Environmental Science & Technology

Article

pubs.acs.org/est

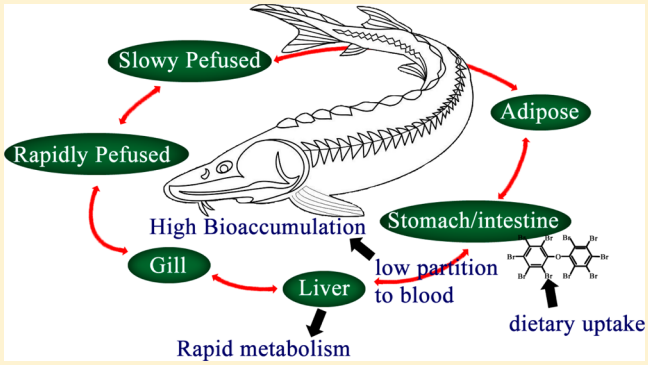
Distribution is a Major Factor Affecting Bioaccumulation of Decabrominated Diphenyl Ether: Chinese Sturgeon (*Acipenser sinensis*) as an Example

Yi Wan, Kun Zhang, Zhaomin Dong, and Jianying Hu*

Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, People's Republic of China

Supporting Information

ABSTRACT: While decabromodiphenyl ether (BDE-209) has very low bioavailability and a rapid biotransformation rate, it exhibits high bioaccumulation in wildlife. To explore the bioaccumulation mechanism of BDE-209 in organisms, its toxicokinetic processes were investigated in Chinese sturgeons from the Yangtze River. Different from less brominated BDEs, lipids did not play an important role in the distribution of BDE-209 with relatively high concentrations detected in liver (54.5 ± 3.3 ng/g wet weight (ww)), gills (47.4 ± 2.9 ng/g ww), and intestine (41.9 ± 3.0 ng/g ww), followed by stomach (21.9 ± 9.0 ng/g ww), muscle (19.1 ± 5.6 ng/g ww), heart (7.5 ± 5.2 ng/g ww), gonad (6.8 ± 4.9 ng/g ww), adipose (4.9 ± 1.2 ng/g ww), and egg (2.8 ± 2.3 ng/g ww). In vitro metabolism of BDE-209 by microsomal fractions of Chinese sturgeon found that BDE-209 was biotransformed rapidly with the rate constant (K) of 0.039 h^{-1} in liver. BDE-126, BDE-154, BDE-188, BDE-184, BDE-183, BDE-202, BDE-201, and BDE-204/197 were observed as debrominated products of BDE-209 after incubation, and their formation rates were 0.026, 0.016, and 0.006 h^{-1} for BDE-126 BDE-184, and BDE-154, respectively. The concentration ratios between heart and intestine for individual PBDEs suggested slow delivery of BDE-209 among tissues after absorption. A Bayesian hierarchical model was further developed to estimate partition coefficients in a physiologically based pharmacokinetic model of BDE-209 in Chinese sturgeon. The estimated partition coefficients between tissues and blood were higher than those of less brominated BDE or PCBs in various animals, suggesting that the low partition ratios from blood to tissues would lead to high bioaccumulation of BDE-209, especially in absorbing organs.



INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants consisting of 209 congeners, which have been commercialized as penta-, octa-, and deca-brominated mixtures.[1] While penta-BDE and octa-BDE mixtures have been nominated for a global ban and phase-out under the Stockholm Convention because of their persistent, bioaccumulative and toxic characteristics,[2] deca-BDE continues to be widely used, comprising approximately 80% of the world market demand for PBDEs. Although some European countries or states in the U.S.A. have recently banned their use,[3] more attention has shifted to deca-BDE mixtures, especially 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209), which is the primary component in the commercial mixture.

In contrast with the less brominated PBDEs, BDE-209 has been considered to be not bioavailable in the aquatic environment due to its high octanol−water partition coefficient (log K_{ow} =12.1) and molecular weight (m/z = 959 amu).[4] However, more and more studies have reported relatively high concentrations of BDE-209 in aquatic organisms including fish, birds, and grizzly bears compared with less brominated PBDEs.[5−7] Biomagnification of BDE-209 via food chains was also observed in the Lake Winnipeg food web.[8] It is well-known that absorption, distribution, metabolism, and excretion are the four pharmacokinetic processes to influence biomagnification of a chemical. Numerous laboratory studies have found that BDE-209 absorption through oral administration is limited.[3] By feeding rainbow trout with BDE-209, the uptake ratio based on the muscle concentration and mean dietary dose was estimated to be 0.02−0.13%,[9] and Stapleton et al. reported that only 0.44% of deca-BDE exposure was accumulated by rainbow trout.[10] BDE-209 also has a high biotransformation rate (half-life: 26 days) compared with less brominated PBDEs (half-life: 58−346 days) in lake trout.[11] Thus, absorption, metabolism and excretion could not reasonably explain the high bioaccumulation of BDE-209 in biota. Tissue distribution is an essential component in toxicokinetics, and partition

Received: December 2, 2012
Revised: February 3, 2013
Accepted: February 6, 2013
Published: February 6, 2013

coefficients between tissues and blood are important indexes to assess the distribution under steady-state conditions.[12] It is necessary to investigate the tissue distribution of BDE-209 for proper understanding the high bioaccumulation of BDE-209 in wildlife. Since the compound hydrophobicity is one of the main factors influencing the distribution index,[13] it can be hypothesized that the extremely high hydrophobicity of BDE-209 would lead to its high partition coefficients. However, available data on the tissue distribution of BDE-209 in aquatic species have been limited to one or two major organs with a lack of information on the partition coefficients of BDE-209 between different tissues and blood.[9,10,14]

Chinese sturgeon, a typical predatory fish, spends approximately 2−3 years in the Yangtze River for reproduction. The fish almost stop feeding during the migration period, thus the oral absorption of BDE-209 could be neglected in the period. Such a special migration habitat provided an opportunity for exploring the major toxicokinetic processes influencing the bioaccumulation of BDE-209 by only considering the metabolism and tissue distribution. In this study, the tissue distribution of high brominated PBDEs (including BDE-201/197, BDE-203, BDE-196, BDE-205, BDE-208, BDE-207, BDE-206 and BDE-209) was investigated by measuring the compounds in thirteen organs, including 8 liver, 8 muscle, 6 heart, 7 gonad, 5 stomach, 7 intestine, 5 adipose, 6 gill, 2 pancreas, 1 kidney, 1 gallbladder, 1 spleen and 15 egg samples from 17 Chinese sturgeon. To explore the key factor influencing the bioaccumulation of BDE-209, a Bayesian hierarchical model (BHM) was developed to estimate the partition coefficients of BDE-209 in various organs of Chinese sturgeon in a physiologically based pharmacokinetic (PBPK) model using the analyzed results. The metabolic kinetics of BDE-209 used in the PBPK model was assessed by in vitro microsomes from the liver of Chinese sturgeon.

