Dioxin-like Potency of OH- and MeO- Analogues of PBDEs' the Potential Risk through Consumption of Fish from Eastern China

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1 Chemical Analysis Procedures

1.1 Identification and Quantification of PBDEs and their analogues

Concentrations of individual polybrominated diphenyl ethers (PBDE), and hydroxylated brominated diphenyl ethers (OH-BDE) were determined by application of an adaptation of the methods[1]. After measuring the length and weight of individual fish, the edible fillet was removed, lyophilized and homogenized. Approximately 5.0 g of dry sample, to which surrogate standard - ^{13}C-BDE-139 and C^{13}-2-HO-BDE-99 was added, was extracted by accelerated solvent extraction (ASE, Dionex ASE-350, Sunnyvale, CA, USA). Extraction was conducted with n-hexane / dichloromethane (DCM) (1:1) as the first extraction solvent at a temperature of 100 ℃ and pressure of 1500 psi, and then the samples were extracted with n-hexane/methyl tert-butyl ether (MTBE) as the second extraction solvent at a temperature of 60 ℃ and pressure of 1500 psi. Two cycles were performed for each solvent and duration of each cycle was 10 min. The extract was concentrated by rotary evaporation to 10 mL, and 2 ml of extract was taken out for gravimetrically lipid content determination. An aliquant of 4 mL of 0.5 M potassium hydroxide (KOH) in 50% ethanol was added to the concentrated extract. Phenolic compounds were separated from the neutrals into an aqueous layer of KOH. The aqueous phase was extracted with 8mL of n-hexane three times (neutral fraction), followed by acidification with 1.5 mL of 2 M hydrochloric acid. Then phenolic compounds were extracted three times with n-hexane/MTBE (9:1; v/v).

For neutral chemicals, the extract was concentrated to near dryness and dissolved in 10 ml of dichloromethane and hexane (V:V=1:1) and acidified with 10 ml of H_2SO_4 to remove the fat. PBDEs and MeO-PBDEs were back extracted with a total of 30 mL dichloromethane and hexane (V:V=1:1) in 3 separate 10 mL extractions. The organic solvent containing PBDEs and MeO-PBDEs was concentrated and passed through a silica gel column for further clean up. The silica gel column was packed with glass-wool, activated silica gel (0.25 g), 44% (w/w) acid silica gel (1.0 g), silica gel (0.25 g), and anhydrous sodium sulfate (0.30 g) from bottom to top in a disposable

Pasteur pipette [2]. The fraction containing PBDEs and MeO-PBDEs was eluted with 15 mL hexane followed by 15 mL n-hexane/dichloromethane(1:1). The elution was concentrated by rotary evaporation and further concentrated to near dryness under a gentle nitrogen flow. Then, 9.6 ng of ^{13}C-PCB-178 was added as the internal injection standard and made up to 100 μL with hexane prior to GC/MS analysis.

For the extract containing the phenolic compounds, the extract was concentrated to near dryness by rotary evaporation and transfer into a 15 ml blown glass vials with 3 ml of n-hexane. The organic solvent containing HO-PBDEs were dried under a gentle nitrogen flow. And then the derivatization process was conducted according to previously published methods [1]. The aqueous solution was extracted with 6 mL of n-hexane three times, and the extracts were subjected to the silica gel chromatography as described above. The column was eluted with 30 mL n-hexane/DCM (1:1), and the elution was concentrated by rotary evaporation and further concentrated to near dryness under a gentle nitrogen flow. Then, 9.6 ng of ^{13}C-PCB-178 was added as the internal injection standard and made up to 100 μL with hexane prior to identification and quantification by use of GC/MS.

1.2 Instrument Conditions

Concentrations of 13 PBDEs and 34 PBDEs analogs were determinzed by use of a Thermo Scientific TSQ Quantum GC (USA), coupled with an Agilent DB-XLB column (15 m × 0.25 mm × 0.25 μm, USA). The mass spectrometer detector was operated in electron impact ionization (EI) mode. Samples and standards were analyzed in selected reaction mode (SRM) mode. Quantification and qualification were processed by SRM modes. The precursor ion and product ions selected in SRM mode for each chemical were based on the mass spectrum of the standard solution. Detailed information about precursor ion, product ions, ions ratio and collision energy are given in Supporting Information (Table S2).

