13	2.38 (nd–4.8)	3.26 (nd–8.74)	9.21 (1.39–18.7)	14.9 (32.4–30.34)
Butterfly ( <i>Pieris rapae</i> Linne)	1 (pooled)	8.87	2.62	9.38	9.38	6.39	18.7
Dragonfly ( <i>Pantala flavescens</i> )	4 (pooled)	14.9 ± 5.49	3.35 ± 0.1	0.15 (nd–0.45)	0.09 (nd–0.19)	0.66 (nd–1.27)	0.91 (0.16–1.85)

<sup>a</sup> Arithmetic mean ± standard deviation. <sup>b</sup> Mean, minimum and maximum concentrations are listed. <sup>c</sup> nd = not detected.

### Quality assurance/quality control (QA/QC)

Laboratory blanks were run for each batch of ten samples. HBCD isomers were not detected in any of the blanks. Duplicate HBCD standard mixtures (5 ng mL<sup>-1</sup> of native  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD) were analyzed for every ten samples to check the instrument's stability and repeatability of the analytical method. The relative standard deviations of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD were 13.7, 10.6 and 3.4%, respectively. The limit of detection (LOD) was defined as the signal-to-noise ratio (S/N) = 3. Their LODs ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD) were 0.25–6.78, 0.06–3.52, 0.15–3.91 ng g<sup>-1</sup> lipid weight (lw) in terrestrial species, and 0.45–32.3, 0.14–10.6 and 0.26–18.6 ng g<sup>-1</sup> lw in aquatic species, respectively. The mean recovery for <sup>13</sup>C<sub>12</sub>- $\gamma$ -HBCD was 94.5 ± 32.9% (mean ± SD, *n* = 37).

### Determination of the trophic level and trophic magnification factor

An isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher, MA, USA) was used to measure the stable isotope ratios of nitrogen. Stable nitrogen isotope abundances ( $\delta^{15}\text{N}$ ) were calculated based on atmospheric nitrogen (air) according to the following equation:

$$\delta^{15}\text{N} = \left[ \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right] \times 1000\text{‰} \quad (1)$$

For individual samples, the TL was determined according to the equation below.<sup>30</sup>

$$\text{TL}_{\text{consumer}} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \Delta\delta^{15}\text{N} + 2 \quad (2)$$

where 2 and  $\delta^{15}\text{N}_{\text{baseline}}$  were the trophic level and  $\delta^{15}\text{N}$  of the reference species, which were zooplankton, the primary consumer.  $\Delta\delta^{15}\text{N}$  is the isotope enrichment factor. Post<sup>30</sup> evaluation studies on the trophic position of consumers using stable isotopes in multiple ecosystem found no significant differences in mean fractionation between the aquatic and terrestrial organisms. Therefore, in the present study, a commonly applied value of 3.4‰ for  $\Delta\delta^{15}\text{N}$  was adopted to calculate the trophic level of both the terrestrial and aquatic species.<sup>31</sup>

Trophic magnification factor (TMF) for the whole food web was based on the slope of linear regression of

logarithmically transformed lipid-normalized HBCD concentrations against TLs.<sup>3</sup>

$$\ln(\text{HBCD}) = a + b \times \text{TL} \quad (3)$$

where *b* is the slope, thus

$$\text{TMF} = e^b \quad (4)$$

A TMF value above 1 indicates a trophic magnification throughout the food web; whereas a TMF value between zero and 1 implies a trophic dilution.<sup>14</sup>

## Results and discussion

### Levels and profiles of HBCD in the terrestrial species

The detection frequencies of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD of all the samples were 87.9, 36.4 and 84.8%, respectively. The concentrations of HBCDs in the terrestrial species are presented in Table 1. The highest  $\Sigma_3$ HBCD concentration (sum of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD) was found in rats (mean value 40.3, range 22.1–51.1 ng g<sup>-1</sup> lw), followed by butterflies (mean value 18.7 ng g<sup>-1</sup> lw) and grasshoppers (mean value 14.9, range 32.4–30.3 ng g<sup>-1</sup> lw). Dragonflies at a higher trophic level had low  $\Sigma_3$ HBCD concentration (mean 0.91, range 0.16–1.85 ng g<sup>-1</sup> lw). The  $\Sigma_3$ HBCD concentration in rats was higher than that in brown rats (*Rattus norvegicus*) (mean value 17, range 1.4–180 ng g<sup>-1</sup> lw) obtained from the Beijing Raptor Rescue Center, China.<sup>1</sup> However, it was difficult to compare HBCD concentrations of terrestrial species since there were few similar studies.