MATERIALS AND METHODS

Sample Collection. The capture of Chinese sturgeon was authorized strictly for artificial propagation for saving this endangered species. The details of the tissue collection were previously described.[15] Eggs were collected before propagation, and 13 organs came from the 17 sturgeon that died between 2003 and 2006 during artificial propagation. After collection, the samples were frozen immediately at −20 °C until analysis for highly brominated PBDEs. The ages of fish were determined by counting growth layers in the cleithrum, as previously described.[16]

In Vitro Microsomal Incubations. Microsomes were isolated from cultured two-year-old Chinese sturgeon according to the method improved by Benedict et al.[17] The isolation included dithiothreitol (DTT) in the homogenization, wash and resuspension buffers to preserve the catalytic activity of reductases and deiodinases, and ethoxyresorunfin-*O*-deethylase (EROD) activity and protein content in the microsomes were determined simultaneously by a fluorescence kit (Genmed Scientific Inc., U.S.). The final reaction volume was 100 μL and contained 50 μL of the microsomal preparation and 3 uL of BDE-209. The concentration in the incubation mixture ranged from 25 to 500 ng/mL. The protein concentration in the reaction vial was 5.5 mg/mL and the CYP1A-mediated EROD activity was 260 pmol/mg/min. Reactions were performed at 25 °C for 24 h with constant agitation. Incubations without chemicals and without microsomes were used as negative controls to assess background contaminants and the possibility of nonenzyme mediated changes in chemical structure. After the incubation, the samples were analyzed immediately for identification of the metabolites and determination of the biotransformation rates. Information on chemicals and reagents used in this study, details of PBDE analysis in tissues and microsomal samples, and quality assurance and quality control (QA/QC) are provided in the Supporting Information (SI).

Process for Evaluating the Partition Coefficient. A BHM was developed to estimate the partition coefficients (P_f) and assimilation efficiency (AE) in a PBPK model of BDE-209 in Chinese sturgeon.[18,19] The procedure consisted of three steps: (1) building a PBPK model of BDE-209 in Chinese sturgeon; (2) developing a BHM which consists of the PBPK model and likelihood calculation; and (3) inversing the P_f and AE using the BHM (Figure 1).

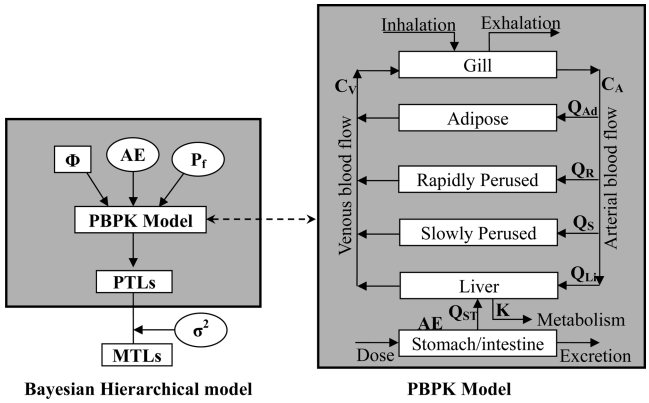


Figure 1. Framework for estimation of the partition factors between blood and various tissues based on monitoring information. P_f: Partition coefficients; AE: Assimilation efficiency; Φ: model parameters except for AE and Pf; MTLs: Measured tissue levels; PTLs: Predicted tissue levels; σ^2: error.

On the basis of the analyzed tissues in the current study, the PBPK model consisted of six tissue compartments perfused by blood: stomach/intestine, adipose, liver, gill, slowly perfused tissues (primarily white muscle) and rapidly perfused tissues (consisting of gonads and heart) (Figure 1). Tissue levels were simulated through the PBPK model by inputting the P_f, AE, and other model parameters (Φ), and then the predicted tissue levels (PTLs) and measured tissue levels (MTLs) were related through a residual error model (Gaussian distribution) with the mean (zero) and variance (σ^2) in the likelihood calculation.

According to Bayesian theory, the posterior probability density function (PPDF) for the objective parameters including the P_f, AE, and σ^2 were obtained from the product of the joint prior probability density function (pPDF) and the likelihood function. A joint prior probability distribution $p(\sigma^2,P_f\text{AE})$ was encoded as $p(\sigma^2,P_f\text{AE}) = p(\sigma^2)p(P_f)p(\text{AE})$. Hence, the PPDF for P_f, AE and σ^2 can be expressed by eq 1.

$$p(\sigma^2, P_f, \text{AE}|C_{\text{MTLs}}) \propto p(C_{\text{MTLs}}|\sigma^2, P_f, \text{AE})p(\sigma^2)p(P_f)p(\text{AE}) \quad (1)$$

The prior distributions were noninformative for $p(\sigma^2)$, $p(P_f)$ and $p(\text{AE})$. With the prior distributions for P_f and AE, the residual error model used in the likelihood function for tissue i (i = 1−5) was expressed as eq 2,

$$\ln(C_{\mathrm{MPTs}-i}) = f(P_f, \mathrm{AE}, \Phi) + \varepsilon_i = \ln(C_{\mathrm{PTLs}-i}) + \varepsilon_i \quad (2)$$

where ε_i is the error in tissue i, which was termed $\varepsilon \approx N(0, \sigma^2)$, and f expresses the PBPK model. With the prior distribution for σ^2, the posterior distribution for the parameters of interest was calculated by applying eq 2 in the likelihood function (eq 3).

$$p(C_{\mathrm{MTLs}}|\sigma^2, P_f, \mathrm{AE}) \propto \prod_{i=1}^{6} p(C_{\mathrm{MTLs}-i}|C_{\mathrm{PTLs}-i}, \sigma^2) \quad (3)$$

Markov Chain Monte Carlo (MCMC) computation was applied to inverse the parameters. The Gibbs and Metropolis Hastings (MH) samplers were used to update the object parameters. On the basis of Bayesian analysis of the hierarchical linear model, the parameter, σ^2, was randomly drawn from the inverse gamma distribution, using the Gibbs sampler. Since the PBPK model is nonlinear, the conditional distributions for P_f and AE have no closed form, thus we sampled the P_f and AE using the steps of the Metropolis algorithm as described previously.[18]

PBPK Model. The PBPK model of BDE-209 in Chinese sturgeon consisted of six tissue compartments (Figure 1). The metabolism of a chemical was set in the liver, and assimilation through uptake and excretion of BDE-209 was set in the stomach and intestine. Chemical fluxes between blood and tissues were considered to be flow-limited, while chemical exchange in gills was modeled as a countercurrent process regulated by flow and diffusion limitations.[20] Since concentrations of BDE-209 in eggs were under the detection limits in most egg samples of Chinese sturgeon, maternal transfer of BDE-209 in female sturgeon was neglected in the PBPK model. The parameters applied in the model are listed in SI Table S1.

