SUPPORTING INFORMATION

For:

Tissue concentrations of Polybrominated compounds in Chinese Sturgeon (*Acipenser sinensis*): Origin, Hepatic Sequestration, and Maternal Transfer

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This supporting information provides detailed descriptions of chemicals and standards used in the analysis, extraction and cleanup of tissue samples and instrument condition. Figures, and tables addressing: details of Chinese sturgeon samples (Table S1); Mean concentrations and ranges of OH-PBDEs and MeO-PBDEs in tissues of Chinese sturgeon (Table S2); Mean concentrations and ranges of PBDEs in tissues of Chinese sturgeon (Table S3); Percentages of brominated compounds relative to the dosing concentration after metabolism with Chinese sturgeon microsomes exposed to PBDEs and 6-MeO-BDE47 (Table S4); Association of concentrations of PBDE congeners with age of Chinese sturgeon (Table S5); Tissue distribution of MeO-PBDEs and $\sum$PBDEs in Chinese sturgeon after lipid normalization (Figure S1); Ratios of concentrations of BDE99 to BDE47 (BDE99/47) and BDE183 to BDE154 (BDE183/154) in Chinese sturgeon tissues (Figure S2); Relationships between the concentrations of 6-OH-BDE47 and the concentration of 6-MeO-BDE47 (Figure S3); Lipid normalized liver to adipose concentration ratios in Chinese sturgeon plotted versus Ah

receptor binding affinities (Figure S4).

Chemicals and Standards. Eleven PBDEs (BDE28, BDE47, BDE66, BDE77, BDE100, BDE99, BDE85, BDE154, BDE153, BDE138 and BDE183), twelve MeO-PBDEs (6-MeO-BDE17, 4-MeO-BDE17, 2'-MeO-BDE68, 6-MeO-BDE47, 5-MeO-BDE47, 4'-MeO-BDE49, 5'-MeO-BDE100, 4'-MeO-BDE103, 4'-MeO-BDE99, 4'-MeO-BDE101, 6-MeO-BDE90, and 6-MeO-BDE85), and nine OH-PBDEs (2'-OH-6'-Cl-BDE7, 6-OH-BDE47, 3-OH-BDE47, 5-OH-BDE47, 2'-OH-BDE68, 4'-OH-BDE49, 2'-OH-6'-Cl-BDE68, 6-OH-BDE90 and 2-OH-BDE123) were selected as target compounds. PBDEs, ^{13}C-PBDEs, and eight MeO-PBDEs standards were obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada). 3-OH-BDE47, 5-OH-BDE47 and 2'-OH-BDE68 were obtained from AccuStandard (New Haven, Connecticut, USA). 6-MeO-BDE17, 4-MeO-BDE17, 6-MeO-BDE90, 6-MeO-BDE85, 2'-OH-6'-Cl-BDE7, 6-OH-BDE47, 4'-OH-BDE49, 2'-OH-6'-Cl-BDE68, 6-OH-BDE90 and 2-OH-BDE123 were synthesized in the Department of Biology and Chemistry, City University of Hong Kong, and purities of all metabolites were >98%. Dichloromethane (DCM), n-hexane, methyl *tert*-butyl ether (MTBE), acetonitrile and methanol were pesticide residue grade obtained from OmniSolv (EM Science, Lawrence, KS, USA). Sodium sulfate, silica gel (60-100 mesh size), aluminum oxide (neutral, 150 mesh size), pyridine (anhydrous, 99.8%), methyl chloroformate (MCF), potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For biochemical analyses, the fluorescence kit was obtained from (Genmed Scientific Inc, USA), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4) and potassium phosphate monobasic (KH_2PO_4), resorufin, ethylenediaminetetraacetic acid (EDTA), and dithiothreitol (DTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other biochemical reagents, including NADPH, were obtained from Sigma-Aldrich and were reagent grade or better unless stated otherwise.

