Supplementary information

Estrogenic activity profiles and risks in surface water and sediments of the Pearl River system, South China, assessed by chemical analysis and *in vitro* bioassay

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Quality control and quality assurance

Recoveries in river water were obtained by spiking 1 L of reservoir water with 5, 100, and 200 ng of seven selected phenolic compounds, while recoveries in sediments were obtained by adding 20, 100, and 500 ng of each compound to 5 g of reservoir sediment. The recoveries in river water for 4-t-OP, 4-NP, BPA, E1, DES and EE2 were between 70%-120% at three spiked concentrations, but E2 had slightly higher recoveries. The limits of detection (LODs) and the limits of quantitation (LOQs) for analytes in river water were defined as 3 and 10 times the standard deviation (SD) of 7 spiked samples at 5 ng/L respectively. LODs were below 1 ng/L and LOQs were below 2 ng/L for most compounds, except for 4-NP which had a higher LOD and LOQ. While the recoveries in sediments were between 70%-120% for most compounds, and the LODs and LOQs were below 1.0 ng/g and 3.0 ng/g for most compounds, the LOD and LOQ of 4-NP were 1.5 ng/g and 4.9 ng/g, respectively. Detailed recoveries, LODs and LOQs data are available in Table S2.

As a quality control, duplicate blank samples and duplicate spiked control samples were analysed simultaneously with the unspiked field samples. Spiked control samples for river water and reservoir sediment were obtained by spiking 1 L of Milli-Q water with 100 ng of each compound, and spiking 5 g of reservoir sediment with 100 ng of each compound . The recoveries for target compounds in the control samples were between 70% -120% in each batch of experiments. For the YES bioassay, spiked control samples were obtained by spiking 1 L of Milli-Q water and 5 g of reservoir sediment with 54.48 ng of E2. The EEQs (estradiol equivalent) for the control samples in water and sediment were between 45 ng to 65 ng in each batch of YES (recombinant yeast estrogen screening) bioassay.

Measured EEQ and calculated EEQ

The calculation approaches for measured EEQs and calculated EEQs were developed from the published methods.[1] In brief, it was assumed that the real sample had the same dose-effect curve as E2; hence it shared the same dose-effect equation as E2. The dose-effect curve of E2 is expressed as the arithmetic means with standard derivations of corrected absorbance data (blank value was subtracted) toward Logarithm of dose (equivalent amount at ng/L). The dose-effect equation of E2 was then fitted using log-logistic model. The equation is expressed as the following:

$$Y = A_2 + \frac{(A_1 - A_2)}{1 + (x / EC_{50})^p} .$$

Here, Y is the corrected absorbance at 540 nm, A_1 is the minimum absorbance, A_2 is the maximum absorbance, EC_{50} is half dose-effect concentration, and p is the Hill slope. So, the measured EEQ a certain sample was calculated by using the ratio EC_{50} of the sample to EC_{50} of E2.

Based on the concentration addition model, calculated EEQ was expressed as the sum of all estrogenic contributions of seven compounds by multiplying their corresponding estradiol equivalency factors (EEFs) and chemical concentration.[2] Then, the EEFs of the target analytes are calculated by the ratios f EC_{50} values, that is EEF(i)= $EC_{50}(i)$ / EC_{50}(E2).[1] The EEFs for seven target compounds were listed in Table S6. The Calculated EEQ of real sample was the addition of values that all individual compound concentration multiplied the corresponding EEFs:

$$\sum \text{Calculated EEQ}(i) = \sum \text{EEF}(i) \times c(i).$$

Derivation of predict no effect concentration (PNEC) of E2

The derivation of PNEC of E2 was carried out as the Technical Guidance Document (TGD) on risk assessment.[3] We collected no observed effect concentrations (NOECs), low observed effect concentrations (LOECs) data on the effects of reproduction and vitellogenin (VTG) concentration tests of fish, daphnia, and some other species in literature. The detailed data were summarized in Table S4. Here, we selected 77 *in vivo* chronic NOECs of reproduction data and set up the species sensitivity distribution curve (Fig. S1), and then fitted by the 4-pamameter logistic regression. The parameters were shown in the following equation:

$$y = -1.346 + \frac{97.17}{1 + (x/68.14)^{-0.6998}}.$$

From this distribution of species sensitivity distribution curve, the hazardous concentration (HC_5) was identified as fifth percentage of all species tested,[4,5] behind which 95% of species would not display adverse effects associated with E2 exposure. The PNEC corresponding to the HC_5 was 1.5 ng/L for E2.