1.3 Quality Assurance/Quality Control

QA/QC was conducted by performing laboratory blanks, GC/MS detection limit

(based on 3S/N) and standard spiked recoveries. Concentrations of target analytes in laboratory blanks were less than 5% of the sample minimum concentration, which demonstrated that samples were free from contamination. The limit of detection (LOD) was defined as the concentration that would result in a signal-to-noise ratio of 3. LOD based on 2.0 g of dry sample and instrument sensitivity, varied from congener to congener, from 30.3 to 123.4 pg/g dry wt. Concentrations less than the LOD were assumed to be not detected in calculating summary statistics. For samples where concentrations of a congener were less than the LOQ, they were reported as not detected. Before sample analysis, matrix spike (n=4) for each target compound had been evaluated. And recoveries ranged from 74.3 to 125.2% for PBDEs and their analogs, respectively. To ensure accuracyof analytical procedures, ^{13}C-labeled BDE-139 and ^{13}C-labeled 2-HO-BDE-99 was used as the internal standard for neutral (PBDEs and MeO-PBDEs) and phenolic compounds (HO-PBDEs), respectively. Recoveries of the ^{13}C-labeled BDE-139 internal standard were between 85.1 and 111.2%.

2 TEQ_{BIO} testing for each biological sample

The procedures of biological samples for H4IIE-*luc* testing were similar to those described by with some modifications[3]. Approximately 10.0 g of dry sample was extracted by accelerated solvent extraction (ASE, Dionex ASE-350, Sunnyvale, CA, USA). Extraction was conducted with dichloromethane (DCM) [4] as the extraction solvent at a temperature of 100 ℃ and pressure of 1500 psi. Two cycles were performed for each sample and duration of each cycle was 10 min. The extract was then concentrated to approximately 5 ml using a rotary evaporator under reduced pressure. To avoid the fat's toxicity to cells, the 5 ml extract was acidified with 5 mL concentrated H_2SO_4 to remove the fat[5]. And the target compounds were back extracted with a total of 30 mL dichloromethane in 3 separate 10 mL extractions. Finally, the extract were collected and concentrated to 150 μL for AhR activity testing.

Cell culture and bioassay had been described in section "2.2 H4IIE-*luc* Cell Culture and Bioassay" of the manuscript.

Supporting Table 1 Samples Information

Samples	n	Location	Time	Mass (g)	Length (cm)
Sinonovaculaconstrzcta	17	Yellow Sea	2011.02.21	8.3-12.1	5.5-7.5
Drepanepunctata	2	Yellow Sea	2011.02.21	200/189.55	22/20.5
Acanthogobius hasta	14	Yellow Sea	2011.02.21	8.1-15.2	6.5-10.9
Suggrundusmeerdervoortii	1	Yellow Sea	2011.02.21	537.65	43.5
Pseudosciaenapolyactis	2	Yellow Sea	2011.02.21	288.83/295.58	24/24.5
HemiculterLeuciclus	16	Yangtze River	2011.06.16	13.6-43.2	10.0-14.0
Pelteobagrusfulvidraco	32	Yangtze River	2011.06.16	11.7-32.6	10.0-14.0
Carassiusauratus	10	Yangtze River	2011.06.16	39.9-81.5	10.0-14.0
CoiliamacrognathosBleeker	9	Yangtze River	2011.06.16	30.0-65.8	20.0-26.0
Silurusspp	2	Yangtze River	2011.06.16	671.3/554.3	44/41
Cyprinuscarpio	3	Yangtze River	2011.06.16	885.3/426.1/420.7	33/26/26.5

Supporting Table 2 Ion pairs, abundance ratio and collision energy of selected reaction mode.