Uptake. Chinese sturgeon is a typical anadromous fish. They live in the East Sea from birth until the initial reproduction of fish at an average age of approximately 14.3 years.[15,16] The maturing adults stop feeding, leave the ocean, and ascend the main channel of the Yangtze River to spawning grounds.[15,16] Prespawners (sturgeons with immature gonads) mature for about one year in the Yangtze River to reproduce during the spawning season of the next year, and then return to the sea after one year.[15,16] Totally, the migration period is about three years. Then sturgeons stay in the sea for three years before their next migration. The average age of the sturgeon investigated in the present study was 22 ± 2.8 years, thus the captured sturgeon had reproduced at most twice in their life. In the migration ascending the Yangtze River, diet uptake of BDE-209 was neglected since Chinese sturgeon almost stop feeding during this period and concentrations of BDE-209 in fish collected from the Yangtze River are all under detection limits.[21] Thus, the sturgeon accumulated BDE-209 through diet in the East China Sea, and the reproduction time was set to 1 or 2 in the PBPK model run separately and differences in the results were shown in a sensitivity analysis (SI). The dietary uptake of BDE-209 (UD_f) per hour during their habitat period in the Ease Sea can be calculated by eq 4,

$$\frac{d\mathrm{UD}_f}{dt} = C_{\mathrm{Dose}} \times \mathrm{BW} \times 0.02 \times \mathrm{AE} \quad (4)$$

where C_{Dose} is the concentration of BDE-209 in diet, BW is the body weight of Chinese Sturgeon, AE is the assimilation efficiency of BDE-209, and the amount of the food dietary uptake for wild Chinese sturgeon was estimated to be 2% of body weight every day.[22,23] Concentrations in diet were the average concentrations of BDE-209 in fishes from the East Sea.[5] BW was estimated by the relationship between BW and age (SI Figure S2) which was developed based on the reported values in previous investigations for Chinese sturgeon.[24−26] AE was estimated by a BHM using the concentrations investigated in the present study (Figure 1).

Uptake of BDE-209 through water exchange in the gill was also considered. The uptake of BDE-209 from water (UD_w) per hour can be calculated by eq 5,

$$\frac{d\mathrm{UD}_w}{dt} = Q_w \times \left(C_{\mathrm{water}} - \frac{C_V}{K_{\mathrm{ow}_{209}}}\right) \quad (5)$$

where C_{water} is the concentration of BDE-209 in water, K_{ow209} is the octanol−water partition factor of BDE-209, C_V is the concentration of BDE-209 in blood vessels, and Q_w is the effective respiratory volume. Concentrations of BDE-209 in water were estimated based on the bioaccumulation factor of the compound[27] and concentrations in biota from the Yangtze River and East Sea.[5,21] Q_w of rainbow trout were applied for Chinese sturgeon due to the lack of data (SI Table S1), and the sensitivity analysis was carried out for the referenced parameters.

Metabolism. The metabolic transformation rate of BDE-209 in the microsomes of Chinese sturgeon was used in the PBPK model. A Michaelis−Menten-type model was used for the results obtained from microsome incubations. The metabolism of BDE-209 (A_M) per hour in liver can be calculated by eq 6,

$$\frac{dA_M}{dt} = \frac{V_{\max} C_{\mathrm{li}}}{K_s + C_{\mathrm{li}}} \quad (6)$$

where C_{li} is the concentration of BDE-209 in liver (ng/g ww), $V_{\max}$ is the apparent maximum reaction rate (ng/g/h) and K_s is the apparent half-saturation constant (ng/g). $V_{\max}$ and K_s were determined from the microsomes of Chinese sturgeon incubated with BDE-209.

Distribution. Distribution of BDE-209 in various tissues is determined by the blood flow to the six compartments and partition coefficients between various tissues and blood. The distribution in tissue i can be calculated by eq 7,

$$\frac{dA_i}{dt} = Q_i \times \left(C_V - \frac{C_i}{P_i \times V_i}\right) \quad (7)$$

where A_i is the amount of BDE-209 in tissue i, Q_i is the blood flow to tissue i, C_V is the blood concentration of BDE-209 in arterial blood, C_i is the concentration of BDE-209 in tissue i, V_i is the volume of tissue i ($V_i = BW \times f_i$, f_i is the fraction of body weight for tissue i) and P_i is the partition coefficient between tissue i and blood. Considering the lack of data for sturgeon, the blood flow in various tissues of rainbow trout (Q_{st}, Q_s, Q_r, Q_{ad}, and Q_{li}) were applied in the present study (SI Table S1), and a sensitivity analysis was carried out (SI Table S2). Tissue volumes (%, fraction of body weight) of Chinese sturgeon were from the previous study.[28] The partition coefficients of BDE-209 in the PBPK model were estimated by BHM. The sensitivity analysis of the referenced parameters in the PBPK model is provided in the SI. The calculation was run on an Intel Pentium 2 × 4 CPU (2.0 GHz) with Red Hat Enterprise Linux 4.

RESULTS AND DISCUSSION

Concentrations among Tissues. Of the nine octa-, nona-, and deca- BDEs, BDE-196, BDE-206, BDE-207, BDE-208, and BDE-209 were detected in the tissues of Chinese sturgeon. As shown in Figures 2 and S1 in SI, the total concentrations of the

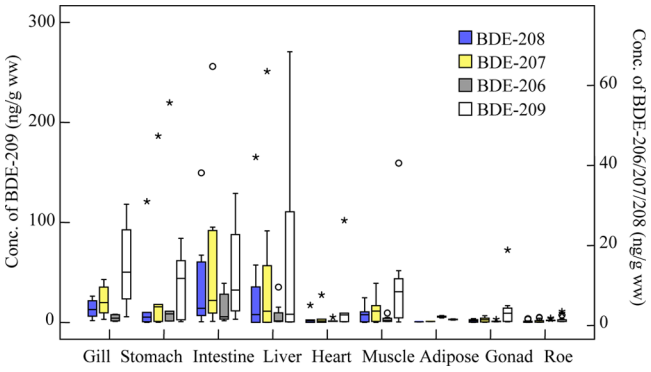


Figure 2. Concentration levels of BDE-206/207/208/209 in vatious tissues of Chinese sturgeon. Kidney, spleen, and gallbladder are not included in the graph due to the limited number of samples. The horizontal line represents the median concentration. The 25th and 75th centiles define the boxes and the whiskers represent the 10th and 90th centiles.