For the isomeric profile, commercial HBCD products in China were dominated by  $\gamma$ -HBCD (77–80%).<sup>22</sup> In the present study,  $\gamma$ -HBCD was predominant in grasshoppers (65.8%) and dragonflies (73.6%), which was similar to the profiles in the commercial products (Fig. 1). The percentage of  $\gamma$ -HBCD in butterflies (34.2%) was still higher than that in frogs (9.33%). He *et al.*<sup>20</sup> presented that the mean percentages of  $\gamma$ -HBCD in spotted dove and Chinese francolin were 72 and 63% collected from an e-waste recycling region in South China. The dominance of  $\gamma$ -HBCD indicated that some species might be exposed to nearby sources of HBCD pollution.<sup>32</sup> It was interesting that the percentage of  $\alpha$ -HBCD in rats (97%) was much higher than

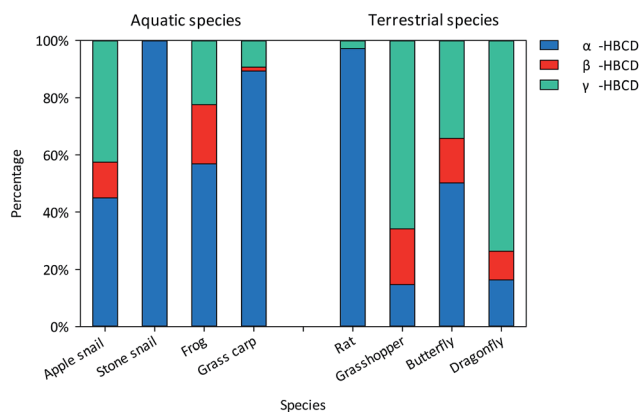


Fig. 1 Isomer profiles of HBCD in the terrestrial and aquatic food webs from an e-waste recycling region in Taizhou, East China.

those in insects (16.3–50.2%). The complex food sources and metabolized properties in rats could induce this result. Abdallah *et al.*<sup>33</sup> confirmed the process of cytochrome P450 enzyme mediated biotransformation of  $\gamma$ -HBCD in rats, inducing enormous increase of  $\alpha$ -HBCD in composition compared to the commercial mixtures. But cytochrome P450 enzymes seem not to play a central role in xenobiotic detoxification in insects, in contrast to mammals, and its expression was diverse in the different developmental stages of the insects.<sup>34,35</sup>

### Levels and profiles of HBCD in the aquatic species

$\Sigma_3$ HBCD concentration in the aquatic species varied from 5.02 ng g<sup>-1</sup> lw in apple snails to 45.9 ng g<sup>-1</sup> lw in grass carps (Table 1). These levels were lower than the data reported in the aquatic species from e-waste recycling areas in China,<sup>18,36</sup> freshwater species near a textile industry in Sweden (65–1808 ng g<sup>-1</sup> lw)<sup>12</sup> and marine species near known HBCD point sources in the Netherlands (9–1110 ng g<sup>-1</sup> lw).<sup>37</sup> They were in the same range in freshwater species from Yangtze River in China (11–330 ng g<sup>-1</sup> lw).<sup>38</sup> Frogs had comparable  $\Sigma_3$ HBCD concentrations (mean 6.28, range 4.22–13.2 ng g<sup>-1</sup> lw) with those in apple snails. Both of them were lower than those in stone snails (mean 13.8, range 8.72–18.9 ng g<sup>-1</sup> lw); while the latter was consistent with Chinese mystery snails (13.9 ng g<sup>-1</sup> lw) reported by Wu *et al.*<sup>18</sup> The  $\Sigma_3$ HBCD concentration of grass carps was lower than those in mud carps (868 ng g<sup>-1</sup> lw) and crucian carps (129 ng g<sup>-1</sup> lw) in the same e-waste recycling region.<sup>18</sup>