Chemicals	Ion pairs for Quantification and Qualification			Collision Energy (eV)
	Parent Ion	Product Ion	Abundance Ratio	
BDE-17	245.88	245.88, 138.85	100/30	20
BDE-28	245.88	245.88, 138.86	100/12	20
BDE-71	325.66	216.79, 218.94	92/100	30
BDE-47	325.66	216.79, 218.95	61/100	30
BDE-66	325.66	216.79, 218.96	100/93	30
BDE-100	405.63	296.60, 405.63	100/44	30
BDE-99	405.63	296.60, 405.64	100/40	30
BDE-85	405.63	296.60, 405.65	100/28	30
BDE-154	483.64	483.64, 402.57	42/100	30
BDE-153	483.64	483.64, 402.58	18/100	30
BDE-138	483.64	483.64, 402.59	5/100	30
BDE-183	563.73	563.73, 485.15	5/100	30
BDE-190	563.73	563.73, 485.15	5/100	30
2'-HO-BDE-7	263.85	155.48, 127.37	70/100	15
3'-HO-BDE-7	401.70	198.25, 183.19	100/10	15
6'-Cl-2'-HO-BDE-7	297.96	126.14, 189.17	100/20	30
6'-HO-BDE-17	341.71	126.30, 235.49	100/4	30
5-Cl-6-HO-BDE-47	455.70	456.51, 347.31, 349.44	100/30/40	20
4-HO-BDE-90	578.56	578.93, 443.86, 390.96	100/40/26	15
2'-HO-BDE-66	419.77	420.25, 313.24	40/100	20
2'-HO-BDE-25	341.88	126.33, 235.49	100/4	30
2'-HO-BDE-28	341.90	233.39, 235.29, 342.52	38/100/1	20
2'-HO-BDE-68	419.75	313.33, 311.33	100/50	20
6-HO-BDE-47	419.76	313.41, 420.45	20/100	25
4'-HO-BDE-49	500.64	365.82, 364.24	100/4	25
6'-Cl-2'-HO-BDE-68	455.71	347.22, 456.14	52/100	20
6-HO-BDE-90	499.64	392.58, 390.99	100/54	30
6-HO-BDE-85	499.60	390.99, 340.09	100/40	25
6-HO-BDE-137	513.52	297.88, 470.69	2/100	25
2'-MeO-BDE-28	435.57	342.12, 340.12	100/44	25
2'-MeO-BDE-68	515.45	422.14, 420.06	40/100	30
6-MeO-BDE-47	515.47	422.14, 420.06	10/100	30
4'-MeO-BDE-49	515.47	356.17, 516.26, 501.12	100/4/12	15
6'-Cl-2'-MeO-BDE-68	549.43	456.17, 454.13, 434.33	55/100/1	25
6-MeO-BDE-90	435.57	420.89, 392.91, 339.95	44/100/22	25
6-MeO-BDE-85	593.38	499.68, 433.95	100/4	25
6-MeO-BDE-137	673.31	579.69, 577.59, 513.83	20/10/100	25
6'-MeO-BDE-17	435.56	341.95, 339.94	50/100	25
5-MeO-BDE-47	515.42	356.12, 516.25	100/2	15

5-Cl-6-MeO-BDE-47	549.40	455.92, 453.88, 390.00	100/80/80	20
3-MeO-BDE-100	433.56	418.92, 390.97	100/20	20
4-MeO-BDE-90	593.35	578.78, 433.84	100/48	15
2-MeO-BDE-123	593.35	499.84, 497.81	100/62	30
C^{13}-BDE-139	495.49	335.78, 415.01	100/30	30
C^{13}-2-HO-BDE-99	511.57	351.82, 402.02, 403.91	60/80/100	30
C^{13}-PCB-178	405.62	370.73, 335.86	80/100	20

Supporting Table 3 Responses caused by OH- and MeO-PBDE in the H4IIE-*luc* assay, relative to the maximum response to 2,3,7,8-TCDD (TCDD-max) and their respective 2,3,7,8-TCDD equivalency factors ($ReP_{H4IIE\text{-}luc}$).

Chemicals	Test Concentrations (ng/ml)	TCDD-max	$ReP_{H4IIE\text{-}luc}$
TCDD		100.00%	
DMSO Control	0	0%	
6'-Cl-2'-HO-BDE-7	2500	13.20%	5.40×10^{-05}
2'-HO-BDE-28	2500	12.70%	1.30×10^{-06}
2'-HO-BDE-68	10000	5.00%	1.27×10^{-10}
6-HO-BDE-47	2500	52.70%	7.63×10^{-05}
5-Cl-6-HO-BDE-47	10000	101.80%	4.00×10^{-04}
6-HO-BDE-85	2500	42.20%	2.20×10^{-04}
6-HO-BDE-90	10000	6.80%	7.35×10^{-12}
2-HO-BDE-123	10000	31.30%	3.32×10^{-06}
4-HO-BDE-90	10000	16.40%	7.23×10^{-07}
6-HO-BDE-137	10000	56.20%	1.91×10^{-04}
3-HO-BDE-100	10000	18.10%	8.96×10^{-07}
2'-HO-BDE-66	10000	35.20%	3.92×10^{-06}
2'-HO-BDE-25	10000	9.80%	1.99×10^{-07}
2'-MeO-BDE-28	10000	25.70%	2.18×10^{-06}
6-MeO-BDE-47	10000	14.50%	1.71×10^{-07}
5-Cl-6-MeO-BDE-47	10000	59.40%	6.48×10^{-05}
6-MeO-BDE-85	10000	37.10%	2.56×10^{-05}
2-MeO-BDE-123	10000	9.60%	2.23×10^{-08}
6-MeO-BDE-137	10000	28.00%	2.68×10^{-06}