Extraction and Cleanup of Tissue Samples Details of our methods for identifying and quantifying PBDE, OH-PBDEs and MeO-PBDEs have been described previously (*26*). Tissues were freeze-dried, then approximately 1-3 g dry weight (dw) subsamples were spiked with a mixture of ^{13}C-labeled PBDE (^{13}C-BDE28, ^{13}C-BDE47, ^{13}C-BDE99, ^{13}C-BDE100, ^{13}C-BDE153, ^{13}C-BDE154 and ^{13}C-BDE183) and 6-OH-BDE17 surrogates, and extracted by accelerated solvent extraction (Dionex ASE-200, Sunnyvale, CA). The extraction employed two 10 min cycles, the first cycle was performed with n-hexane/dichloromethane (DCM) (1:1) at 100 °C and 1500 psi, followed by a second cycle with n-hexane/methyl tert-butyl ether (MTBE) (1:1) at of 60°C and pressure of 1000 psi. The two extraction fractions were combined and rotary evaporated to near dryness. The extract was then transferred to 15 ml glass tubes by 8 mL hexane, and 4 mL 0.5 M KOH in 50% ethanol was added. The aqueous layer (KOH) was extracted with 8 mL of n-hexane three times (neutral fraction). After extraction, 1.5 mL of 2 M HCl was added to 15 ml tubes and the phenolic compounds were extracted with n-hexane/MTBE (9:1; v/v) three times (phenolic fraction).

The neutral fraction was concentrated to approximately 2 mL and loaded onto a column of 1 g Na_2SO_4 and 8 g acidified silica (48% H_2SO_4) and eluted with 15 mL of n-hexane and 10 mL of DCM. The eluate was further purified on a neutral alumina column (4 g of sodium sulfate, 4 g of neutral alumina, 4 g of sodium sulfate). The first fraction eluted from the alumina column with 20 mL of hexane was discarded. The second fraction, which contained PBDEs and MeO-PBDEs, was obtained by elution with 25 mL of 60% DCM in n-hexane. The eluate was evaporated to dryness under a gentle stream of nitrogen, then 30 µl nonane and 10 µl internal standards (^{13}C-BDE138) were added for analysis of PBDEs and MeO-PBDEs.

After dried by a gentle stream of nitrogen, the phenolic fraction was re-dissolved in 480 µL of derivatization solvent (acetonitrile/methanol/water/pyridine (5:2:2:1; v/v/v/v)) and then

40 μL of methyl chloroformate (MCF) was added. The reaction mixture was shaken on a vortex at room temperature for 1 h and then diluted with 1.2 mL of pure water. The aqueous solution was extracted three times with 6 mL volumes of n-hexane. Extracts were concentrated and subjected to acidified silica gel chromatography as described above, eluted with 30 mL of n-hexane and 30 mL of DCM. The eluate was concentrated to 40 μL for OH-PBDE analysis.

Instrumental Conditions. Identification and quantification of PBDEs congeners were performed using a Hewlett-Packard 5890 series II high-resolution gas chromatograph interfaced to a Micromass® Autospec® high-resolution mass spectrometer (HRGC-HRMS) (Micromass®, Beverly, MD). Chromatographic separation was achieved on a DB-5MS capillary column (30 m length, 0.25 mm ID, 0.1 μm film thickness, Agilent, Carlsbad, CA). A splitless injector was used and the injector was held at 285°C. The interface temperature was 320°C, and ion temperature was 285°C. The carrier gas was helium. The electron ionization energy was 37 eV and the ion current was 750 μA. Data acquisition was conducted in selected ion monitoring mode. For PBDEs, the temperature program was from 110°C (10min) to 250°C at the rate of 25°C/min, then increased to 260°C at the rate of 1.5°C/min, and then to 323°C (15 min) at a rate of 25°C/min. For OH-PBDEs, the temperature program was from 150°C (2min) to 320°C (2min) at the rate of 10°C/min. For MeO-PBDEs, the temperature program was from 150°C (2 min) to 245°C (2 min) at 2°C/min, and then increased to 320°C (2 min) at 30°C/min.

106 **SUPPORTING INFORMATION TABLE S1.** Details of Chinese Sturgeon Samples.

Sample Code	date of collection	age (year)	wt (kg)	body length (cm)	Tissue collected[a]
A0403	2005	24	260	280	E
A0406	2004	18	174	245	E, L, M, H, Go, St, P
A0408	2004	22	230	258	E
A0410	2004	17	140	246	E, L, M, H, Go, St, I, A, Gb
A0412	2004	24	230	287	E, L, M, H, Go, St, I, Gi, P
A0414	2004	25	263	285	E, L, M, I, A, Gi
A0438	2006	26	334	290	E
A0439	2006	21	223	262	E, L, M, H, Go, St, I, A, Gi, S
A0440	2006	18	176	250	E
A0441	2006	25	240	300	E
A0444	2005	23	224	270	E
A0445	2005	18	187	237	L, M, H, Go, I, Gi
A0447	2005	19	192	247	E, L, M, H, Go, I, A, Gi
A0449	2005	22	252	275	E
A0452	2005	23	207	282	E
A0500	2005	22	227	261	E
A0466	2003	24	254	285	L, M, Go, St, I, A, Gi, K

