

1 **Supporting Information**

2 **Modulation of Benzo[a]pyrene-induced Toxic Effects in Japanese Medaka (*Oryzias***
3 ***latipes*) by 2,2',4,4'-Tetrabromodiphenyl Ether**

4 Yanbin ZHAO, Kai LUO, Zhanlan FAN, Chong HUANG, and Jianying HU*

5 MOE Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences,
6 Peking University, Beijing 100871, China

7
8 **Address for Correspondence**

9 Prof. Dr. Jianying HU

10 College of Urban and Environmental Sciences

11 Peking University, Yi Fu Second Building

12 Beijing 100871 China

13 TEL & FAX: 86-10-62765520

14 Email: hujy@urban.pku.edu.cn

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19 **Chemicals and Reagents for Analysis.** BaP, BDE-47, and their surrogate standards,
20 perylene-*d*₁₄ and PCB153 were obtained from AccuStandard (New haven, Connecticut, USA).
21 BaP-3sulfate were obtained from Midwest Research Institute, NCI Chemical Carcinogen
22 Repository (Kansas City, MO, USA). E2 and E2-*d*₃ were obtained from Wako Pure Chemical
23 Industries, Ltd. (Tokyo, Japan). All solvents (dichloromethane, methanol, acetonitrile and
24 hexane) were HPLC grade purchased from Fisher Scientific (New Jersey, USA). Sodium
25 chloride, sodium sulfate and aluminum oxide were analytical grade and heated at 400°C for 4
26 hours before use.

27 **GC-MS Analysis of BaP and BDE47.** Identification and quantification of BDE47 and
28 BaP were performed using a gas chromatography-electron capture negative ionization mass
29 spectrometry (GC-ENCI-MS) (Shimadzu QP 2010 plus, Japan) and a gas
30 chromatography-electron impact-mass spectrometry (GC-EI-MS) (Alilent 6890N GC; Alilent
31 5975C inert XL MSD), respectively. Chromatographic separation was achieved on a DB-5MS
32 capillary column (30m × 0.25 mm × 0.1 μm film thickness; J&W Scientific, USA). A splitless
33 injector was used and the injector was maintained at 250°C. For BaP analysis, the temperature
34 program was from 110°C (1 min) to 180°C at the rate of 10°C/min, then increased to 220°C
35 (5 min) at the rate of 5°C/min, and then to 310°C (5 min) at a rate of 20°C/min. For BDE47
36 analysis, the temperature program was from 110°C (1 min) to 180°C at the rate of 10°C/min,
37 then increased to 220°C (5 min) at the rate of 5°C/min, and then to 310°C (5 min) at a rate of
38 20°C/min. The interface and ion temperatures were 320°C and 280°C, respectively. The
39 carrier gas was helium at a constant flow rate of 2 ml/min. Data acquisition was conducted in
40 selected ion monitoring mode.

41 **UPLC-MS/MS for BaP-3-Sulfate Analysis.** The concentrations of BaP-3-Sul in each
42 exposure groups were determined every two weeks through the exposure period. Equal
43 volumes (15 ml) of test water samples collected from both chambers of each exposure group
44 were combined, and then extracted through the SPE C18 cartridges (500mg, 6cc, Waters
45 Sep-Pek), which were preconditioned with 6 mL methanol and 6 mL distilled water. The
46 cartridges were dried under nitrogen flow and then the target analytes were subsequently
47 eluted with 6 mL methanol. The eluates were evaporated to dryness under a gentle stream of
48 nitrogen and reconstituted with 0.2 ml of methanol for UPLC-MS/MS analysis. The recovery
49 for spiked samples was $95.85 \pm 0.42\%$, and the MDL was 0.0007 ng/L.

