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Supporting Information

for

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

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This supporting information provides detailed descriptions of (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 (Figure S1), (5) relationship between body weight and age of Chinese sturgeon (Figure S2), (6) physiological and anatomical parameters used in PBPK model (Table S1), and (7) sensitivity analysis of PBPK model referenced by rainbow trout (Table S2).

25 **Chemicals and Reagents.** Eight PBDEs (BDE-201/197, BDE-203, BDE-196, BDE-205,
26 BDE-208, BDE-207, BDE-206 and BDE-209) and PCB-209 standards were obtained from
27 Wellington Laboratories Inc. (Guelph, Ontario, Canada). Pesticide residue grade
28 dichloromethane (DCM), *n*-hexane, methyl tert-butyl ether (MTBE), acetonitrile and
29 methanol were obtained from Fisher Inc. (USA). Sodium sulfate, silica gel (60-100 mesh
30 size), aluminum oxide (neutral, 150 mesh size), and potassium hydroxide (KOH) were
31 purchased from Sigma-Aldrich (St. Louis, MO, USA). For biochemical analyses, the
32 fluorescence kit was obtained from (Genmed Scientific Inc, USA), and sodium phosphate
33 dibasic (Na₂HPO₄), sodium phosphate monobasic (NaH₂PO₄) and potassium phosphate
34 monobasic (KH₂PO₄), resorufin, ethylenediaminetetraacetic acid (EDTA), and dithiothreitol
35 (DTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other biochemical
36 reagents, including NADPH, were obtained from Sigma-Aldrich and were reagent grade or
37 better unless stated otherwise.

38 **Extraction and Cleanup of Samples.** Tissues were freeze-dried, and then approximately 1-3
39 g dry weight (dw) subsamples were spiked with PCB-209, and extracted by accelerated
40 solvent extraction (Dionex ASE-200, Sunnyvale, CA). The extraction employed two 10 min
41 cycles, the first cycle was performed with *n*-hexane/dichloromethane (DCM) (1:1) at 100°C
42 and 1500 psi, followed by a second cycle with *n*-hexane/methyl tert-butyl ether (MTBE) (1:1)
43 at of 60°C and pressure of 1000 psi. The two extraction fractions were combined and rotary
44 evaporated to near dryness. The extract was then transferred to 15 ml glass tubes by 8 mL
45 hexane, and 4 mL 0.5 M KOH in 50% ethanol was added. The aqueous layer (KOH) was
46 extracted with 8 mL of *n*-hexane three times. The extract was concentrated to approximately

47 2 mL and loaded onto a column of 1 g Na₂SO₄ and 8 g acidified silica (48% H₂SO₄) and
48 eluted with 15 mL of n-hexane and 10 mL of DCM. The eluate was further purified on a
49 neutral alumina column (4 g of sodium sulfate, 4 g of neutral alumina, 4 g of sodium sulfate).
50 The first fraction eluted from the alumina column with 20 mL of hexane was discarded. The
51 second fraction, which contained PBDEs, was obtained by elution with 25 mL of 60% DCM
52 in n-hexane. The eluate was evaporated to dryness under a gentle stream of nitrogen, and then
53 40 µl hexane were added for analysis of the high brominated PBDEs.

54 For microsomal samples, each of the microsomal reaction mixture samples was spiked
55 with PCB-209 followed by addition of 2 mL pure water. The aqueous layer was extracted
56 with 2 mL n-hexane/MTBE (1:1; v/v) three times. The extract was evaporated to dryness
57 under a gentle stream of nitrogen, and then 50 µl hexane were added for gas
58 chromatography-electron capture negative ionization mass spectrometer (GC-ENCI-MS)
59 analysis.

60 **Instrumental Conditions.** Identification and quantification of high brominated PBDEs were
61 performed using a GC-ENCI-MS (Shimadzu QP 2010 plus, Japan). Chromatographic
62 separation was achieved on a VF-5MS capillary column (15 cm × 0.25 mm × 0.1 µm film
63 thickness; J&W Scientific, USA). A splitless injector was used, and the injector was held at
64 290°C. The temperature program was from 120°C (2 min) to 310°C (5 min) at a rate of
65 25°C/min. The transfer line temperature and the ion source temperature were maintained at
66 280°C and 260°C, respectively. High pressure injection was applied with the pressure of 300
67 psi hold for 1 min. The carrier gas was helium at a constant flow rate of 5 ml/min. Data
68 acquisition was conducted in selected ion monitoring mode.