Dominance of  $\alpha$ -HBCD was observed in the aquatic organisms, especially for stone snails (only  $\alpha$ -HBCD was detected) (Fig. 1).  $\alpha$ -HBCD in grass carps, frogs and apple snails accounted for 89.8, 55.8 and 44.9% of  $\Sigma_3$ HBCDs, respectively.  $\alpha$ -HBCD was reported to have a remarkable contribution to  $\Sigma_3$ HBCDs in invertebrates, fish (marine and freshwater), marine mammals, and cormorants,<sup>4</sup> species from the Arctic marine food web<sup>16</sup> and Chinese coastal cities.<sup>39</sup> Wu *et al.*<sup>18</sup> also found increasing percentage of  $\alpha$ -HBCD and decreasing  $\gamma$ -HBCD against the ascending TLs in aquatic species. Haukås *et al.*<sup>13</sup> investigated five marine species in a Norwegian coastal area, and found that  $\alpha$ -HBCD appeared to be the most prominent isomer in the

majority of biotic samples, particularly in the upper species. The dominance of  $\alpha$ -HBCD can be explained by the metabolic biotransformation between individual isomers in exposed aquatic system,<sup>4,37</sup> since *in vitro* studies of rainbow trout (*Oncorhynchus mykiss*),  $\alpha$ -HBCD appeared the most resistant to metabolic degradation.<sup>33</sup> Tomy *et al.*<sup>3</sup> also observed a higher rate of biomagnification for the  $\alpha$ -HBCD isomer compared to the  $\gamma$ -HBCD isomer in the Lake Ontario food web.

### Trophic transfer of HBCD

**HBCD in the terrestrial food web.** In the present study negative correlation was observed between  $\ln \Sigma_3$ HBCD concentrations *versus* TLs (Adj  $R^2 = 0.52$ ,  $p = 0.11$ ) (Fig. 2). The TMF derived from the slope of the fitted equation was 0.10, suggesting a trophic dilution. Similar trends were observed for individual isomers ( $\alpha$ -HBCD: Adj  $R^2 = 0.53$ ,  $p = 0.16$ , TMF = 0.08 and  $\gamma$ -HBCD: Adj  $R^2 = 0.12$ ,  $p = 0.56$ , TMF = 0.47). Since only limited species in the food web were analyzed, and all the relationships were non-significant, the uncertainties of TMFs existed. Furthermore, little information is available on HBCDs in the terrestrial food chain. The biodilution of  $\alpha$ -HBCD in the present study was consistent with those in the sparrow–kestrel food chain<sup>1</sup> and gill-breathing poikilotherms from a coastal food web, Western Norway.<sup>13</sup> The TMF for  $\gamma$ -HBCD in the present study was comparable to that of 0.5 in a marine food

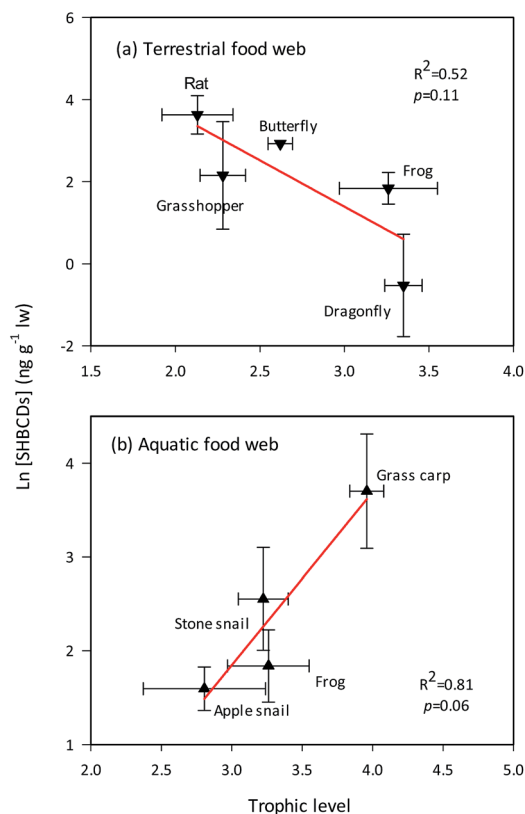


Fig. 2  $\Sigma_3$ HBCD concentrations *versus* trophic level (TL) in the terrestrial and aquatic food webs. Linear regression equations: (a)  $\ln [\Sigma_3\text{HBCDs}] = -2.26 \times \text{TL} + 8.17$  (Adj  $R^2 = 0.52$ ,  $p = 0.11$ ); (b)  $\ln [\Sigma_3\text{HBCDs}] = 1.85 \times \text{TL} - 3.69$  (Adj  $R^2 = 0.81$ ,  $p = 0.07$ ).