50 The LC apparatus was an Acquity Ultra Performance LC (Waters, USA). Acquity
51 UPLC® BEH C8 column (100×2.1 mm, $1.7 \mu\text{m}$ particle size) (Waters, USA) was used for
52 separation. The column was maintained at 40°C at a flow rate of 0.3 mL/min, and the
53 injection volume was 5 μL . Methanol and ultrapure water containing 0.1% NH_4OH (v/v) were
54 used as mobile phases. Methanol was initially increased linearly from 10% to 50% within 1.0
55 min, and then increased to 100% at 4 min and kept for 1.0 min, followed by a decrease to
56 initial conditions of 10% and held for 2 min to allow for equilibration. Mass spectrometry was
57 performed using a Premier XE tandem quadrupole mass spectrometer (Waters, USA)
58 equipped with a Z-Spray ionization (ESI) source in the negative ion mode. The optimized
59 parameters were as follows: source temperature, 110°C ; desolvation temperature, 350°C ;
60 capillary voltage, 2.50 kV; desolvation gas flow, 800 L/h; cone gas flow, 50 L/h.

61 **UPLC-MS/MS for Estrogen Analysis.** The whole medaka fish (about 0.4 g wet weight)
62 was freeze-dried and spiked with 10 ng E2-d₃ as surrogate standard. The sample was then

63 extracted with 8 mL of methanol/acetonitrile (1:1, v/v) three times by ultrasonication of 10
64 min. The homogenate was centrifuged at 6000 r/s for 10 min and the supernatants were
65 decanted into an eggplant-shaped flask to be combined. The collected extract was dried using
66 a rotary evaporator and redissolved with 6 mL of methanol/methyl tert-butyl ether (1:1, v/v).
67 The solution then was normally passed through a NH₂-SPE cartridge preconditioned with 6
68 mL of methanol/methyl tert-butyl ether (1:1, v/v). The filtrate was collected, then dried and
69 redissolved in 0.2 mL of ethyl acetate and 1.8 mL of hexane. The mixed solution was applied
70 to silica cartridges (3 mL, 500 mg, Waters) which had been preconditioned with 4 mL
71 water-saturated ethyl acetate and 4 mL hexane/ethyl acetate (90:10, v/v). After the cartridges
72 were rinsed with 3 mL of hexane/ethyl acetate (90:10, v/v), the fraction containing 17 β -E2
73 was eluted with 3 mL of hexane/ethyl acetate (38:62, v/v). The elution was dried and
74 redissolved with 1 mL hexane-methylene chloride (DCM) (1:1, v/v), and then passed through
75 the preconditioned Florisil cartridges (6 mL, 1 g, Waters). 10 mL of a mixture of
76 hexane-DCM (1:1, v/v) were discarded and the fraction containing all estrogens was eluted
77 with 6 mL of acetone-DCM (1:9, v/v). The solution was evaporated to dryness under a gentle
78 stream of nitrogen and reconstituted with 0.2 mL of methanol for LC-ESI-MS/MS analysis.
79 The recovery of E2-d₃ was 82% \pm 6%, and the recovery of E2 for spiked samples was
80 88% \pm 11%. The MDL of E2 were 0.2 ng/g dry weight (dw).

81 The LC apparatus was an Acquity Ultra Performance LC (Waters, USA). Acquity
82 UPLC® BEH C8 column (100 \times 2.1 mm, 1.7 μ m particle size) (Waters, USA) was used for
83 separation. The column was maintained at 40°C at a flow rate of 0.3 mL/min and the injection
84 volume was 5 μ L. Methanol and ultrapure water were used as mobile phases. Methanol was

85 initially increased linearly from 10% to 50% in 0.5 min, to 80% in the next 5.5 min, to 100%
86 in the following 1.0 min, and kept for 1.0 min. The column was then equilibrated for 3.0 min.

87 Mass spectrometry was performed using a Quattro Premier™ XE detector (Waters, USA)
88 which was operated with ESI in the negative ion (NI) mode. The detection conditions of the
89 mass spectrometer were as follows: capillary voltage, 3.0 kV; source temperature, 110°C;
90 desolvation temperature, 400°C; desolvation gas flow, 800 L/h; and cone gas flow, 50 L/h.
91 Finally, the data acquisition was performed under time-segmented conditions based on the
92 chromatographic separation of the target compounds to maximize sensitivity of detection
93 (Table S1).