107 a) E: egg; L: liver; M: muscle; H: heart; Go: gonad; St: stomach; I: intestine; A: adipose; Gi:
108 gill; K: kidney; Gb: gallbladder; P: pancreas; S: spleen;

SUPPORTING INFORMATION TABLE S2. Mean Concentrations and Ranges of OH-PBDEs and MeO-PBDEs (pg/g ww) in Tissues of Chinese Sturgeon.

Chemicals	Eggs n=15	Liver n=8	Gonad n=7	Adipose n=5	Heart n=6	muscle n=8	Intestine n=7	Stomach n=5	Gill n=6	Pancreas n=2	Gall bladder n=1	Spleen n=1	Kidney n=1
lipid content (%)	35 (24-50)	13 (7.1-29)	3.5 (1.2-6.3)	66 (46-89)	3.8 (2.3-5.9)	1.9 (0.5-3.6)	2.6 (1.0-5.4)	1.2 (0.8-1.7)	2.2 (1.4-3.4)	6.8 (5.1-8.6)	23	3.3	32
6-OH-BDE47	180 (17-1200)	170 (35-580)	35 (18-54)	17 (N.D.-38)	38 (9.3-81)	5.2 (N.D.-18)	15 (N.D.-41)	7.7 (N.D.-20)	8.5 (N.D.-26)	7.1 (5.4-8.8)	58	64	19
2-OH-BDE68	N.D.	2.9 (N.D.-7.7)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5-OH-BDE47	3.2 (N.D.-17)	16 (N.D.-39)	N.D.	14 (N.D.-62)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	N.D.	N.D.
4-OH-BDE49	8.8 (N.D.-75)	15 (N.D.-59)	4.2 (N.D.-17)	N.D.	4.4 (N.D.-16)	N.D.	N.D.	N.D.	N.D.	N.D.	56	N.D.	N.D.
4-MeO-BDE17	0.6 (N.D.-3.2)	1.2 (N.D.-4.1)	0.3 (N.D.-0.7)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2'-MeO-BDE68	14 (1.5-45)	4.5 (N.D.-12)	0.8 (N.D.-2.4)	16 (7.1-29)	0.6 (N.D.-2.4)	0.4 (N.D.-0.9)	0.4 (N.D.-1.4)	N.D.	N.D.	N.D.	N.D.	N.D.	1.9
6-MeO-BDE47	110 (15-370)	25 (N.D.-69)	6.3 (1.3-24)	120 (36-210)	3.3 (N.D.-9.5)	1.3 (N.D.-3.7)	1.5 (N.D.-6.9)	N.D.	2.2 (N.D.-6.7)	N.D.	12	N.D.	12
5-MeO-BDE47	0.5 (N.D.-4.9)	0.7 (N.D.-2.0)	N.D.	1.1 (N.D.-4.8)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5'-MeO-BDE100	1.2 (N.D.-3.6)	1.4 (N.D.-3.1)	N.D.	1.8 (N.D.-4.8)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a]ND, not detected.

SUPPORTING INFORMATION TABLE S3. Mean Concentrations and Ranges of PBDEs (ng/g ww) in tissues of Chinese Sturgeon.

