ENANTIOMER-SPECIFIC ACCUMULATION, DEPURATION, METABOLIZATION AND ISOMERIZATION OF HEXABROMOCYCLODODECANE DIASTEREOMERS IN MIRROR CARP FROM WATER

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Introduction

HBCDs have drawn growing concern in the international community because of their persistent, bioaccumulative and toxic properties[1].Three dominant diastereomers (α-, β-, and γ-) comprise almost all of the HBCDs in technical products, and related research usually differentiates among the specific diastereomers due to their different environmental behaviors, which arecaused by their different water solubility, polarity, and dipole moment[2]. In recent studies, selective absorption and bioisomerization of the three diastereomers have been found in organisms exposed to HBCDs via diets[3-5]. Each of the three HBCD diastereomers has a pair of enantiomers, and though data are still deficient, it has been reported that organisms selectiveone enantiomer preferentially over its corresponding antipode in field studies and laboratory experiments [6-9].Moreover, HBCDs may be transformed in organisms, and metabolic products from both debromination and hydroxylation pathways have been observed[10-13].

Aquatic organisms primarily accumulate chemical pollutants in two ways, direct uptake from water and uptake from diet. Until now, no study has examined the accumulation of HBCDs by aquatic organisms via water exposure, most likely because of the low water solubility of HBCD diastereomers and great quantity of expensive HBCD diastereomer standards that would be needed for a water exposure experiment.Very little is known about diastereomer and enantiomer selectivity, transformation potential, and metabolites of HBCDs during direct uptake from water.

Materials and methods

The d_{18}-labeled α-, β- and γ-HBCD were from Wellington Laboratories (Andover, MA, USA) and used for calibration during the analysis. Native standards were obtained from AccuStandard (New Haven, CT, USA) and used as standards during analysis. Spiked standards were obtained from seperation of technical products by HPLC system. One-year-old mirror carp (*Cyprimuscarpiomorphamoblis*)were obtained from a localbreeder. The dried samples were Soxhlet extracted and cleaned up by concentrated sulfuric acid and silica column before analyzed by HPLC-MS/MS.

Data obtained during the depuration phase was fitted to a first-order decay curve (eq 1) according to Du et al. [3]

$$C_t = C_0 e^{-k_2 t} \quad (1)$$

Data obtained during the uptake phase were fitted to the following model(OECD-302):

$$C_f(t) = C_w(t)\frac{k_1}{k_2}(1 - e^{-k_2 t}) \quad (2)$$

The kinetic BCF_k was calculated by eq 3:

$$BCF_k = \frac{k_1}{k_2} \quad (3)$$

The growth-corrected kinetic BCF_{kg}has been proposed in the literatureto normalize the dilution effects of growth (eq 4):

$$BCF_{kg} = \frac{k_1}{k_2 - k_g} \quad (4)$$

In this study, k_g was assigned as zero due to that no significant gain was observed in whole body weight during

the experiment period. Hence, the BCF_{kg} was not adopted in this study.
The lipid-normalized kinetic BCF_{KL} was calculated by eq 5 (Ln is mean lipid content based on wet weight)

$$BCF_{KL} = \frac{0.05}{L_n} \cdot BCF_K \quad (5)$$

Depuration half-time ($t_{1/2}$) was calculated by eq 6:

$$t_{1/2} = \frac{\ln 2}{k_2} \quad (6)$$

Results and discussion

Table 1. Bioaccumulation and depuration parameters of HBCD diastereomers in exposure