94 **TABLE S1.** Parameters for Analyzing Estrogens by LC-ESI-MS/MS

Compound	MRM transition	Cone voltage (V)	Collision energy (eV)
17 β -estradiol (E2)	271 > 145	60	48
	271 > 183		38
17 β -estradiol-d ₃ (E2-d ₃)	274 > 185	58	46

95

96 **TABLE S2.** Primers and their Amplicon Size (L) and Efficiency (E) for Quantitative
 97 Real-time PCR.

Gene symbol	Gene full name	Accession No.	Sequences (first row, forward primers; second row, reverse primers)	L (bp)	E (%)
RPL-7	Ribosomal protein L7	DQ118296	5'-CGCCAGATCTTCAACGGTGTAT-3' 5'-AGGCTCAGCAATCCTCAGCAT-3'	72	99
VTG-1	Vitellogenin 1	AB064320	5'-CTCCAGCTTTGAGGCCATTAC-3' 5'-ACAGCACGGACAGTGACAACA-3'	81	97
P53	tumor protein 53	U57306.1	5'-TCTGAAAACCGAGGGTCTGG-3' 5'-TTTTTGGGCTGCGTTTTCTG-3'	117	98
CYP11	cytochrome P450 cholesterol side-chain cleavage	EF537029	5'-TTGCCGTGAGTCTGCAAAGATA-3' 5'-AAAGTCCCAGCCGGGATGT-3'	73	95
CYP17	P-450 17alpha- hydroxylase/ C17,20-lyase	D87121	5'-CCCCTGGTTACAGATTTTTCCC-3' 5'-TGCAGCAGCTGGTCTCTAACTG-3'	78	96
17β-HSD 1	17-beta hydroxysteroid dehydrogenase type 1	EF530597	5'-CTTGCTGGAATGAAAGCACA-3' 5'-TGAAAGGAAGCCCATGGAGTC-3'	80	96
17β-HSD 3	17-beta hydroxysteroid dehydrogenase type 3	EF530598	5'-TCTTATACAGGCAGTGGCTCCA-3' 5'-GGTAAAAAGGTCACCCTGTTGG-3'	81	91
CYP19A	cytochrome P450, family 19, subfamily A	D82968	5'-GCGTAGAGCCCTTTTCGATGA-3' 5'-TGCGGCCCGTATTCAAGAT-3'	80	93
20β-HSD	20-beta-hydroxysteroid Dehydrogenase	EF537021	5'-GATGTGGACAGCATCAGCACTG-3' 5'-AGTCGTGTCTGCCACCTTGAAC-3'	108	101
CYP1A	cytochrome P450 1A	AY297923	5'-ATCGGCCTGAATCGAAATCC-3' 5'-TGTGTCCCTTGTGTGCAGTGT-3'	132	98
CYP1B	cytochrome P450 1B	JF894387	5'-GCTGTTTCTCTTCGTGGCATT-3' 5'-CGATGTCATAGGCGTGAGGTTT-3'	119	93
CYP2A	cytochrome P450 2A	EF546459	5'- ATATGGGATCGGGATCAGCAA-3' 5'- CCGCAGCGTCGTCAGAGTG-3'	70	102
CYP2C	cytochrome P450 2C	NP*	5'-AGGAGAAAATGCAGGAGGAGATC-3' 5'-GTGAGGGAGGCTGAAAGGTGT-3'	146	96
CYP3A	cytochrome P450 3A	AF105018	5'-AGGAAACAGAGATCCCCTTCGA-3' 5'-AGGCACCAGCTTCAGAAAGATG-3'	80	94
EHPX1	Epoxide hydrolase1	NP	5'-CCTTCTACGAGTTCTACGGGATTC-3' 5'-ATGTTGGTCGTGATGAGGGAG-3'	232	95
EHPX2	Epoxide hydrolase2	NP	5'-GGTTTTCTGTCCAGCATTTACTCC-3' 5'-CTGGCCTGGCTTTCTTTACAC-3'	125	97
GSTA	Microsomal glutathione S-transferase alpha	NP	5'-TGAATTTGATGAGATGTATTTGAC-3' 5'-TTTTGCTTTACTCTGAATGTTGTCC-3'	298	95
GSTM	Microsomal glutathione S-transferase 1	NP	5'-CATCACCAGAGGGTCTTTTGTC-3' 5'-AGCCAGGATGTAGGAAATCGTGT-3'	256	104
GSTP1	Microsomal glutathione S-transferase P	NP	5'-AATGGCAATGACTCTGGCTTACT-3' 5'-AGCTTACCACAGACATAAAACTTG-3'	115	94
UGT1A	UDP glucuronosyltransferase 1 family, polypeptide A1	EF546456	5'-TGACCTTTTAGCCCATCCCA-3' 5'-GCAGATGCCCTCATAGATTCCA-3'	76	101
UGT2A	UDP-glucuronosyltransferase 2 family, polypeptide A	EF546458	5'-AGATCTGCCCGCTGACTTAGCT-3' 5'-CATTCTGTGGCAGCCAATCAA-3'	140	90
SULT1	sulfotransferase 1	EF546450	5'-AGAGAATCCTCGCCGTGAAGTT-3' 5'-CCACGATTTGGTTGATGACCTC-3'	83	91