web from the Canadian arctic<sup>16</sup> and 0.3 in a coastal aquatic food web near a HBCD point source, Western Norway.<sup>13</sup> Yu *et al.*<sup>1</sup> reported the biodilution of  $\alpha$ - and  $\gamma$ -HBCD in the Eurasian tree sparrow–common kestrel food chain, with corresponding biomagnification factors (BMFs) of 0.07 and 0.28. The similar trend was found in the brown rat–common kestrel food chain with a BMF of  $\alpha$ -HBCD (0.21). However, biomagnification of  $\alpha$ -HBCD was observed in the food chains of Eurasian tree sparrow–owl and brown rat–owl, with BMFs of 5.5 and 16.<sup>1</sup> Meanwhile, He *et al.*<sup>20</sup> demonstrated the positive correlation between the log-transferred concentration and  $\delta^{15}\text{N}$  values from grain to spotted dove (the terrestrial phytophagous bird), implying the potential biomagnification, and the BMFs of  $\alpha$ - and  $\gamma$ -HBCD were 2.8–75 and 7.1–51, respectively. Sun *et al.*<sup>19</sup> found trophic magnification of  $\alpha$ -HBCD in three terrestrial birds at the e-waste and rural sites, but no relationship between  $\gamma$ -HBCD and  $\delta^{15}\text{N}$  was observed at the e-waste site.

The trophic dilution of  $\alpha$ -HBCD and  $\Sigma_3\text{HBCDs}$  could be contributed to the specific food web. The concerned terrestrial species in this study included insects and small mammals. These species, especially the insects, appeared to grow incrementally, then the growth dilution was greater than the accumulation, inducing the trophic dilution of HBCD in the terrestrial food web. The other factors, such as the rate constants for respiratory uptake and loss, dietary uptake, egestion losses, biotransformation losses, and ecological and physiological attributes of species also affected the tendencies.<sup>40,41</sup>

**HBCD in the aquatic food web.** In contrast to the trophic dilution of  $\Sigma_3\text{HBCDs}$  in the terrestrial food web, a positive correlation was observed in the aquatic food web (Adj  $R^2 = 0.81$ ,  $p = 0.06$  for  $\ln \Sigma_3\text{HBCDs}$  versus TLs) (Fig. 2). Similar situation was also obtained for  $\alpha$ -HBCD (Adj  $R^2 = 0.80$ ,  $p = 0.10$ ), but not for  $\gamma$ -HBCD. The calculated TMF was 6.36 for  $\Sigma_3\text{HBCDs}$ , and 13.6 for  $\alpha$ -HBCD, respectively. These values were higher than those in the freshwater food web in the same e-waste region, where the TMFs were 2.19 and 1.82 for  $\alpha$ -HBCD and  $\Sigma_3\text{HBCDs}$ .<sup>18</sup> It was also higher than the estimated ranges of constrained

TMFs in aquatic food webs reported by Marvin *et al.*,<sup>6</sup> who showed that TMFs changed by isomers and food webs from 0.3 to 2.2, and were always statistically non-significant. The trophic magnifications of  $\alpha$ - and  $\Sigma\text{HBCDs}$  have been reported in various aquatic food webs (freshwater, marine, coastal, limnic food webs) (Table 2).<sup>3,13–17</sup> Law *et al.*<sup>15</sup> demonstrated the trophic magnification of  $\beta$ - and  $\gamma$ -HBCD in the aquatic food web in the Lake Winnipeg, Canada. However, the trophic dilution of  $\gamma$ -HBCD was found in the marine food web in Eastern Canadian Arctic<sup>16</sup> and coastal aquatic food web in a HBCD point source, Western Norway.<sup>13</sup>