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Fig. S1:

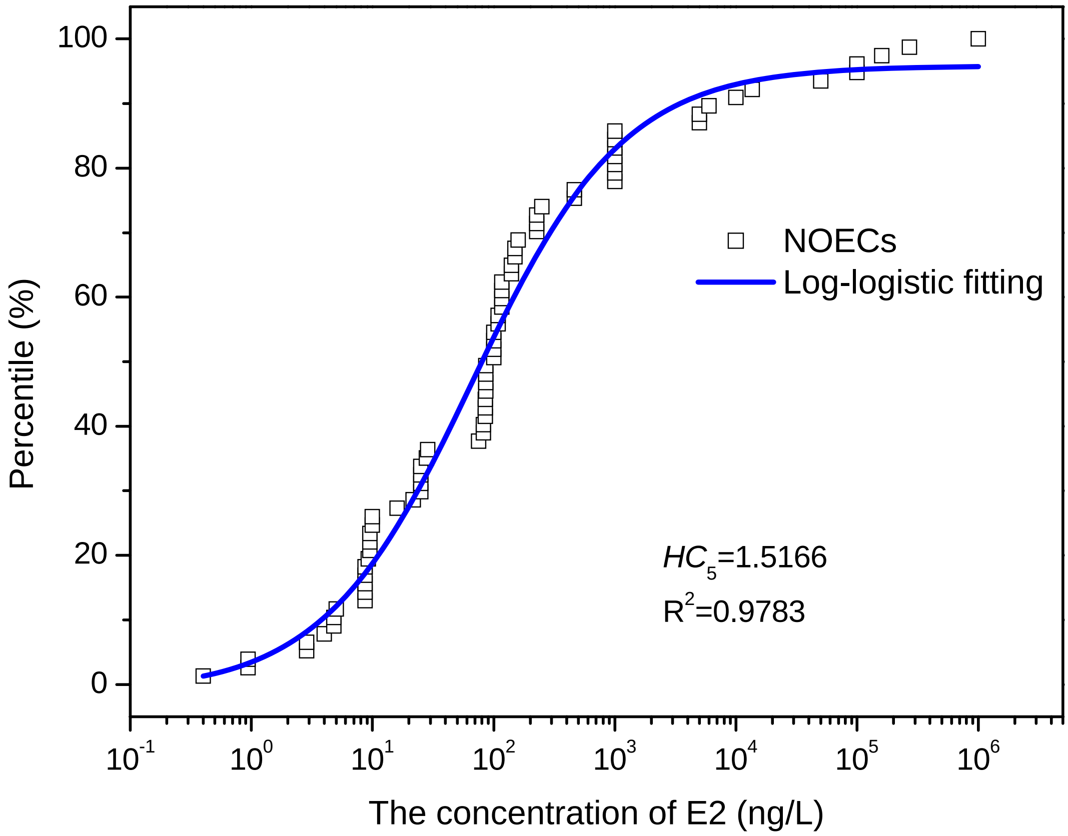

Fig S1. Estimating of predicted no-effect concentration (PNEC) from species sensitivity distribution (SSD) curve using *in vivo* predicted no-effect concentrationa (NOECs) data. The curve was fitted using log-logistic model. HC_5 means 95% of species will not display adverse estrogenic effect below the concentration of E2.

Table S1

The surface water and sediment parameters of sampling sites.[a]

Sites	T (ºC)	pH	Conductivity (µs/cm)	DO [b] (mg/L)	TOC_{dry} [c] (%)	TOC_{wet} [d] (%)
S0	31.1	7.27	86.7	10.5	1.23	1.40
S1	31.0	7.45	112	14.6	2.61	0.30
S2	31.1	7.24	166	13.81	0.90	0.07
S3	30.2	6.92	257	3.07	2.46	2.17
S4	31.1	7.03	343	3.86	3.95	2.70
S5	31.2	7.10	383	5.11	3.26	5.81
S6	32.8	7.14	446	3.14	2.95	2.84
S7	30.6	7.16	340	4.53	2.40	2.32
S8	30.8	7.33	230	11.15	1.44	2.04
S9	32.4	7.31	325	5.12	1.91	1.94
S10	31.9	7.28	266	4.23	2.82	1.91
S11	31.3	7.10	360	4.67	6.01	4.97
S12	30.9	7.26	675	1.04	NA [e]	3.80
S13	31.4	7.34	458	2.14	6.55	2.79
S14	30.8	7.31	549	0.99	7.45	3.18
R1	32.9	7.27	843	0.33	NA	0.56
R2	32.9	7.09	720	0.13	NA	6.24
W1	31.8	7.09	723	6.82	NA	NA
W2	31.8	7.08	799	6.81	NA	NA
W3	30.8	7.06	624	7.93	NA	NA
W4	30.7	7.23	463	3.42	NA	NA

[a] The data of temperature, pH, conductivity and DO listed here were from the wet season (September 10-12, 2008); [b] Dissolved oxygen; [c] Total organic carbon of freeze-dried sediment in dry season; [d] Total organic carbon of freeze-dried sediment in wet season; [e] Not analyzed.
R1 and R2 are two urban streams in the city area.