98 *Nucleotide sequence which was identified in our lab and not published

99 **TABLE S3.** Mean Numbers of Spawned Eggs per Female per Day, Prevalence of Skeletal Deformation, Protein Content of Egg, VTG-1
 100 Transcription Level and E2 Concentration.

Single BaP exposure groups									
Measured BaP Concentration (µg/L)	ND ^a	0.033	0.034	0.235	1.602	11.356			
Egg number /female /day	18.11±1.57	16.60±1.41	12.76±1.14	13.46±1.51	10.84±0.88	7.96±0.92			
Prevalence of skeletal deformation (%)	0.02±0.01	0.04±0.01	0.10±0.02	0.08±0.02	0.12±0.03	0.22±0.03			
Protein content per/egg (µg)	89.19±3.10	84.79±2.54	78.82±4.43	80.39±2.15	68.76±2.09	60.91±3.32			
BaP and BDE47 coexposure groups									
Measured BaP Concentration (µg/L)	ND	ND	ND	ND	1.206	0.950	0.564	0.464	
Measured BDE47 Concentration (µg/L)	ND	0.039	0.771	5.377	ND	0.053	0.439	2.584	
Egg number /female /day	14.86±1.16	13.62±1.72	14.47±1.99	15.18±1.23	8.23±0.88	8.01±1.15	12.29±0.60	13.80±1.19	
Prevalence of skeletal deformation (%)	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.10±0.02	0.09±0.01	0.19±0.02	0.16±0.02	
Protein content per/egg (µg)	89.91±3.61	87.33±2.44	90.82±3.71	85.44±3.87	63.11±3.15	59.72±3.32	90.98±3.69	97.12±3.50	
mRNA expression of VTG1	1.000±0.134	1.413±0.115	1.708±0.133	1.562±0.156	0.597±0.131	1.052±0.166	1.159±0.208	1.655±0.157	
Concentration of 17β-E ₂ (ng/g d.w.)	3.22±0.30	3.31±0.34	2.61±0.30	2.93±0.26	0.83±0.16	0.68±0.35	2.25±0.57	3.07±0.65	

101 Data are presented as means±standard errors. ^aND: not detected.

102 **TABLE S4.** Relative mRNA Expressions of Steroidogenesis Enzyme Genes and *p53* Gene in Gonad and Phase I and Phase II Metabolism
 103 Enzyme Genes in Liver of Medaka Fish ^a.