Supporting Table 4 PBDEs analogs detected in other publications.

Number	Samples	Tissue	Chemicals	Reference
1	Human	Serum	4′-HO-BDE-17, 6-HO-BDE-47, 3-HO-BDE-47, 4′-HO-BDE-49, 4-HO-BDE-42 4-HO-BDE-90	6
2	Human	Breast Milk	2’-MeO-BDE-28, 4’-MeO-BDE-17, 2’-MeO-BDE-75, 6-MeO-BDE-47, 2’-MeO-BDE-74, 6’-MeO-BDE-66, 4’-HO-BDE-17, 2’-HO-BDE-75, 6-HO-BDE-47, 2’-HO-BDE-74, 6’-HO-BDE-66	7
3	Human	Blood	4-HO-BDE-42, 3-HO-BDE-47, 5-HO-BDE-47, 6-HO-BDE-47, 4’-HO-BDE-49, 5’-HO-BDE-99, 6’-HO-BDE-99	8
4	Human	Serum	4’-HO-BDE17, 5-HO-BDE47, 6-HO-BDE47, 4’-HO-BDE49	9
5	Human		6-HO-BDE-47	10
6	Salmo	Blood	5-Cl-6-HO-BDE-47, 5-Cl-6-MeO-BDE-47, 6’-HO-BDE-49, 6’-MeO-BDE-49, 4’-HO-BDE-49, 3’-Cl-6’-HO-BDE-49, 6’-Cl-2’-HO-BDE-68, 6’-Cl-2’-MeO-BDE-68, 6-MeO-BDE-90, 6-HO-BDE-47,	11
7	Fish	Plasma	2’-MeO-BDE-68, 6-MeO-BDE-47, 2’-HO-BDE-68, 6-HO-BDE-99, 2’-HO-BDE-68, 6-HO-BDE-47, 3-HO-BDE-47, 5-HO-BDE-47, 4’-HO-BDE-49, 4-HO-BDE-42, 6-HO-BDE-90, 6-HO-BDE-99, 6-HO-BDE-85, 2-HO-BDE-123	12
8	Glaucous Gulls and Polar Bears	Plasma	2’-MeO-BDE-28, 4-MeO-BDE-42, 6-MeO-BDE-47, 3-MeO-BDE-47, 4’-MeO-BDE-49, 6-MeO-BDE-90, 6-MeO-BDE-99, 4-HO-BDE-42, 6-HO-BDE-47, 3-HO-BDE-47, 4’-HO-BDE-49, 6’-HO-BDE49, 2’-HO-BDE-68	13
9	Bald Eaglet	Plasma	6’-HO-BDE-49, 6-HO-BDE-47, 4’-HO-BDE-49	14
10	Beluga whales	Blood, Milk and Blubber	6′-HO-BDE-49, 2′-HO-BDE-68, 2′-HO-BDE-75, 6-HO-BDE-90, 6-MeO-BDE-17, 2′-MeO-BDE-28, 4-MeO-BDE-42, 5-MeO-BDE-47, 6-MeO-BDE-47, 6′-MeO-BDE-49, 6′-MeO-BDE-66, 2′-MeO-BDE-68, 6-MeO-BDE-90, 6-MeO-BDE-99	15
11	Harbour seals and harbour porpoises	Serum	2’-MeO-BDE-68, 6-MeO-BDE-47	16
12	Bird	Serum	3-HO-BDE-47, 2'-HO-BDE-68, 4'-HO-BDE-17, 6-HO-BDE-47, 4'-HO-BDE-49, 3-MeO-BDE-47, 6-MeO-BDE-47	17
13	Japanese amberjack and scalloped hammerhead shark	Blood	6-HO-BDE-47, 6-MeO-BDE-47	18
14	bottlenose dolphins	Plasma	3’-HO-PBDE-7, 6’-HO-PBDE-17, 2’-HO-PBDE-28, 4’-HO-PBDE-17, 3’-HO-PBDE-28, 6’-HO-PBDE-49,	19