69 **Quality Assurance and Quality Control (QA/QC).** Concentrations of high brominated
70 PBDEs were quantified by the internal standard isotope-dilution method with mean relative
71 response factors determined from standard calibration runs. High brominated PBDEs were
72 quantified in sample extracts relative to PCB-209. Recoveries of PCB-209 were $74.9 \pm 38.8\%$
73 in all samples. All equipment rinses were carried out with acetone and hexane to avoid
74 sample contamination. A laboratory blank was incorporated in the analytical procedures for
75 every batch of 12 samples. The method detection limits (MDL) were set to be the mean
76 concentration plus three times the standard deviation in the blank samples, in which only
77 BDE-209 was detected. The MDLs for the other compounds, which were not detected in
78 blank samples, were set to the instrumental minimum detectable amounts. The detection
79 limits were 0.01 ng/g ww for BDE-204/197, BDE-203 and BDE-205, 0.02 ng/g ww for
80 BDE-206, 0.1 ng/g ww for BDE-208, 0.2 ng/g ww for BDE-207, 1.0 ng/g ww for BDE-196,
81 and 2 ng/g ww for BDE-209.

82 **Sensitivity Analysis of Parameters in PBPK Model.** Due to the lack of available data, we
83 apply some parameters of rainbow trout (arterial blood flow to tissues, effective respiratory
84 volume and cardiac output) to the PBPK model of Chinese sturgeon, and therefore sensitivity
85 analysis was performed for the parameters in PBPK models. The sensitivity coefficient (s)
86 was calculated by Equation (1):

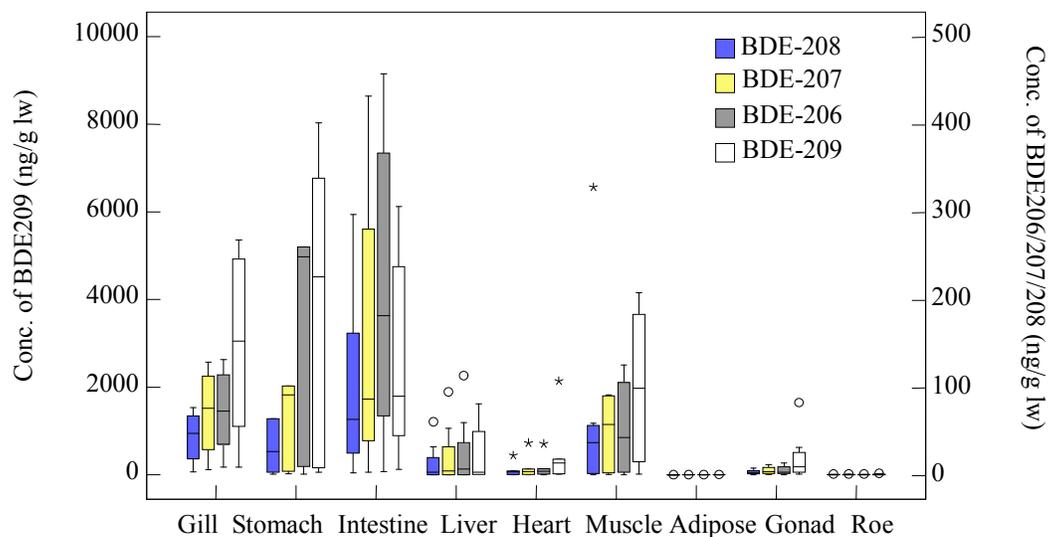
$$87 \quad s = \frac{(f(x + \Delta \times x, \Phi) - f(x, \Phi)) / f(x, \Phi)}{\Delta} \quad (1)$$

88 where x is the objective parameters; Δ is the changing scale of the objective parameter and
89 was set as 1%; Φ is the model default parameter family that excluded the objective
90 parameter; f is the PBPK model for estimating the concentration in tissues. In this study, the

91 parameters of PBPK model were performed for sensitivity analysis in turn. The parameter
92 with a sensitivity coefficient over 0.1 is usually considered as a sensitive parameter, which
93 means varying the sensitivity parameter value by 1% has a 0.1% impact on the response.¹ The
94 results of sensitivity analysis were showed in Table S2. It was found that the average
95 sensitivity coefficients of blood flow to liver (Q_{li}), adipose (Q_{ad}), richly perfused tissue (Q_r),
96 stomach/intestine (Q_{st}), poorly perfused tissue (Q_s), effective respiratory volume (Q_{cc}) and
97 cardiac output (Q_{ww}) were 0.00008, -0.0001, -0.0003, 0.0002, 0.01, 0.002 and -0.009,
98 respectively. This indicated that the referenced parameters had slight effects on the predicted
99 concentrations of BDE-209 in various tissues by PBPK model.