	DMSO	0.039µg/L BDE47	0.771 µg/L BDE47	5.377 µg/L BDE47	-	0.053 µg/L BDE47	0.439 µg/L BDE47	2.584 µg/L BDE47
					1.206 µg/L BaP	0.950 µg/L BaP	0.564 µg/L BaP	0.464 µg/L BaP
<u>Steroidogenesis Enzyme Genes</u>								
<u>Female</u>								
CYP11	1.00±0.14	1.06±0.08	1.36±0.18	1.15±0.23	1.29±0.39	1.03±0.53	1.15±0.18	0.82±0.33
CYP17	1.00±0.44	0.87±0.12	0.85±0.05	0.73±0.17	0.92±0.23	0.63±0.30	0.43±0.15* [#]	0.57±0.21
17β-HSD1	1.00±0.23	1.18±0.16	1.57±0.31	1.65±0.42	0.49±0.35*	0.56±0.39	0.92±0.18	1.51±0.16[#]
17β-HSD3	1.00±0.18	1.42±0.41	0.78±0.18	0.72±0.51	0.96±0.20	0.69±0.30	0.67±0.56	0.38±0.31* [#]
CYP19A	1.00±0.58	1.67±0.27	1.09±0.27	0.90±0.11	1.16±0.42	0.72±0.56	0.54±0.20	0.62±0.35
20β-HSD	1.00±0.31	1.02±0.77	0.77±0.76	0.73±0.88	0.62±0.83	1.41±0.20	0.96±0.30	0.80±0.69
<u>Male</u>								
CYP11	1.00±0.21	1.13±0.13	0.96±0.10	0.81±0.25	1.01±0.32	0.77±0.20	1.14±0.14	1.32±0.20
CYP17	1.00±0.38	1.68±0.22	1.10±0.14	0.94±0.13	1.09±0.39	0.80±0.05	0.97±0.37	0.75±0.25
17β-HSD1	1.00±0.42	0.72±0.38	1.41±0.22	1.60±0.50	0.58±0.24*	0.73±0.65	1.37±0.38	1.80±0.47[#]
17β-HSD3	1.00±0.19	1.91±0.45	1.64±0.33	0.89±0.20	1.70±0.47	1.30±0.21	0.78±0.35	0.97±0.13
CYP19A	1.00±0.23	0.63±0.28	0.83±0.04	0.32±0.09*	0.83±0.41	0.95±0.45	0.94±0.23	0.88±0.41
20β-HSD	1.00±0.32	0.59±0.68	0.85±0.21	0.65±0.13	0.88±0.21	0.88±0.22	0.88±0.40	0.74±0.44
<u>Phase I Metabolism Enzyme Genes</u>								
<u>Female</u>								
CYP1A	1.00±0.54	1.19±0.41	0.62±0.35	0.71±0.55	3.06±0.37*	3.70±0.91*	2.33±0.63*	1.63±0.31[#]
CYP1B	1.00±0.44	1.43±0.19	1.14±0.36	0.70±0.25	1.54±0.26	1.73±0.52	0.88±0.21	1.07±0.24
CYP2A	1.00±0.24	0.77±0.72	0.89±0.51	0.69±0.13	1.66±0.54	1.64±0.94	1.25±0.62	0.76±0.21
CYP2C	1.00±0.37	1.18±0.55	1.02±0.50	0.72±0.31	1.55±0.39	1.36±0.15	0.76±0.32	0.50±0.17[#]
CYP3A	1.00±0.27	1.62±0.49	2.01±0.37*	2.29±0.46*	0.80±0.37	0.64±0.17	2.75±0.59* [#]	3.10±1.06* [#]
EHPX1	1.00±0.19	1.64±0.32	1.80±0.29*	1.34±0.20	1.73±0.15	1.53±0.52	1.17±0.24	1.30±0.13
EHPX2	1.00±0.22	1.32±0.07	1.39±0.30	1.17±0.13	1.62±0.11	2.65±0.33*	1.64±0.67	0.96±0.02
<u>Male</u>								