detected compounds in liver, gills, and intestine were 54.5 ± 3.3, 47.4 ± 2.9, and 41.9 ± 3.0, ng/g ww, respectively, followed by stomach (21.9 ± 9.0 ng/g ww), muscle (19.1 ± 5.6 ng/g ww), heart (7.5 ± 5.2 ng/g ww), gonad (6.8 ± 4.9 ng/g ww), adipose (4.9 ± 1.2 ng/g ww), and egg (2.8 ± 2.3 ng/g ww). Concentrations of BDE-209 in the muscle of Chinese sturgeon were lower than those in fish collected from the East China Sea (260.1 ng/g ww) and Yellow Sea (161.5 ng/g ww)[5] where the sturgeon stay before they mature for reproduction, and the possible reason could be that Chinese sturgeon stay in the Yangtze River for a period of approximately one year before reproduction and concentrations of BDE-209 in fishes from the Yangtze River were all below the detection limits.[21] The BDE-209 concentration in the liver of Chinese sturgeon was higher than those in the liver of common sole, bib, and whiting (3.4 to 37.2. ng/g ww) from the western Scheldt estuary, which is subjected to a variety of suspected PBDE sources, such as a brominated flame retardant manufacturing plant.[29] Conclusively, concentrations of BDE-209 in Chinese sturgeon are relatively high compared with other fish worldwide.

When concentrations of highly brominated PBDEs were expressed on a lipid weight basis, relatively high total concentrations were found in intestine (2500 ± 4.3 ng/g lw), gills (2200 ± 3.6 ng/g lw), stomach (1900 ± 12 ng/g lw), and muscle (1200 ± 10 ng/g lw), much higher than gonad (219 ± 4.2 ng/g lw), heart (210 ± 5.6 ng/g lw), liver (112 ± 10 ng/g lw), adipose (7.6 ± 1.4 ng/g lw), and roe (8.3 ± 2.3 ng/g lw) (Figure 2). Different from the less brominated PBDEs,[15] concentrations of octa-, nona-, and deca-BDEs were low in tissues with relatively high lipid content (e.g., roe, adipose), although the highly brominated BDEs all have high log K_{ow} values (11.2 for nona-BDEs, 12.1 for deca-BDE), suggesting that lipid may not play an important role in the distribution of the compound. The relatively high concentrations of BDE-209 observed in muscle compared with liver were similar to the distribution of BDE-209 in rainbow trout injected intraperitoneally with the chemical,[14] but different from that in rainbow trout fed with deca-BDE treated food for 21−49 days by Kierkegaard et al.[9] and Stapleton et al.[10] in which BDE-209 was mostly concentrated in liver tissue on both a wet and lipid weight basis. The possible reason causing the distribution of BDE-209 in Chinese sturgeon was that the sturgeons were in a depuration period similar to the trout injected with BDE-209, since the maturing female adults ascend the main channel of the Yangtze River to spawn for about 1−2 years before reproduction, and they almost stop feeding during the migration period. Additionally, by comparing the concentrations of BDE-209 in the liver and muscle of rainbow trout during the depuration period reported by Kierkegaard et al,[9] it can be found that BDE-209 was reduced by 75% in muscle but by 97% in liver within 71 days, further demonstrating that the rapid metabolism of BDE-209 in liver tissues would cause relatively low concentrations of the compound in liver after a period of depuration. It should be noted that concentrations of octa-, nona-, and deca- BDEs were under detection limits in most eggs. The extremely low maternal transfer of BDE-209 was also observed in its placental transfer in human,[30] suggesting that the compound poses low risks to the offspring of Chinese sturgeon.

BDE-209 was the predominant congener in all tissues except for adipose and egg, accounting for 72−85% of the total concentrations followed by BDE-207 (5.4−13%), BDE-208 (3.9−8.6%), BDE-206 (1.3−5.7%), and BDE-196 (0.6−4.3%) (Figure 3). In adipose and egg, the contribution of BDE-196

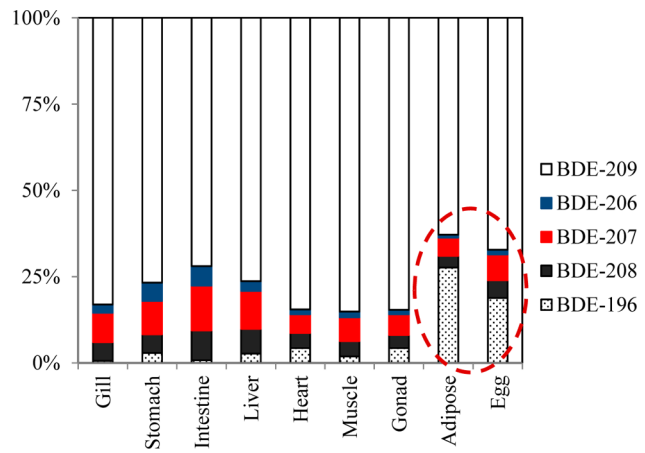


Figure 3. Profiles of highly brominated PBDEs in different tissues of Chinese sturgeon.

became high (19−28%), resulting in a relatively low proportion of BDE-209 (63−67%). The different profiles of highly brominated BDEs in adipose and egg could be due to the different partition coefficients among different congeners.[9]

Metabolism of BDE-209. To assess the role of metabolism on the bioaccumulation of BDE-209 and the potential sources of highly brominated BDEs detected in Chinese sturgeon, in vitro metabolism of BDE-209 by microsomal fractions of Chinese sturgeon were conducted. As shown in Figure 4, one penta-BDE (BDE-126), one hexa-BDE (BDE-154), three hepta-BDEs (BDE-188, BDE-184, and BDE-183) and three octa-BDEs (BDE-202, BDE-201, and BDE-204/197) were detected in microsomes of Chinese sturgeon incubated with BDE-209 while no debrominated products were found in the heat-inactivated microsomes. Previous studies have found hexa- to octa- BDEs (BDE-155, BDE-154, BDE-184, BDE-188, BDE-