The variant TMFs could be attributed to several aspects: first, as reported previously, different species within different food webs could induce diverse biotransformation and metabolism for HBCD. Zhang *et al.*<sup>17</sup> demonstrated the TMF values of 2.44 and 1.68 for the  $\Sigma_3\text{HBCDs}$  in the limnic and marine food webs from Tianjin, China, respectively. Haukås *et al.*<sup>13</sup> reported that the TMF of  $\alpha$ -HBCD in the homeotherms (3.6) was larger than that in poikilotherms (0.3). Homeotherms have generally more efficient metabolism and higher energy requirements than poikilotherms, inducing different rates of contaminant accumulation with trophic levels.<sup>13</sup> Secondly, the factors that affect HBCD concentrations in the environmental matrices may also influence the TMF values. Due to different physicochemical properties of the three HBCD isomers, the variant TMF values in the same food web were expected. For example, Law *et al.*<sup>15</sup> reported that TMF values of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\Sigma_3\text{HBCDs}$  were 1.4, 1.3, 2.2 and 1.8 in a freshwater aquatic food web, respectively. Finally, different concentration units (wet, dry or lipid weight basis) demonstrated variable TMFs. Haukås *et al.*<sup>13</sup> presented that the TMFs of  $\alpha$ -HBCD based on lipid and wet weight were 2.1 versus 9.1, and 0.3 versus 0.9 for  $\gamma$ -HBCD, respectively.

## Conclusions

In the present study, the trophic transfer of HBCDs was systematically investigated in both the terrestrial and aquatic

Table 2 Trophic magnification factors (TMFs) for hexabromocyclododecane

No.	Food web categories	Locations	Years	Trophic Magnification Factor (TMF)				Reference
				$\alpha$ -HBCD	$\beta$ -HBCD	$\gamma$ -HBCD	$\Sigma_3\text{HBCDs}$	
1	Freshwater pelagic food web	The Lake Ontario	2004				6.3 <sup>c</sup>	3
2	Aquatic food web	Lake Winnipeg, Canada	2006	1.4	1.3	2.2	1.8	15
3	Marine food web	Eastern Canadian Arctic	2008	2.1		0.5		16
4	Coastal aquatic food web	A point source, Western Norway	2010	2.6 (9.1) <sup>a,d</sup>		0.3 (0.9) <sup>a,d</sup>		13
5	Freshwater food web	E-waste region, South China	2010	2.19 <sup>d</sup>			1.82 <sup>b,d</sup>	18
6	Marine food webs	Tianjin, China	2013	1.74			1.68	17
7	Limnic food webs	Tianjin, China	2013	2.58			2.44	17
8	Freshwater pelagic food web	Lake Maggiore, Northern Italy	2014				1.8 <sup>b</sup>	14
9	Three terrestrial passerine birds	E-waste site, Qingyuan, South China	2012	>1				19
10	Three terrestrial passerine birds	Rural site, the Pearl River Delta, South China	2012	>1		>1		19
11	Freshwater food web	E-waste region, Taizhou, East China	2016	13.6 <sup>b</sup>			6.36 <sup>b</sup>	This study
12	Terrestrial food web	E-waste region, Taizhou, East China	2016	0.08	0.04 <sup>b</sup>	0.47 <sup>b</sup>	0.10 <sup>b</sup>	This study

<sup>a</sup> The TMFs in the parentheses were calculated based on wet weight. <sup>b</sup> Not statistically significant (0.05). <sup>c</sup> Based on wet weight. <sup>d</sup> The TMF values were calculated based on individual sample concentrations versus the trophic level.

food webs from an e-waste recycling region in Taizhou, East China. HBCD isomers were detected in the majority of species. Higher percentage of  $\alpha$ -HBCD existed in the aquatic species, compared to the varied ratio of  $\alpha$ - and  $\gamma$ -HBCD along with TLs in the terrestrial species. Moreover, the two systems demonstrated opposite trophic transfer tendency. The trophic dilution of  $\Sigma_3$ HBCDs in the terrestrial food web was different from the trophic magnification in the aquatic system. This could be contributed to variant organisms with unlike metabolisms in the different ecosystems. However, the non-significant relationships between concentrations and trophic levels indicated the uncertainties of demonstrated tendencies, due to the short range of food webs and limited species. Further research is necessary to expand the sample numbers and investigate the internal mechanism of trophic transfer for HBCD in food webs.

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