Chemicals	Eggs	Liver	Gonad	Adipose	Heart	Muscle	Intestine	Stomach	Gill	Pancreas	Gall bladder	Spleen	Kidney
	n=15	n=8	n=7	n=5	n=6	n=8	n=7	n=5	n=6	n=2	n=1	n=1	n=1
BDE28	1.6	1.2	0.2	2.4	0.1	0.05	0.03	0.01	0.05	0.04	1.2	0.02	0.1
	(0.2-4.0)	(0.1-3.2)	(0.05-0.5)	(0.8-4.8)	(0.05-0.3)	(0.02-0.1)	(0.01-0.1)	(N.D.-0.01)	(0.01-0.1)	(0.03-0.1)			
BDE47	14	15	1.7	29	1.7	0.5	0.3	0.1	0.4	0.5	20	0.2	0.7
	(1.6-48)	(0.3-49)	(0.5-4.2)	(4.1-85)	(0.5-4.1)	(0.2-1.3)	(0.03-0.8)	(N.D.-0.1)	(0.04-0.8)	(0.4-0.7)			
BDE66	0.3	0.2	0.04	0.4	0.03	0.01	0.01	0.001	0.01	0.01	0.1	0.01	0.03
	(0.04-1.0)	(0.01-0.8)	(0.0-0.1)	(0.1-0.9)	(0.01-0.1)	(N.D.-0.03)	(N.D.-0.02)	(N.D.-0.004)	(N.D.-0.03)	(N.D.-0.02)			
BDE77	0.005	0.01	0.002	0.009	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.002
	(N.D.-0.1)	(N.D.-0.03)	(N.D.-0.005)	(N.D.-0.03)									
BDE100	2.5	3.8	0.3	5.7	0.4	0.1	0.1	0.02	0.1	0.1	4.4	0.1	0.2
	(0.3-10)	(0.1-13)	(0.1-0.8)	(1.0-16)	(0.1-0.8)	(0.1-0.3)	(0.01-0.2)	(N.D.-0.03)	(0.01-0.2)	(0.1-0.2)			
BDE99	0.3	0.2	0.03	0.5	0.03	0.03	0.02	0.02	0.02	0.02	0.2	0.02	0.02
	(0.02-0.7)	(0.03-0.7)	(0.01-0.1)	(0.2-1.0)	(0.02-0.1)	(0.01-0.1)	(0.01-0.04)	(N.D.-0.04)	(0.01-0.03)	(0.01-0.02)			
BDE85	0.2	N.D.	N.D.	N.D.	N.D.	0.05	N.D.	0.1	N.D.	N.D.	N.D.	N.D.	N.D.
	(N.D.-0.7)					(N.D.-0.2)		(N.D.-0.2)					
BDE154	2.2	3.6	0.4	3.5	0.4	0.1	0.1	0.1	0.1	0.2	1.7	0.04	0.2
	(0.3-5.1)	(0.1-11)	(0.1-1.0)	(1.7-5.6)	(0.1-0.8)	(0.1-0.2)	(0.02-0.2)	(0.02-0.2)	(0.02-0.3)	(0.1-0.4)			
BDE153	0.5	1.0	0.1	1.1	0.1	0.04	0.03	0.1	0.03	0.1	1.0	0.02	0.04
	(0.1-1.3)	(0.02-2.6)	(0.03-0.2)	(0.2-2.4)	(0.04-0.2)	(0.01-0.1)	(N.D.-0.1)	(N.D.-0.1)	(N.D.-0.1)	(0.03-0.1)			
BDE183	N.D.	0.03	N.D.	N.D.	N.D.	0.01	0.05	1.3	0.01	0.04	N.D.	N.D.	N.D.
		(N.D.-0.2)				(N.D.-0.1)	(N.D.-0.2)	(N.D.-6.1)	(N.D.-0.02)	(N.D.-0.1)			

[a]ND, not detected.

SUPPORTING INFORMATION TABLE S4. Percentages of brominated compounds relative to the dosing concentration after metabolism with Chinese sturgeon microsomes exposed to PBDEs and 6-MeO-BDE47 (%)

	Exposed group (chemicals)				
	BDE47[a]	BDE99 [a]	BDE154 [a]	BDE183 [a]	6- MeO-BDE47 [a]
6-OH-BDE47	N.D.[b]	N.D.	N.D.	N.D.	N.D.
6-MeO-BDE47	N.D.	N.D.	N.D.	N.D.	106 ± 7
5-MeO-BDE47	N.D.	N.D.	N.D.	N.D.	N.D.
4′-MeO-BDE49	N.D.	N.D.	N.D.	N.D.	N.D.
5′-MeO-BDE100	N.D.	N.D.	N.D.	N.D.	N.D.
4′-MeO-BDE103	N.D.	N.D.	N.D.	N.D.	N.D.
4′-MeO-BDE99	N.D.	N.D.	N.D.	N.D.	N.D.
4′-MeO-BDE101	N.D.	N.D.	N.D.	N.D.	N.D.
BDE28	N.D.	N.D.	N.D.	N.D.	N.D.
BDE47	99 ± 5	52 ± 2	N.D.	N.D.	N.D.
BDE66	N.D.	N.D.	N.D.	N.D.	N.D.
BDE100	N.D.	N.D.	N.D.	N.D.	N.D.
BDE119	N.D.	N.D.	N.D.	N.D.	N.D.
BDE99	N.D.	28 ± 1	N.D.	N.D.	N.D.
BDE85	N.D.	N.D.	N.D.	N.D.	N.D.
BDE154	N.D.	N.D.	109 ± 6	73 ± 5	N.D.
BDE153	N.D.	N.D.	N.D.	N.D.	N.D.
BDE183	N.D.	N.D.	N.D.	6 ± 1	N.D.