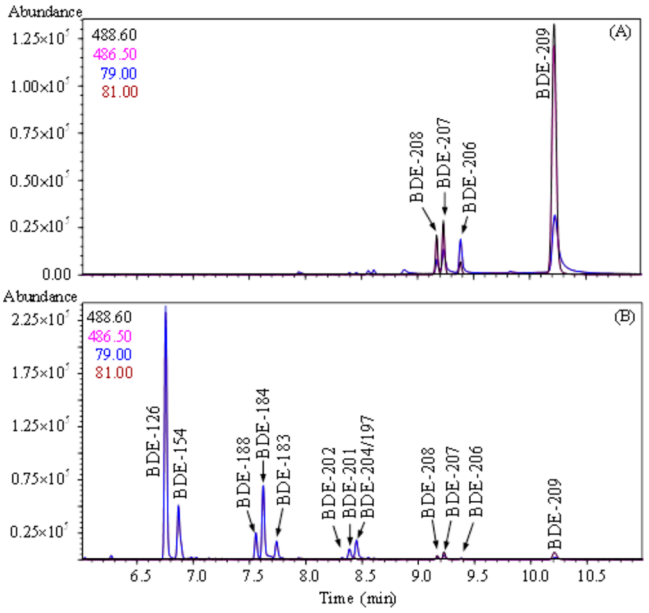


Figure 4. GC/NCI-MS chromatograms (*m/z* 79/81/488.6/486.5) in microsomes of Chinese sturgeon exposed to BDE-209. (A) Control and (B) Exposure.

202, BDE-201, and BDE-197) as debrominated products in hepatic microsomes of juvenile carp incubated with BDE-209 and only one octa-BDE (BDE-202) was found in hepatic microsomes of rainbow trout.[31,32] Compared to common carp and rainbow trout, sturgeon could generate more products (e.g., BDE-126 and BDE-183), which was possibly due to species-specific differences in the enzyme system responsible for the debromination of PBDEs, as exemplified by the differences among rainbow trout, common carp, and chinook salmon.[32]

After a 24-h incubation, the liver microsomes of Chinese sturgeon biotransformed 96−97% of the BDE-209 when the exposure concentrations ranged from 25 to 500 ng/mL. A calculation was performed using the Michaelis−Menten-type model (eq 8) for the results obtained from microsome incubations:

$$\frac{dS}{dt} = \frac{V_{max}S}{K_s + S} \quad (8)$$

where S is the concentration of a compound in incubation (ng/mL), V_{max} is the apparent maximum reaction rate (ng/mL/h), and K_s is the apparent half-saturation constant (ng/mL). In the current study, a significant linear relationship was observed between BDE-209 concentration and the biotransformation rates (Figure 4a). These results indicated that the incubation concentrations of BDE-209 were much lower than the half-saturation constant ($S \ll K_s$), thus the first-order kinetics between biotransformation rates and BDE-209 incubation concentrations were modeled as shown in eq 9,

$$\frac{dS}{dt} = \frac{V_{max}S}{K_s} = KS \quad (9)$$

where K is the biotransformation rate constant (h^{-1}), and was calculated to be 0.039 h^{-1} (Table 1), suggesting that BDE-209 would be biotransformed at a rapid rate (half-life: 18 h) in the liver of Chinese sturgeon. Unfortunately, the biotransformation rate in microsome incubation using the Michaelis−Menten-type model is not available for BDE-209 in other aquatic species. The depletion percentages of BDE-209 in the microsomal biotransformation were reported by two previous studies,[31,33] which reported that greater depletion in rainbow trout and common carp (40.3−67.4% of 15 pmol of BDE209 after 24 h)[31] and relatively lower depletion in polar bear, beluga whale, ringed seal, and laboratory rat (14−25% of 30 pmol BDE209 after 1.5 h) [33] at protein concentration of 1 mg/mL. The depletion percentage of BDE-209 in Chinese sturgeon (65.6% of 25 pmol of BDE209 after 24 h) was similar to that of rainbow trout and common carp, whereas the protein concentration in the present study (5.5 mg/mL) was fiver times higher than that in the previous study.[31] Thus, the biotransformation rate of BDE-209 in Chinese sturgeon should be lower than those in rainbow trout and common carp.

Table 1. Biotransformation/Formation Kinetics of BDE-209, BDE-208, BDE-207, BDE-206, and Their Debrominated Products in Microsomes of Chinese Sturgeon[a,b,c]

compounds	$K = V_{max}/K_s$	r^2	$t_{1/2}$
biotransformation rate constant			
BDE-209	0.039	0.9993	18
BDE-208	0.034	0.9562	20
BDE-207	0.029	0.943	24
BDE-206	0.04	0.9735	17
formation rate constant			
BDE-126	0.026	0.9967	27
BDE-154	0.006	0.9926	116
BDE-188	0.005	0.9919	139
BDE-184	0.016	0.9924	43
BDE-183	0.004	0.9973	173
BDE-202	0.0003	0.9995	2310
BDE-201	0.0024	0.9955	289
BDE-204/197	0.005	0.9888	139

[a] $t_{1/2}$ = 0.693/K. [b] $t_{1/2}$ of product formation is the time for forming the products at twice of initial concentration. [c] Units = ng/mL/h for V_{max}, ng/mL for K_s, h^{-1} for K and h for $t_{1/2}$.

While BDE-206, BDE-207 and BDE-208 were debrominated products of BDE-209, these compounds were also detected with relatively high abundance in the control samples (Figure 3a), indicating that the deca-BDE contains small amounts of the nona-BDEs as impurities.[34] A significant linear relationship was observed between the biotransformation rates and the concentrations of BDE-206, BDE-207, and BDE-208, suggesting that these compounds were readily biotransformed in the incubation and the relatively low r^2 values were possibly due to the contribution of the debromination of BDE-209 (Table 1). Although the accuracy of the K values of the nona-BDEs would be influenced by the BDE-209 debromination, the r^2 of the linear kinetics was still high (>0.9), suggesting that the concentrations of produced nona-BDEs were much lower than those of the impurities. On the basis of the kinetics results, the biotransformation rate of BDE-206 was the highest, which is consistent with the low proportion of the compound detected in Chinese Sturgeon (Figure 3), although this compound was the predominant nona-BDE in the deca-PBDE technical flame-retardant mixtures.[35]