CYP1A	1.00±0.77	1.20±0.14	1.46±0.48	1.80±0.31	1.66±0.37	1.39±0.63	3.55±0.81* #	5.28±1.13* #
CYP1B	1.00±0.33	0.88±0.20	1.04±0.12	1.42±0.33	0.76±0.40	0.81±0.16	1.45±0.68	1.93±0.25* #
CYP2A	1.00±0.42	0.60±0.47	0.93±0.11	1.33±0.11	0.82±0.22	0.78±0.68	0.93±0.81	0.74±0.26
CYP2C	1.00±0.47	0.72±0.13	0.84±0.04	0.99±0.18	0.69±0.18	0.43±0.18*	1.07±0.45	0.77±0.09
CYP3A	1.00±0.56	1.24±0.37	1.83±0.19*	2.05±0.24*	0.75±0.20	0.90±0.49	1.70±0.26	1.86±0.17* #
EHPX1	1.00±0.11	0.88±0.20	1.08±0.23	0.88±0.15	1.20±0.20	0.24±0.33* #	1.06±0.07	0.83±0.41
EHPX2	1.00±0.16	0.96±0.06	1.20±0.27	0.88±0.09	0.61±0.24	0.49±0.34	1.12±0.11	1.05±0.40

Phase II Metabolism Enzyme Genes

Female

GSTA	1.00±0.37	1.39±0.40	1.91±0.45	2.11±0.30*	0.48±0.22*	0.34±0.43*	2.27±0.45* #	1.71±0.18* #
GSTM	1.00±0.73	1.15±0.48	1.44±0.62	1.29±0.55	1.42±0.15	1.35±0.85	0.62±0.08	1.21±0.31
GSTP1	1.00±0.32	2.70±0.26*	5.55±0.32*	2.88±0.17*	1.38±0.33	2.31±0.33* #	3.44±0.23* #	2.85±0.23* #
UGT1A	1.00±0.23	1.10±0.34	1.12±0.08	0.97±0.33	1.43±0.22	1.86±0.12*	1.24±0.15	0.98±0.15
UGT2A	1.00±0.26	0.52±0.38*	0.55±0.48*	0.25±0.38*	0.93±0.58	1.95±0.02*	0.44±0.32*	0.66±0.42
SULT1	1.00±0.33	1.60±0.41	1.30±0.48	1.09±0.56	1.38±0.21	1.71±0.40	0.92±0.18	0.85±0.24

Male

GSTA	1.00±0.24	0.99±0.27	1.39±0.35	1.37±0.27	0.44±0.29*	0.33±0.51*	1.40±0.25* #	1.13±0.39
GSTM	1.00±0.62	1.90±1.02	1.15±0.19	1.57±0.27	0.58±0.45	0.43±0.36	1.55±0.20	1.78±0.98
GSTP1	1.00±0.25	0.96±0.32	1.67±0.21	1.63±0.29	0.56±0.42	0.38±0.26*	2.99±0.13* #	2.15±0.21* #
UGT1A	1.00±0.41	0.81±0.46	1.18±0.06	1.49±0.29	0.76±0.38	0.56±0.91	2.01±1.14	1.12±0.38
UGT2A	1.00±0.32	0.28±0.37*	0.31±0.12*	0.38±0.40*	0.70±0.80	0.18±0.64* #	0.67±0.58	0.62±0.83
SULT1	1.00±0.21	0.80±0.25	0.89±0.33	0.74±0.26	0.61±0.43	0.44±0.71	1.25±0.60	0.98±0.62

Gene Involved in DNA Damage

Female

P53	1.00±0.46	1.30±0.12	1.24±0.18	1.27±0.23	1.10±0.24	1.34±0.27	0.99±0.46	0.95±0.20
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Male

P53	1.00±0.23	1.01±0.07	0.99±0.37	1.22±0.57	0.96±0.31	1.07±0.22	1.64±0.26*	2.36±0.49* #
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^a Relative mRNA expression was calculated using RPL-7 as the internal control. The fold-change values are relative to expression in controls. Data are presented as means ± standard deviation (n=6). *: statistically significant differences between results of exposure group and results of the DMSO control. #: statistically significant differences between results in co-exposure of BaP and BDE47 and results of the 25 µg/L single BaP exposure group. -: The BaP control.