			2’-HO-PBDE-68, 6-HO-PBDE-47, 3-HO-PBDE-47, 5-HO-PBDE-47, 4’-HO-PBDE-49, 4-HO-PBDE-42, 6-HO-PBDE-90, 6-HO-PBDE-99, 4-HO-PBDE-90, 2-HO-PBDE-123, 6-HO-PBDE-85, 6-HO-PBDE-137	
15	ringed seals	Liver and Plasma	2′-HO-BDE-68, 6-HO-BDE-47, 3-HO-BDE-47, 6-HO-BDE-90, 4′-HO-BDE-49	20
16	Water		6′-HO-BDE-49, 2′-HO-BDE-68, 6-HO-BDE-47, 3-HO-BDE-47, 5-HO-BDE-47, 4′-HO-BDE-49, 4-HO-BDE-42, 6-HO-BDE-90, 6-HO-BDE-99, 4-HO-BDE-90, 2-HO-BDE-123, 6-HO-BDE-85, 6-HO-BDE-137	21
17	Sediment		6-HO-BDE-47, 2-HO-BDE-68, 5-HO-BDE-47, 4-HO-BDE-49, 3-HO-BDE-47	22
18	Red Alga and Cyanobacteria		2’-HO-BDE-68, 6-HO-BDE-47, 6-HO-BDE-90, 6-HO-BDE-99, 2-HO-BDE-123, 6-HO-BDE-85, 6-HO-BDE-137, 2’-MeO-BDE-68, 6-MeO-BDE-47, 6-MeO-BDE-85, 6-MeO-BDE-137	23
19	Blood of Japanese Terrestrial Mammals		2'-HO-BDE-28, 2'-HO-BDE-68, 6-HO-BDE-47, 5-HO-BDE-47, 4-HO-BDE-49, 3-HO-BDE-154	24
20	Human Blood		3-OH-BDE-100, 3'-OH-BDE-100, 3-OH-BDE-99, 4'-OH-BDE-101, 3-OH-BDE-154, 3'-OH-BDE-154, 3-OH-BDE-153, 4-OH-BDE-187, 4'-OH-BDE-17, 4-OH-BDE-42, 6-OHBDE-47, 3-OH-BDE-47, 4'-OH-BDE-49, 4-OH-BDE-90	25
21	Marine Sponges and Fish Samples		2'-MeO-BDE68, 6-MeO-BDE47, 2,2'-diMeO-BB80, 2',6-diMeO-BDE68, 2'-OH-BDE68, 6-OH-BDE47, 2,2'-diOH-BB80, 2',6-diOH-BDE68	26