Similar to the biotransformation, the formation rates of debrominated products (BDE-126, BDE-154, BDE-188, BDE-184, BDE-183, BDE-202, BDE-201, and BDE-204/197) all increased linearly (r^2 = 0.9888−0.9995) with the incubation concentrations of BDE-209 (Figure 5b and Table 1). The K

dx.doi.org/10.1021/es304926r | Environ. Sci. Technol. 2013, 47, 2279−2286

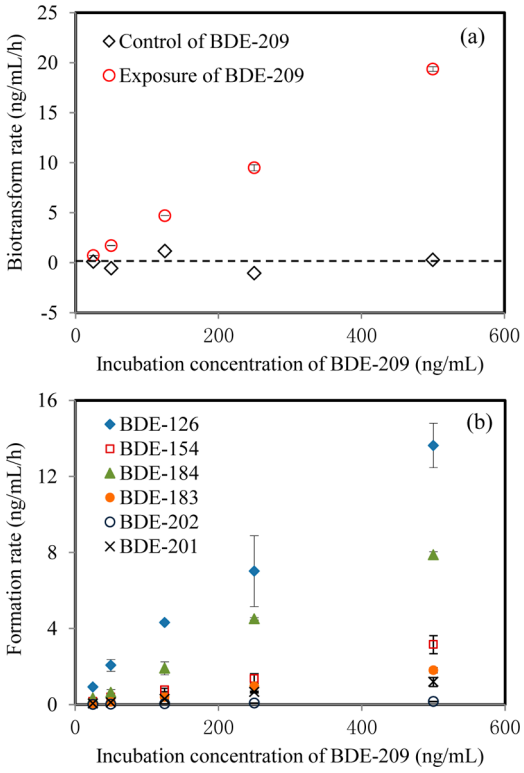


Figure 5. In vitro biotransformation rates of BDE-209 and formation rates of some debrominated products in liver microsomes of Chinese sturgeon incubated with 25−500 ng/g BDE-209 for 24 h at 25 °C.

values, representing the formation rate constant (h^{-1}) for the debrominated products, were the highest for BDE-126 (0.026) followed by BDE-184 (0.016), and BDE-154 (0.006) (Table 1), further demonstrating that the three compounds were the major biotransformation products of BDE-209 in liver tissues. Of the highly brominated PBDEs detected in wild Chinese sturgeon, BDE-196, which was not present in the microsome incubations of BDE-209, would be accumulated directly from environment, since this compound is one of the major components of octa-PBDE technical flame-retardant mixtures.[35]

Blood Perfusion of BDE-209 in Various Tissues. The above investigation indicated that Chinese sturgeon accumulated relatively high concentrations of BDE-209 after they ascend the main channel of the Yangtze River for about one year although oral absorption of BDE-209 could be neglected in the migration period and biotransformation rate of BDE-209 in liver is rapid. Thus metabolism should not be the reason accounting for the high concentrations of BDE-209 in Chinese sturgeon. The partition of BDE-209 from the blood to the other tissues can be reflected by the concentration ratios between heart and intestine since heart is a richly perfused tissue and intestine is the initial absorption tissue to accumulate BDE-209. It is interesting to note that the heart/intestine concentration ratios of PBDEs from tri- to hex-BDEs decreased with increasing logKow, and a flat trend between the concentration ratios and log K_{ow} was observed for hepta- to deca-BDEs (Figure 6). This suggested that the low partition ratios of the hepta- to deca-BDEs from blood to tissues would lead to slow delivery of the pollutants to metabolically active organs and high bioaccumulation in absorbing organs.

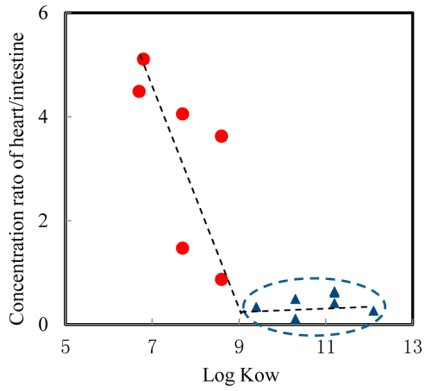


Figure 6. Relationship between log K_{ow} and the concentration ratios of PBDEs between heart and intestine; circled dots: hepta- to deca-BDEs.

A Bayesian method was further applied to estimate partition coefficients and AE in the PBPK model of BDE-209 using the concentrations investigated in the present study (Figure 1). The MCMC simulation runs started at dispersed initial points in the parameter space and each run simulated 20 000 times, and converged to the corrected scale reduction factors (R) of 1.0. The sensitivity analysis shows that the inversion results are stable as described in the SI. The AE of BDE-209 in Chinese sturgeon was 0.005 ± 0.002 (Table 2), which is similar to that

Table 2. Assimilation Efficiency and Partition Coefficients (P_f) Evaluated by Bayesian Hierarchical Model in Sturgeon with One Time of Reproduction in Their Life[a]

parameters	term	parameter value	95% confidence interval
stomach/intestine:blood	P_{st}	40.4 ± 12.0	20.6−67.3
slowly perfused tissue:blood	P_s	22.3 ± 4.4	13.1−29.3
richly perfused tissue:blood	P_r	9.0 ± 2.9	4.6−15.0
adipose:blood	P_{ad}	4.6 ± 0.9	2.7−5.9
liver:blood	P_{li}	14.2 ± 4.1	7.4−23.1
assimilation efficiency	AE	0.005 ± 0.002	0.003−0.009

[a]The influence of reproduction time on estimated parameters was assessed in the SI.

reported in rainbow trout by Kierkegaard (0.02−0.13% [9]) and Stapleton (0.44% [8]), further confirming that accumulation of BDE-209 through oral administration is limited. The partition coefficients of stomach/intestine:blood, poorly perfused tissue:blood, richly perfused tissue:blood, adipose:blood and liver:blood in Chinese sturgeon were 40.4 ± 12.0, 22.3 ± 4.4, 9.0 ± 2.9, 4.6 ± 0.9 and 14.2 ± 4.1, respectively (Table 2). The partition coefficients of poorly perfused tissue:blood (22.3 ± 4.4) and liver:blood (14.2 ± 4.1) in Chinese sturgeon were much higher than those of PCB-52 in rainbow trout (0.2−2), BDE-47 in rat (2−10) and BDE-47, −99, −100, and −153 in harbor porpoises (6.8−7.9).[36−39] Taking the results of the BHM and the above correlation between heart/intestine concentration ratios and log K_{ow} together, we can conclude that the partition coefficients of BDE-209 between tissues and blood were very high compared to those of low brominated PBDEs and some PCBs, which would result in the slow distribution of BDE-209 to metabolically active organs and therefore leading to high accumulation in absorbing organs. In addition, while the partition coefficient of gill (richly perfused

dx.doi.org/10.1021/es304926r | Environ. Sci. Technol. 2013, 47, 2279−2286

tissue):blood was estimated to be much lower than that of stomach/intestine:blood, the concentrations of BDE-209 in gill were slightly higher than stomach/intestine tissues. This is possibly due to the fact that gill could accumulate BDE-209 through direct contact with sediments via inhalation of suspended sediment as exemplified by other persistent organic pollutants such as PCB,[40] while PBPK model designed in this study did not include uptake of sediment in gill.