[a] Data represent mean±standard deviation of technical triplicates. N.D.: concentrations less than the detection limit; dosing concentration was 150 ng/mL for BDE47, BDE99, BDE154, BDE183 and 6-MeO-BDE47 as described in materials and methods..

SUPPORTING INFORMATION TABLE S5. Association of concentrations of PBDE Congeners (pg/g ww) with age of Chinese sturgeon: Ln [PBDE concentration] = a× age+ b (R^2, *p* value).

	a	b	R^2	*p*
BDE28	-0.115	9.49	0.51	0.03
BDE47	-0.163	12.7	0.66	0.01
BDE100	-0.161	10.9	0.69	0.01
BDE99	-0.175	8.86	0.45	0.05
BDE154	-0.098	9.51	0.37	0.08
BDE153	-0.164	9.10	0.56	0.02
∑PBDEs	-0.158	13.0	0.65	0.01

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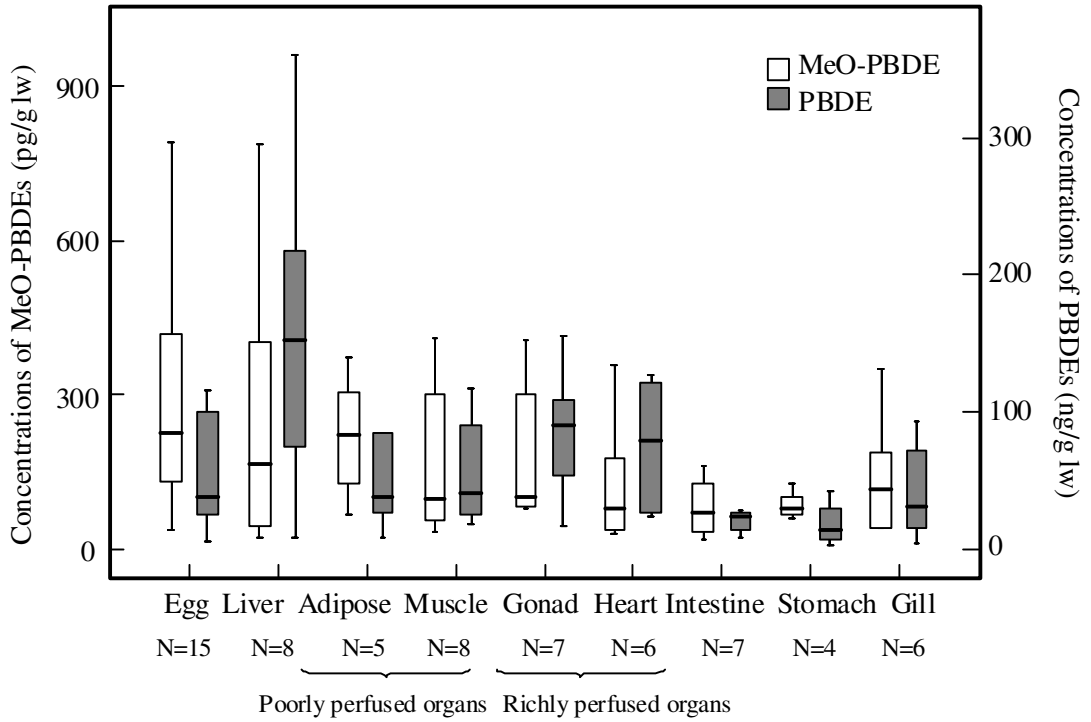

