Supporting Information

Biosensor Medaka for Monitoring Intersex Caused by Estrogenic Chemicals

Yanbin ZHAO, Chen WANG, Shuang XIA, Jieqiong JIANG, Rui HU, Guanxiang YUAN, and Jianying HU*

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

Address for Correspondence

Prof. Dr. Jianying HU

College of Urban and Environmental Sciences

Peking University, Yi Fu Second Building

Beijing 100871 China

TEL & FAX: 86-10-62765520

Email: hujy@urban.pku.edu.cn

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Analytical Processes for Actual Chemical Concentrations in Exposure Tanks. The actual concentrations for 17α-EE_2 and 17β-E_2 exposure groups were determined by using SPE concentration combined with UPLC-ESI-MS/MS. In brief, the water samples (1 L for vehicle control groups, 0.5 ng/L, 1 ng/L and 2 ng/L EE_2 exposure groups and 500 mL for 4 ng/L, 8 ng/L, 16 ng/L and 32 ng/L EE_2 exposure groups; 1 L for vehicle control groups and 5 ng/L 17β-E_2 exposure groups and 500 mL for 10 ng/L, 20 ng/L and 40 ng/L 17β-E_2 exposure groups) were collected from each exposure tanks and then were spiked with 10 ng of 17α-EE_2-d_4 or 10 ng of 17β-E_2-d_3, respectively, (Wako Pure Chemical Co.) as internal standard and were extracted through HLB cartridges (6 mL, 500 mg, Waters) at a flow rate of 5-10 mL/min. The cartridge was preconditioned with 6 mL of ethyl acetate (Fisher Chemical Co.), 6 mL of acetonitrile (Fisher Chemical Co.) and 12 mL of distilled water. Then the cartridges were rinsed with 10 mL of distilled water and dried under a flow of nitrogen. Target analytes were subsequently eluted with 15 mL of ethyl acetate. The eluates were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 0.5 mL of methanol (Fisher Chemical Co.) for UPLC-ESI-MS/MS analysis. The recoveries of 17α-EE_2-d_4 and 17β-E_2-d_3 were 86±13% and 82±6%, respectively. The method detection limits (MDL) of 17α-EE_2 and 17β-E_2 for 1 L water samples were both at 0.1 ng/L.

For analyzing actual 4-NP concentrations, 100 μL water samples were collected from each exposure tanks and then were spiked with 100 μL of 10 μg/L 4-n-NP (Wako Pure Chemical Co.) in methanol as internal standard for UPLC-ESI-MS/MS analysis, and the MDL of 4-NP for water samples was 1.0 μg/L.

The LC apparatus was an Acquity Ultra Performance LC (Waters). For EE_2 and 17β-E_2, Acquity UPLC® BEH C8 column (100 × 2.1 mm, 1.7 μm particle size) (Waters) was used for separation. The column was maintained at 40°C at a flow rate of 0.3 mL/min and the injection volume was 5 μL. Methanol and ultrapure water were used as mobile phases.

Methanol was initially increased linearly from 10% to 50% in 0.5 min, to 80% in the next 5.5 min, to 100% in the following 1.0 min, and kept for 1.0 min. The column was then equilibrated for 3.0 min. For 4-NP, a Waters Acquity UPLC® BEH C18 column (100×2.1 mm, 1.7 µm particle size) coupled with a Waters Acquity UPLC® BEH C18 column (50×2.1 mm, 1.7 µm particle size) was used for separation. The guard column was used for decreasing contamination from UPLC system. The column was maintained at 40°C at a flow rate of 0.15 ml/min and the injection volume was 5µL. Methanol and water were used as mobile phases. Methanol was initially increased linearly from 20% to 50% in 0.5 min, to 90% in the next 3.0 min, to 100% in the following 1.5 min, and kept for 1.0 min. The column was then equilibrated for 3.0 min.