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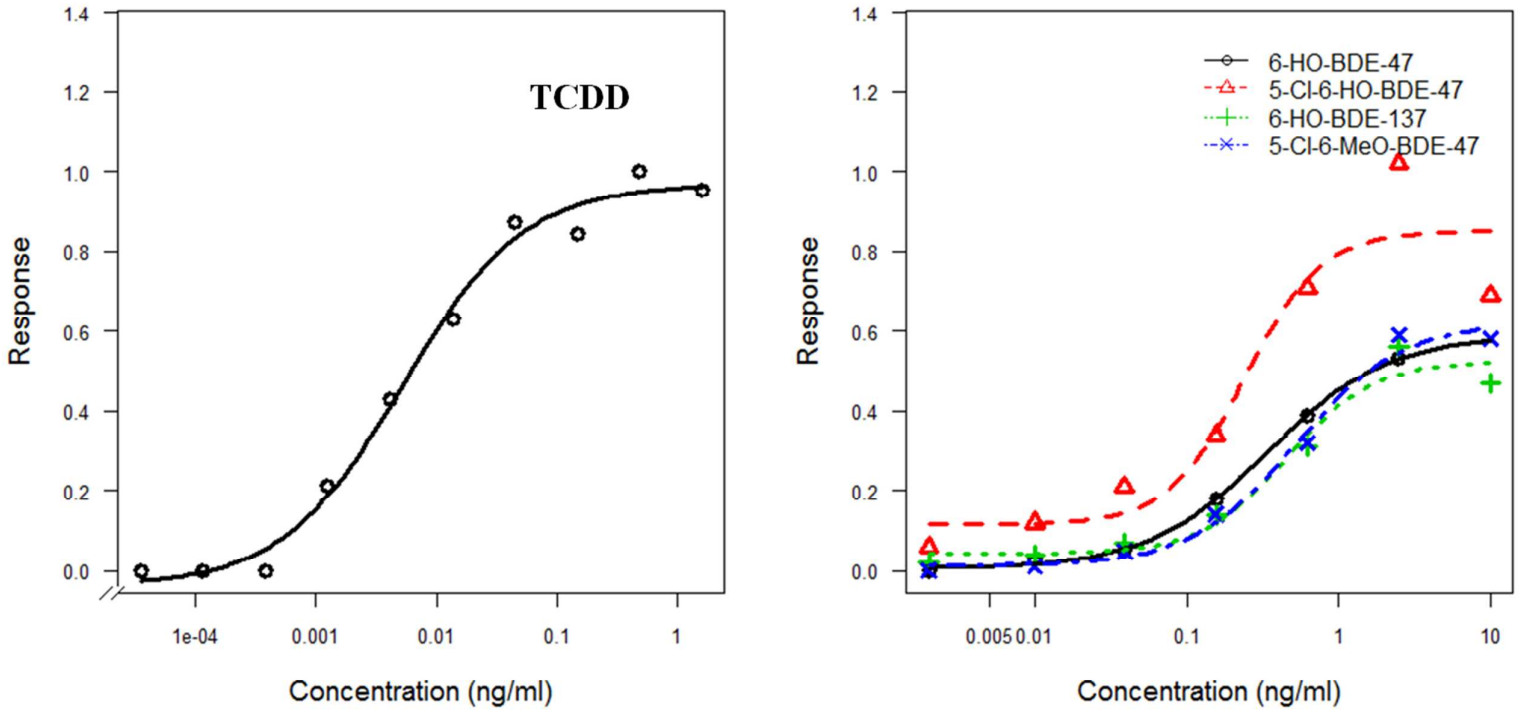
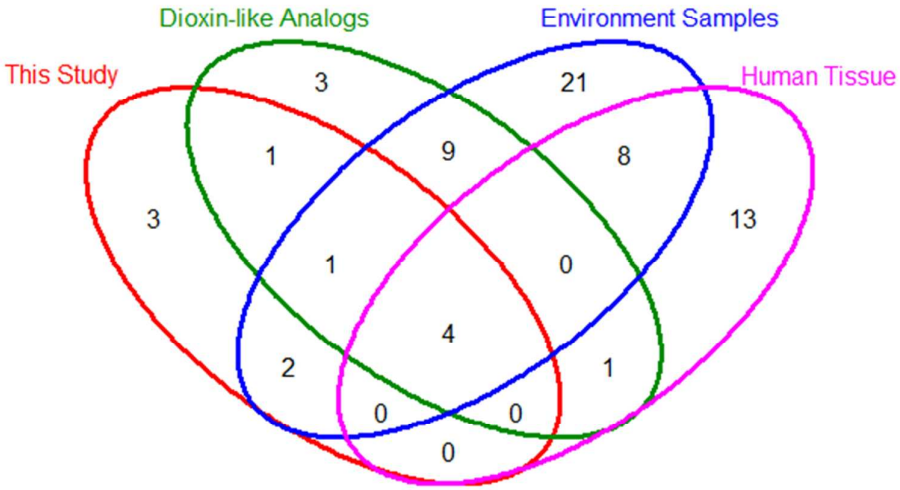
Supporting Figure 1 Responses and a fitted curve for TCDD and 4 analogues of PBDEs that resulted in luciferase expression that exceeded 50 % of TCDD-max. Individual values and mean are plotted along with the fitted curve.



Supporting Figure 2 Number of PBDEs analogues detected in our study (marked with red "This Study"), with a dioxin-like activity (marked with green "Dioxin-like Activity"), detected in the previous publications (marked with blue "Environment Samples"), and detected in human tissues (marked with pink "Human Tissues").



Supporting Figure 3 Profiles of concentrations of PBDEs (A) and HO- and MeO-analogues of PBDEs (B) in fishes from the Yangtze River and marine organisms from the Yellow Sea, china.