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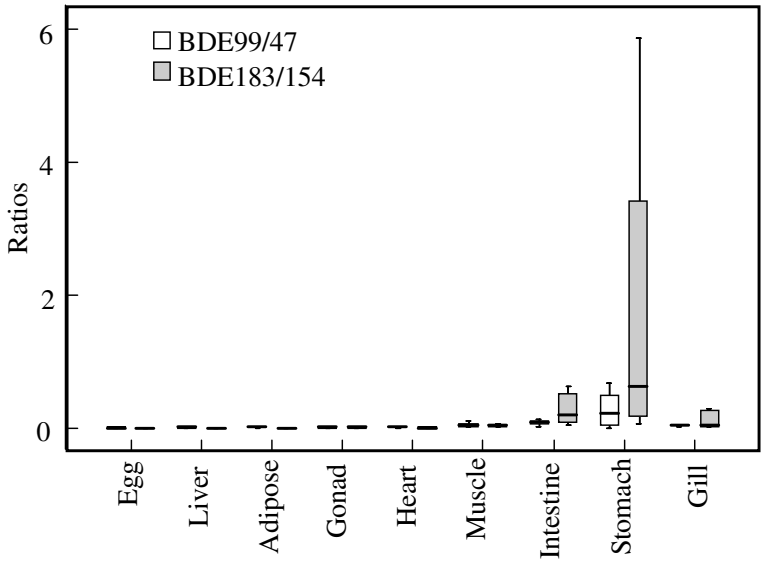
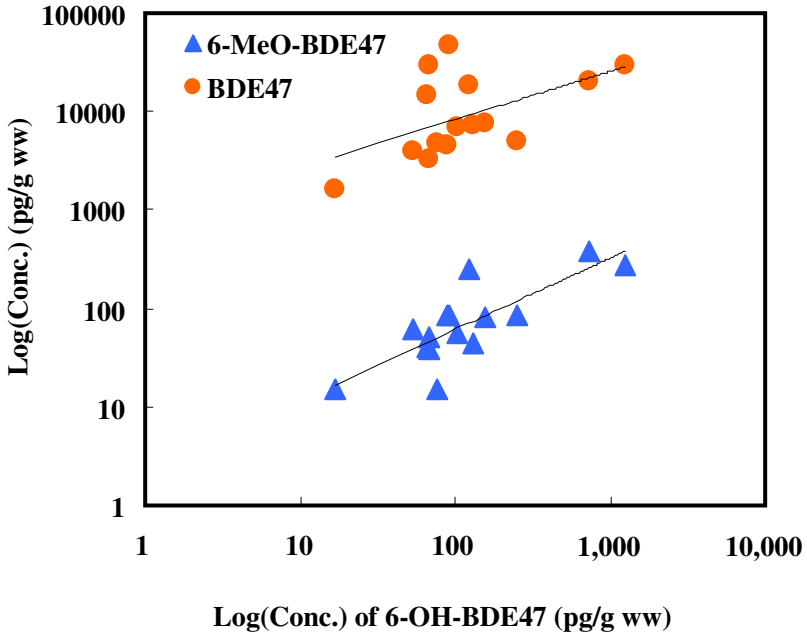
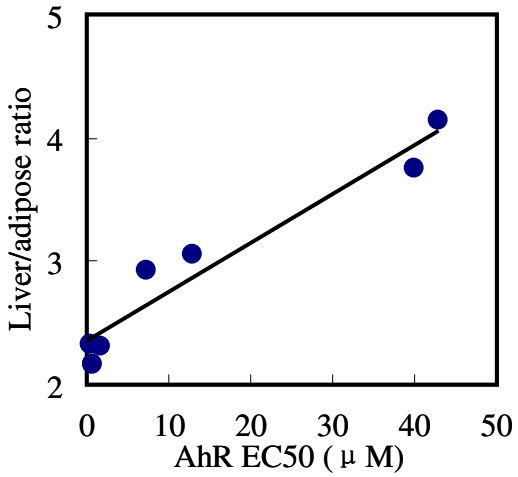
129 **SUPPORTING INFORMATION FIGURE S1**. Levels of MeO-PBDEs and ∑PBDEs in
130 various tissues in Chinese sturgeon after lipid normalization. The horizontal line represents
131 the median concentration. The 25th and 75th centiles define the boxes and the whiskers
132 represent the 10th and 90th centiles.



SUPPORTING INFORMATION FIGURE S2. Ratios of concentrations of BDE99 to BDE47 (BDE99/47) and BDE183 to BDE154 (BDE183/154) in Chinese sturgeon tissues.



SUPPORTING INFORMATION FIGURE S3. Relationships between the concentrations of 6-OH-BDE47 and the concentration of 6-MeO-BDE47 (R^2=0.534, p=0.003) and BDE47 (R^2=0.25, p=0.069).



SUPPORTING INFORMATION FIGURE S4. Lipid normalized liver to adipose concentration ratios in Chinese sturgeon plotted versus Ah receptor binding affinities.