Since the low assimilation efficiency of BDE209 have been reported in fish and rat,[3,9,10] and biotransformation rate of the compound in Chinese sturgeon was relatively low compared to other fishes, distribution as a major factor for affecting bioaccumulation of BDE-209 observed in Chinese sturgeon could be a general phenomenon to aquatic species. Thus, our study showed for the first time that the distribution is in fact a key factor influencing the bioaccumulation of BDE-209 in organisms, and lipid did not play an important role in the distribution of the compound. More bioaccumulation studies should focus on this process in the future.

■ ASSOCIATED CONTENT

Supporting Information

Text, figures, and tables addressing (1) chemicals and reagents, (2) PBDE analytical procedure, instrument condition and quality assurance and quality control, (3) sensitivity analysis, (4) concentrations of highly bromine BDEs based on lipid weight in various tissues, (5) relationship between body weight and age of Chinese sturgeon, (6) physiological and anatomical parameters used in PBPK model, and (7) sensitivity analysis of referenced parameters in the PBPK model. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: 86-10-62765520. E-mail: hujy@urban.pku.edu.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support for this study was obtained from the National Natural Science Foundation of China (41171385) and the Special Scientific Research Funds for Environmental Protection Commonweal Section (201309027). We thank Qiwei Wei and Luoxin Li (Yangtze River Fisheries Research Institute, Chinese Academy of Fisheries Science, Ministry of Agriculture of China) for providing samples of eggs and tissues from Chinese sturgeon.

■ REFERENCES

(1) Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications, their use pattern in different countries/regions, and possible modes of release. *Environ. Int.* **2003**, *29*, 683−689.

(2) *ENDS. Nine POPs added to Still Toothless Treaty*; Environmental Data Services (ENDS): London, 2009; Vol. *412*, pp 37−38.

(3) Costa, L. G.; Giordano, G. Is decabromodiphenyl ether (BDE-209) a developmental neurotoxicant? *NeuroToxicology* **2011**, *32*, 9−24.

(4) Hardy, M. L. A comparison of the properties of the major commercial PBDPO/PBDE product to those of major PBB and PCB products. *Chemosphere* **2002**, *46*, 717−728.

(5) Liu, Y. P.; Li, J. G.; Zhao, Y. F.; Wen, S.; Huang, F. F.; Wu, Y. N. Polybrominated diphenyl ethers (PBDEs) and indicator polychlorinated biphenyls (PCBs) in marine fish from four areas of China. *Chemosphere* **2011**, *83* (2), 168−174.

(6) Chen, D.; Mai, B. X.; Song, J.; Sun, Q. H.; Luo, Y.; Luo, X. J.; Zeng, E. Y.; Hale, R. C. Polybrominated diphenyl ethers in birds of prey from Northern China. *Environ. Sci. Technol.* **2007**, *41* (6), 1828−1833.

(7) Christensen, J. R.; Macduffee, M.; Macdonald, R. W.; Whiticar, M.; Ross, P. S. Persistent organic pollutants in British Columbia grizzly bears: Consequence of divergent diets. *Environ. Sci. Technol.* **2005**, *39* (18), 6952−6960.

(8) Law, K.; Halldorson, T.; Danell, R.; Stern, G.; Gewurtz, S.; Alaee, M. Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. *Environ. Toxicol. Chem.* **2006**, *25*, 2177−2186.

(9) Kierkegaard, A.; Balk, L.; Tjarnlund, U.; De Wit, C. A.; Jansson, B. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **1999**, *33* (10), 1612−1617.

(10) Stapleton, H. M.; Alaee, M.; Letcher, R. J.; Baker, J. E. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. *Environ. Sci. Technol.* **2004**, *38* (1), 112−119.

(11) Tomy, G. T.; Palace, V. P.; Halldorson, T.; Braekevelt, E.; Danell, R.; Wautier, K.; Evans, B.; Brinkworth, L.; Fisk, A. T. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). *Environ. Sci. Technol.* **2004**, *38* (5), 1496−1504.

(12) Nichols, J. W.; Bonnell, M.; Dimitrov, S. D.; Escher, B.; Han, X.; Kramer, N. Bioaccumulation Assessment Using Predictive Approaches. *Integr. Environ. Assess. Manage.* **2009**, *5*, 577−597.

(13) Kleinow, K. M.; Nichols, J. W.; Hayton, W. L.; McKim, J. M.; Barron, M. G. Toxicokinetics in fishes. In *The Toxicology of Fishes*; Digiulio, R. T., Hinton, D. E., Eds.; CRC Press: Boca Raton (FL), 2008, pp 55−152.

(14) Feng, C. L.; Xu, Y. P.; He, Y.; Luo, Q.; Zha, J. M.; Wang, Z. J. Debrominated and methoxylated polybrominated diphenyl ether metabolites in rainbow trout (*Oncorhynchus mykiss*) after exposure to decabromodiphenyl ether. *J. Environ. Sci.* **2010**, *22*, 1425−1434.

(15) Zhang, K.; Wan, Y.; Giesy, J. P.; Lam, M. H. W.; Steve, W.; Jones, P. D. Tissue concentrations of polybrominated compounds in Chinese Sturgeon (*Acipenser sinensis*): Origin, hepatic sequestration and maternal transfer. *Environ. Sci. Technol.* **2010**, *44* (5), 5781−5786.

(16) Wei, Q. W.; Ke, F. E.; Zhang, J. M.; Zhuang, P.; Luo, J. D.; Zhou, R. Q.; Yang, W. H. Biology, fisheries, and conservation of sturgeons and paddlefish in China. *Environ. Biol. Fish.* **1997**, *48* (1−4), 241−256.

(17) Benedict, R. T.; Stapleton, H. M.; Letcher, R. J.; Mitchelmore, C. L. Debromination of polybrominated diphenyl ether-99 (BDE-99) in carp (*Cyprinus carpio*) microflora and microsomes. *Chemosphere* **2007**, *69* (6), 987−993.

(18) Lyons, M. A.; Yang, R. S. H.; Mayeno, A. N.; Reisfeld, B. Computational toxicology of chloroform: Reverse dosimetry using Bayesian interference, Markov chain Monte Carlo simulation, and human biomonitoring data. *Environ. Health Persp.* **2008**, *116* (8), 1040−1046.

(19) Dong, Z. M.; Hu, J. Y. Development of lead source-specific exposure standards based on aggregate exposure assessment: Bayesian inversion from biomonitoring information to multipathway exposure. *Environ. Sci. Technol.* **2012**, *46*, 1144−1152.