Table S2

Recoveries (%), limit of detection and limit of quantitation of seven selected chemicals in surface water.

Compounds [a]	Spiked concentrations [b]			LOD (ng/L) [c]	LOQ (ng/L) [c]
	5 ng/L	100 ng/L	200 ng/L		
4-t-OP	99±4	75±4	74±11	0.3	1.0
4-NP	92±22	115±13	74±5	2.0	7.0
BPA	118±8	103±1	105±3	0.7	2.0
E1	86±4	96±5	90±8	0.2	0.5
DES	73±4	71±3	71±2	0.2	0.5
E2	135±12	145±5	163±34	0.3	1.0
EE2	131±4	101±2	105±6	0.2	0.7

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; E1: estrone; E2: estradiol; DES: diethylstilbestrol; EE2: 17α-Ethinylestradiol.

[b] mean ± standard deviation (n = 4).

[c] LOD: limit of detection; LOQ: limit of quantitation.

Table S3

Recoveries (%), limit of detection and limit of quantitation of seven selected chemicals in sediments.

Compounds [a]	Spiked concentrations [b]			LOD (ng/g) [c]	LOQ (ng/g) [c]
	5 ng/g	20 ng/g	100 ng/g		
4-t-OP	100±6	80±3	75±3	0.3	0.9
4-NP	81±12	96±16	85±6	1.5	4.9
BPA	97±13	88±2	89±1	0.8	2.6
E1	114±6	91±7	95±2	0.3	1.1
DES	152±12	133±12	134±7	0.7	2.3
E2	76±15	83±18	106±14	1.1	3.5
EE2	89±9	92±12	113±7	0.8	2.5

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; E1: estrone; E2: estradiol; DES: diethylstilbestrol; EE2: 17α-Ethinylestradiol.

[b] mean ± standard deviation (n = 4).

[c] LOD: limit of detection; LOQ: limit of quantitation.

Table S4

List of available toxicity data from literature for 17β-estradiol (E2) on fish and other species.

Groups	Test species	Reproductive end point	Duration (days)	NOEC [a] (ng E2/L)	LOEC [b] (ng E2/L)	VTG NOEC [c] (ng E2/L)	Reference No.
Copepod	*Acartia tonsa*	Egg production	10	13600	23000		6
Cladoceran	*Daphnia magna*	Reproduction	21	270000			7
Brown trout	*Salmo trutta*	Sex ratio	274	10			8
Copepod	*Nitocra spinipes*	Larval development	18	160000			9
Copepod	*Nitocra spinipes*	Reproduction	18	50000			9
Copepod	*Nitocra spinipes*	Sex ratio	18	5000	50000		9
Female adult zebrafish	*Danio rerio*	Body length	21	4.8	16.5		10
Female juvenile zebrafish	*Danio rerio*	Body length	21	9.2	21.6		10
Male adult zebrafish	*Danio rerio*	Body length	21	>82			10
Male juvenile zebrafish	*Danio rerio*	Body length	21	>109		25	10
Female larval zebrafish	*Danio rerio*	Body length	21	>117			10
Male larval zebrafish	*Danio rerio*	Body length	21	>117			10
Female adult zebrafish	*Danio rerio*	Body weight	21	4.8	16.5		10
Female juvenile zebrafish	*Danio rerio*	Body weight	21	21.6	109		10
Male adult zebrafish	*Danio rerio*	Body weight	21	>82		5	10
Male juvenile zebrafish	*Danio rerio*	Body weight	21	>109			10
Female larval zebrafish	*Danio rerio*	Body weight	21	>117			10