100 The influence of reproduction time on the predicted AE and P_f in the PBPK models was
101 also assessed. The estimated AE of BDE-209 in Chinese sturgeon with two times of migration
102 in their life was 0.006 ± 0.002 (0.004-0.012). And the partition coefficients of
103 stomach/intestine: blood, poorly perfused tissue: blood, richly perfused tissue: blood,
104 adipose: blood and liver: blood in Chinese sturgeon with two times of migration were
105 40.4 ± 12.5 (18.7-68.1), 23.1 ± 4.7 (11.7-29.6), 9.0 ± 2.6 (4.2-14.5), 4.5 ± 1.0 (2.4-5.9) and
106 14.3 ± 4.2 (6.7-23.6), respectively. These values are very similar to those evaluated in sturgeon
107 with the case assuming that the Chinese sturgeon investigated had only one time of
108 reproduction or migration (Table 2), suggesting the reproduction times had slight effects on
109 the estimated assimilation efficiency and partition coefficients.

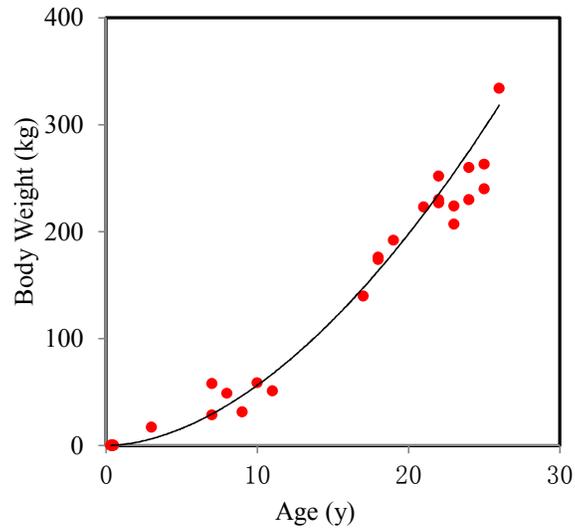
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FIGURE S1. Concentration levels of BDE-206/207/208/209 in various tissues of Chinese sturgeon. Kidney, spleen and gallbladder are not included in the graph due to the limited number of samples. The horizontal line represents the median concentration. The 25th and 75th centiles define the boxes and the whiskers represent the 10th and 90th centiles.

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FIGURE S2. Relationship of body weight and age of Chinese sturgeon based on the data from ref²⁻⁴. $BW = 0.87 \times \text{age}^{1.82}$, $r^2=0.9909$.

134 **TABLE S1** Physiological and Anatomical Parameters used in a PBPK Model for BDE-209 in
 135 Chinese Sturgeon.

Parameters	Term		Reference
Body weight (kg)	BW	$= 0.87 \times \text{age}^{1.82}$	2-4 ^a
Tissue volume (% , fraction of body weight)			
Stomach and intestine	f_{st}	0.025	5
Slowly perfused tissue	f_s	0.9	5
Richly perfused tissue	f_r	0.014	5
Adipose	f_{ad}	0.04	5
Liver	f_{li}	0.021	5
Arterial blood flow to tissue (% , fraction)			
Stomach and intestine	Q_{st}	0.174	6 ^b
Slowly perfused tissue	Q_s	0.652	6 ^b
Richly perfused tissue	Q_r	0.111	6 ^b
Adipose	Q_{ad}	0.034	6 ^b
Liver	Q_{li}	0.029	6 ^b
Effective respiratory volume (L/h/kg)	Q_{ww}	7.4 ^c	6 ^b
Cardiac output (L/h/kg)	Q_{cc}	2.4 ^c	6 ^b

136 ^a The relationship between body weight and age was obtained based on the data reported previously for
 137 Chinese sturgeon (Figure S2).

138 ^b The physiological parameters of rainbow trout was applied to sturgeon due to the lack of the data.

139 ^c The Q_c and Q_w of rainbow trout was applied to sturgeon: $Q_c = Q_{cc} \times BW^{0.75}$; $Q_w = Q_{ww} \times BW^{0.75}$.

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144 **TABLE S2.** Sensitivity Analysis of PBPK Parameters Referenced from Rainbow Trout⁶.

Parameters	sensitivity coefficient (s^*)				
	C_{st}	C_s	C_r	C_{ad}	C_{li}
Q_{lif}	-0.0001	0.00002	-0.0001	-0.0001	0.0007
Q_{ff}	-0.00003	0.00003	-0.00004	-0.0004	-0.0003
Q_{rf}	-0.0001	0.0001	-0.0002	-0.0001	-0.001
Q_{stf}	-0.0015	-0.0006	-0.001	-0.001	0.005
Q_{sf}	0.016	0.013	0.015	0.016	0.002
Q_{cc}	0.0008	-0.0009	0.001	0.0008	0.008
Q_{ww}	-0.009	-0.009	-0.009	-0.009	-0.009

145 *s: sensitivity coefficient for C_{st} , C_s , C_r , C_{ad} and C_{li} which were the concentrations of
 146 BDE-209 in stomach and intestine, slowly perfused tissue, richly perfused tissue, adipose and
 147 liver predicted by PBPK model.

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