	R_1^2	k_1	R_2^2	k_2	BCF_k	Lipid	BCF_{KL}	$t_{1/2}$	BR
α-									
Gill	0.89	103	0.94	0.012	8.58×10^3	1.24	3.45×10^4	57.76	
Viscera	0.94	691	0.99	0.060	1.15×10^4	1.69	3.42×10^4	11.55	
Muscle	0.83	128	0.94	0.023	5.57×10^3	0.91	3.07×10^4	30.14	
Skin	0.96	160	0.78	0.025	6.40×10^3	0.71	4.52×10^4	27.73	
β-									β to α
Gill	0.98	44.7	0.99	0.139	322	1.24	1.29×10^3	4.99	53.0
Viscera	0.99	91.8	0.99	0.143	642	1.69	1.90×10^3	4.85	92.9
Muscle	0.94	38.5	0.99	0.206	187	0.91	1.03×10^3	3.36	87.0
Skin	0.99	28.8	0.88	0.141	204	0.71	1.44×10^3	4.92	79.1
γ-									γ to α
Gill	0.96	29.9	0.85	0.126	237	1.24	0.95×10^3	5.50	98.0
Viscera	0.97	80	0.82	0.137	584	1.69	1.73×10^3	5.06	97.0
Muscle	0.94	31.8	0.92	0.144	221	0.91	1.22×10^3	4.81	98.6
Skin	0.96	32	0.95	0.145	227	0.71	1.61×10^3	4.78	96.2

k_1($L \cdot kg^{-1} \cdot d^{-1}$), uptake rate constant; k_2(d^{-1}), depuration rate constant; BCF_k, kinetic bioconcentration factor; Lipid(%); BCF_{KL}, lipid-normalised and growth-corrected BCF_k; $t_{1/2}$(d), depuration half-time； BR(%): bioisomerization rate represents the ratio of α-HBCD to sum of α and β/γ-HBCD in β/γ group, repectively.

We found that the BCF_{KL} values (calculated from kinetic data and calibrated by lipid content) of α-HBCD in different tissues of the carp were in the range of $3.07\text{-}4.52 \times 10^4$, much higher than those of β-HBCDs ($1.03\text{-}1.90 \times 10^3$) and γ-HBCD ($0.95\text{-}1.73 \times 10^3$), as was true for $t_{1/2}$. The order of BCF_K for α-, β-and γ-HBCD in different tissues was viscera > gill > skin > muscle. β-HBCD and γ-HBCD were transformed to α-HBCD, with 50.0-92.9% and 96.2-98.6% bioisomerization ratesatthe end of experiment, respectively. No isomerization products from α-HBCD were found.