(20) Nichols, J. W.; Fitzsimmons, P. N.; Whiteman, F. W.; Dawson, T. D.; Babeu, L.; Juenemann, J. A physiologically based toxicolokinetic model for dietary uptake of hydrophobic organic compounds by fish, I. Feeding studies with 2,2′,5,5′-tetrachlorobiphenyl. *Toxicol. Sci.* **2004**, *77*, 206−218.

(21) Xian, Q. M.; Ramu, K.; Isobe, T.; Sudaryanto, A.; Liu, X. H.; Gao, Z. S.; Takahashi, S.; Yu, H. X.; Tanabe, S. Levels and body distribution of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in freshwater fishes from the Yangtze River, China. *Chemosphere* **2008**, *71*, 268−276.

(22) Xiao, H.; Wen, Z. H.; Hu, Y. P. Suitable feeding rate of juvenile Chinese sturgeon *Acipenser sinensis*. *Fisher. Sci. Technol. Inform.* **1998**, *6*, 248−253.
(23) Xiao, H.; Zhu, X.; Shi, X. T.; Lu, X. B.; Zhang, D. Z.; Rao, J.; Jian, J. L. Compensatory growth and body composition in juvenile Chinese sturgeon *Acipenser sinensis* following temporary food deprivation. *J. Appl. Ichthyol.* **2011**, *27*, 554−557.
(24) Liu, W.; Wei, Q. W.; Wen, H.; Jiang, M.; Wu, F.; Shi, Y. Compensatory growth in juvenile Chinese sturgeon (*Acipenser sinensis*): Effects of starvation and subsequent feeding on growth and body composition. *J. Appl. Ichthyol.* **2011**, *27*, 749−754.
(25) Wei, Q. W.; Zhang, X. Y.; Zhang, X. F.; Liu, J. Y.; Yang, D. M.; Zhu, Y. J.; Chen, X. H.; Wu, X. X.; Li, L. X.; Liu, J. W. Acclimating and maintaining Chinese sturgeon *Acipenser sinensis* in a large public aquarium environment. *J. Appl. Ichthyol.* **2011**, *27*, 533−540.
(26) Chen, X. H.; Zhang, Y. Z.; Wei, Q. W.; Liu, Z. G.; Li, X. L.; Qiu, S. Observations on gonadal development of 7−11 year old Chinese sturgeon *Acipenser sinensis* cultured in freshwater. *J. Appl. Ichthyol.* **2011**, *27*, 648−650.
(27) Wang, Y. W.; Liu, X. M.; An, L.; Wang, T.; Zhang, Q. H.; Wang, P.; Fu, J. J.; Jiang, G. B. Effect of municipal sewage treatment plant effluent on bioaccumulation of polychlorinated biphenyls and polybrominated diphenyl ethers in the recipient water. *Environ. Sci. Technol.* **207**, *41*, 6026−6032.
(28) Peng, H.; Zhang, K.; Wan, Y.; Hu, J. Y. Tissue distribution, maternal transfer, and age-related accumulation of dechloranes in Chinese sturgeon. *Environ. Sci. Technol.* **2012**, *46*, 9907−9913.
(29) Voorspoels, S.; Covaci, A.; Schepens, P. Polybrominated Diphenyl Ethers in Marine Species from the Belgian North Sea and the Western Scheldt Estuary: Levels, Profiles, and Distribution. *Environ. Sci. Technol.* **2003**, *37*, 4348−4357.
(30) Frederiksen, M.; Vorkamp, K.; Mathiesen, L.; Mose, T.; Knudsen, L. E. Placental transfer of the polybrominated diphenyl ethers BDE-47, BDE-99, and BDE-209 in a human placenta perfusion system: An experimental study. *Environ. Health* **2010**, *9*, 32.
(31) Stapleton, H. M.; Brazil, B.; Holbrook, R. D.; Mitchelmore, C. L.; Benedict, R.; Konstantinov, A.; Potter, D. In vivo and in vitro debromination of decabromodiphenyl ether (BDE-209) by juvenile rainbow trout and common carp. *Environ. Sci. Technol.* **2006**, *40*, 4653−4658.
(32) Roberts, S. C.; Noyes, P. D.; Gallagher, E. P.; Stapleton, H. M. Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. *Environ. Sci. Technol.* **2012**, *45*, 1999−2005.
(33) McKinney, M. A.; Dietz, R.; Sonne, C.; De Guise, S.; Skirnisson, K.; Karlsson, K.; Steingrimsson, E.; Letcher, R. J. Comparative hepatic microsomal biotransformation of selected PBDEs, including decabromodiphenyl ether, and decabromodiphenyl ethane flame retardants in Arctic marine-feeding mammals. *Environ. Toxicol. Chem.* **2011**, *30*, 1506−1514.
(34) Noyes, P. D.; Hinton, D. E.; Stapleton, H. M. Accumulation and debromination of decabromodiphenyl ether (BDE-209) in juvenile fathead minnows (*Pimephales promelas*) induces thyroid disruption and liver alterations. *Toxicol. Sci.* **2011**, *122*, 265−274.
(35) La Guardia, M. J.; Hale, R. C.; Harey, E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penat-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environ. Sci. Technol.* **2006**, *40*, 6247−6254.
(36) Nichols, J. W.; Fitzsimmons, P. N.; Whiteman, F. W.; Dawson, T. D.; Babeu, L.; Juenemann, J. A physiologically based toxicolokinetic model for dietary uptake of hydrophobic organic compounds by fish, I. Feeding studies with 2,2′,5,5′-tetrachlorobiphenyl. *Toxicol. Sci.* **2004**, *77*, 206−218.
(37) Nichols, J. W.; Fitzsimmons, P. N.; Whiteman, F. W.; Dawson, T. D.; Babeu, L.; Juenemann, J. A physiologically based toxicolokinetic model for dietary uptake of hydrophobic organic compounds by fish, II. Simulation of chronic exposure scenarios. *Toxicol. Sci.* **2004**, *77*, 206−218.
(38) Weijs, L.; Yang, R. S. H.; Covaci, A.; Das, K.; Blust, R. Physiologically based pharmacokinetic (PBPK) models for lifetime exposure to PCB 153 in male and female harbor porpoises (*Phocoena phocoena*): Model development and evaluation. *Environ. Sci. Technol.* **2010**, *44*, 7023−7030.
(39) Emond, C.; Raymer, J. H.; Studabaker, W. B.; Garner, C. E.; Birnbaum, L. S. A physiologically based pharmacokinetic model for developmental exposure to BDE-47 in rats. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 290−298.
(40) Craig Barber, M.; Suarez, L. A.; Lassiter, R. R. Modeling bioaccumulation of organic pollutants in fish with an application in Lake Ontario Salmonids. *Can. J. Fish. Aquat. Sci.* **1991**, *48*, 318−337.