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

Table S1. Parameters for Analyzing EE2, 17β-E2 and 4-NP by UPLC-ESI-MS/MS

Compound	MRM transition	Cone voltage (V)	Collision energy (eV)
17α-ethynylestradiol (EE_2)	279>133 279>159	30	21 21
17α-ethynylestradiol-d_4 (EE_2-d_4)	283>135	30	21
17β-estradiol (17β-E_2)	255 > 159 255 > 133	33	20 20
17β-estradiol-d_3 (17β-E_2-d_3)	258 > 159	33	20
4-nonylphenol (4-NP)	219.2>132.9 219.2>147.6	40 40	24 16
4-n-NP	219.2>105.8	40	24

TAGTTATTACTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACG
GTACCTTCAACTTGTGTGGGGTAGATGTAGCTTAACAAGCTTCAGTTTTGAAGGTGGTACACT
GGAAATAAACACTTTACCTCTGATACACCCCCGCTTTCTCCACTGACCTTGCTGTGATTGGCC
AAACGCTCCCCTTTGATGTGCACATCCATCTTTGCAAAAGTTCCATTTTAAATCTTTTTGCGCG
ACAAATGGGATGGATCGTTGCAACACTGACTCTATGTTAAATGGGTGCCCCCCCCCCCCCCCC
CCCCACAAGGAATGAAGGCCTGTGCTTCGGAGATTTGAGGCTAGGTAAATGAGCTGCAAAAA
AACCCATTGTGTGCCAAAGGTTGTATGGTTTACTCTGAAATGCTTAGGAATGGTATAGGCCCTT
CAATCTTACCTGAATTTAAGACTTTAGTACATGAGTATTAAATCAGATTATTCCAAAGTAAATG
CATGTATAGAAGCCAATTTAGACTTGTTTTGCACAACTTTGATTACCTTAAGCTTCCGAATTTG
TTCAGGGGGGCTGCACTTGACTCTCTAAGCTTGTATTTTTGGTTGAACCAAATAACCATTGAA
GCTAAATGCTTTAACTGCACAGCTCTCATGCAATAGTCTTGATTTTGCTGTTAAAGGTGCCAGT
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AAATCAATTTTTAAAATGTTATCTGCCATTGAGTTGCATTAATTTTAAATGTGGAAGATCTCATC
TTTCAAATTTGTGTAAGTTTTACACATACCAAAGAAAACGCCAAACATTTTTTAAATGATGGA
CCAAATGAGAACTAGATGTTTCTAACCTGTCGTATTAAAATTCCCACCCATCCTTCCATGTCTT
CAGAACTCACTGAATCCCTTTTTGGGTCACGGGGTTGCTGGAGCCAATCCAGCTACTGTTGTG
CAAAGGTGGGATACACCCTGAACACTTCACCAGCCTGTTGCAAGGCCACACATTCACAGCCA
CACCTAGCAGAAATTGAGAGATACCAATTAACCCATGAGCCTTTGTTTTTGAACCAAGAAAGG
AAACCAGAGTGTGAAAACCCACGCATGCTCGAGGAAATCACGCAAACCACACAAAGGATCC
AGCCAGAATTCAAACCAGGGCCTTCTCACTGTGAAGCAAGATCGCTAACAAGTACAGCACCG
TACACCATCTTCCTAAGGAATTTTCTCCTCATTAAAATGTGGCGGAAATAAATGCCATTAAAGT
TTGTAAAGAAAATGTTTTTTTTACAAAAGGCCTTACAAAATCATGTGCTGTACACACCATTCTT
CAGTTCAAACAAATATAATGAAAGTAATGACATTAAGAATTGTTTCTGTCTTTAGGTTGTTATT
TCAACTTTAATACAGAATTATTGCTGTCAATACTATTTTGACTGAAAATATATGTGTAAGAACA
AAAAGCACATGACACCAAGATGTGAAAAATTAAAGTAAAAAAATGTTTTTGACATGAGATGT
TGCAACAAGACTCTGAAGTATAGATTTTTCTGGGGTTTTGTTAAAGTAAACGTTTAAAATTTTA
GAAATGAGAAAATATTTTTGATCCAAAACACATCATTTAATTCTGTAAACATTGGCTTAACCAG
GCATTCAAAACAAAACATATTTGTCTAAGAACTTCTGTTCATGTGAACTTGGTGACAGGTTTA
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TTAACAATTTTCTCCAATCAGCTTTGCGGATCAGATATAAGCAGCAGGATGAAGGCATTGGAA
CATGTCACTGAGTTTAGTCTTGGGCATCAGTCAATAGCAACCAGCAGACCCGGGATCCACCG
GTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGA
GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC
ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCC
CACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGA
AGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGG
TGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAA

GCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCA
TCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCAC
TACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG
CACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGT
TCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

Figure S1: Part of DNA sequences of pMOSP1-EGFP construct. “-” indicates the DNA sequence (2067 bp) of the proximal promoter region of OSP1 gene in medaka fish (orange red strain, *Oryzias latipes*); “~” indicates the DNA sequence of EGFP.