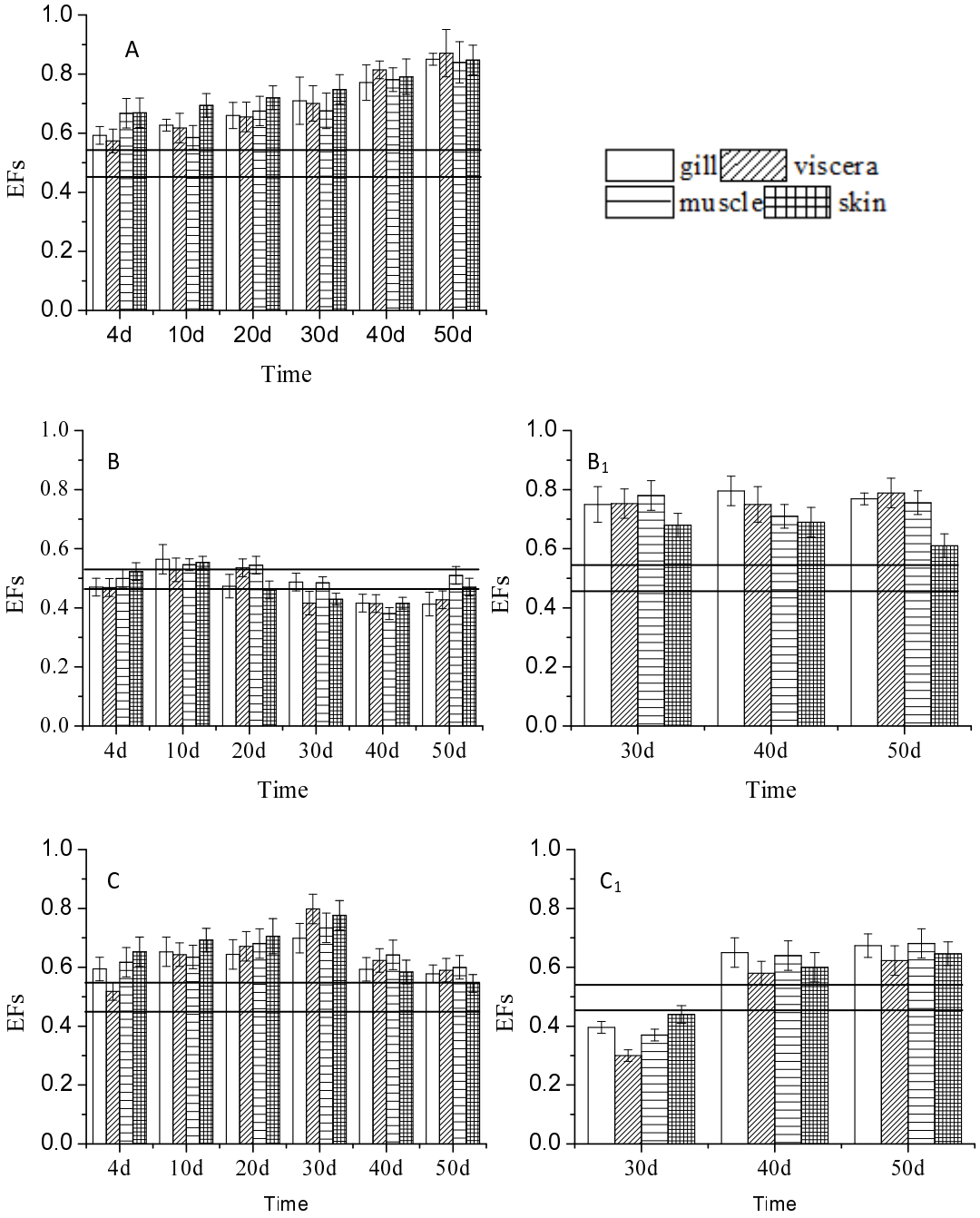

Fig. 1 Enantiomer fractions of α- (A), β- (B), and γ-HBCD(C); α-HBCD isomerized from β- (B_1),and from γ-HBCD(C_1), double lines represent confidence interval of standard EFs.

For α-HBCD, its EFs in all tissues were significantly higher than those in the standards, as was true of γ-HBCD. β-HBCD showed no significant differences during the first20 d; after that its EFs were slightly lower than in the standards. As for α-HBCD isomerized from β-HBCD and γ-HBCD (Fig. 3B_1 and C_1), the EFs of α-HBCD were also significantly higher than those in the standards except the α-HBCD isomerized from γ-HBCD on the 30 d. These results demonstrate that there must be a selective enrichment of the (+)compared to (-)α-HBCD and a selective enrichment of the (+)relative to (-)γ-HBCD in the mirror carp. In contrast, only 8 samples in the

β-HBCD group presented significant (-)β-HBCD selective enrichment after 20 d, which may be caused by transformations to (+)α-HBCD from (+)β-HBCD.
Metabolites were monitoring by MRM mode in HPLC-MS/MS.In all of the samples, tetrabromocyclododecadiene (TBCDi) was most frequently detected, with a detection rate of 38.1%. Other detected metabolites were tetrabromocyclododecene (TBCDe), tribromocyclododecadiene (TriBCDi), tribromocyclododecatriene (TriBCDie), and dibromocyclododecadiene (DBCDi), with detection rates of 14.3%, 9.52%, 9.52%and 9.52%, respectively. The metabolite pentabromocyclododecene (PBCDe) reported in the literature was not detected.Polar metabolites were not detected in this study, most likely because those were removed by the concentrated sulfuric acid in sample clean-up. New metabolites such as TBCDi, TriBCDi and TriBCDie were reported in the mirror carp for the first time.

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