Male larval zebrafish	*Danio rerio*	Body weight	21	>117			10
Poppe	*Eurytemora affinis*	Survival	10	6000	18000		11
Female japanese medaka	*Oryzias latipes*	Fecundity	60	150			12
Japanese medaka	*Oryzias latipes*	Spawn times	60	150		100	12
Japanese medaka	Oryzias latipes	Hatching rate	14	140.6			13
Japanese medaka	Oryzias latipes	Survival percentage of larvae	14	140.6			13
Crustacean	*Tisbe battagliai*	Reproductive output	21	>100000			14
Java-medaka	*Oryzias javanicus*	Fecundity	187	9.5	16		15
Java-medaka	*Oryzias javanicus*	Hepatosomatic index	187	159	243		15
Java-medaka	*Oryzias javanicus*	Reproduction	180	9.5	16	16	15
Java-medaka	*Oryzias javanicus*	The number of eggs	187	16	68		15
Java-medaka	*Oryzias javanicus*	Total length	187	9.5	16		15
Embryo zebrafish	*Danio rerio*	Expression of ER [d] alpha	3	100	250	1000	16
Larval zebrafish	*Danio rerio*	Expression of ER alpha	7	100	250	250	16
Embryo zebrafish	*Danio rerio*	Expression of ER beta	3	1000	>1000		16
Larval zebrafish	*Danio rerio*	Expression of ER beta	7	1000	>1000		16
Male adult zebrafish	*Danio rerio*	Expression of hepatic ER beta	7	250	500	100	16
Cladoceran	*Ceriodaphnia dubia*	Neonates produced	7	5000	50000		17
Cladoceran	*Ceriodaphnia dubia*	Survival	7	1000000			17
Japanese medaka	*Oryzias latipes*	Fertility of eggs	21	227	463	29.3	18

Male Japanese medaka	*Oryzias latipes*	GSI [e]	21	227	463		18
Female Japanese medaka	*Oryzias latipes*	Hatchability from F1 embryos	21-31	463	/		18
Japanese medaka	*Oryzias latipes*	Sex ratios	60	>463	/		18
Japanese medaka	*Oryzias latipes*	Total number of eggs from F0	21	227	463		18
Cladoceran	*Daphnia magna*	Reproduction	25	>100000			19
Larval minnow	*Gobiocypris rarus*	Body weight	21	25	100	25	20
juvenile minnow	*Gobiocypris rarus*	Body weight	42	>1000		25	20
Adult minnow	*Gobiocypris rarus*	Body weight	171	>1000		5	20
Larval minnow	*Gobiocypris rarus*	Length	21	25	100		20
juvenile minnow	*Gobiocypris rarus*	Length	42	>1000			20
Adult minnow	*Gobiocypris rarus*	Length	171	>1000			20
Juvenile minnow	*Gobiocypris rarus*	Sex ratio	42	5	25		20
Larval minnow	*Gobiocypris rarus*	Testes-ova	21	25	100		20
Juvenile minnow	*Gobiocypris rarus*	The development of gonads	42	25	100		20
Cladoceran	*Diaphanosoma celebensis*	Reproduction	3G [f]	1000			21
Japanese medaka	*Oryzias latipes*	Body weight	90	10	100		22
Japanese medaka	*Oryzias latipes*	Testes-ova	90	0.4	4		22
Japanese medaka	*Oryzias latipes*	Sex ratio	100	75	150		22
Japanese medaka	*Oryzias latipes*	Total length	90	100	1000		22
Male fathead minnow	*Pimephales promelas*	Gonad weight	21	100	320	32	23
rotifer	*B. calyciflorus.*	Reproduction	4	10000			24
sand goby	*Pomatoschistus minutus*	Egg production	7	4	97		25
Medaka	*Oryzias latipes*	Body weight of F0	60	0.939	2.86		26
Medaka	*Oryzias latipes*	Embryological	98-100	8.66			26

		abnormalities of F1					
Medaka	*Oryzias latipes*	Female GSI of F0	71-100	27.9			26
Medaka	*Oryzias latipes*	Fertility of F0	71-100	2.86	8.66	0.939	26
Medaka	*Oryzias latipes*	Male GSI of F0	71-100	8.66	27.9	2.86	26
Medaka	*Oryzias latipes*	sex ratio of F1	56	2.86	8.66		26
Medaka	*Oryzias latipes*	sex ratio of F0	60	8.66	27.9		26
Medaka	*Oryzias latipes*	Testis-ova of F0	60	8.66	27.9		26
Medaka	*Oryzias latipes*	Total length of F0	60	0.939	2.86		26
Medaka	*Oryzias latipes*	Total number of eggs from F0	71-100	8.66	27.9		26
Male zebrafish	*Danio rerio*	GSI	21	>85.9		24.5	27
Female zebrafish	*Danio rerio*	GSI	21	>85.9		24.5	27
Male medaka	*Oryzias latipes*	GSI	21	>85.1		<8.94	27
Female medaka	*Oryzias latipes*	GSI	21	>85.1		28.2	27
Male medaka	*Oryzias latipes*	Number of papillary processes	21	>85.1			27
Male fathead minnow	*Pimephales promelas*	GSI	21	>86		28.6	27
Female fathead minnow	*Pimephales promelas*	GSI	21	>86		8.77	27
Male fathead minnow	*Pimephales promelas*	Number of nuptial tubercles	21	28.6	86		27

[a] No observed effect concentration; [b] Lowest observed effect concentration; [c] VTG, vitellogenin, only for fish study, ; [d] ER, estrogen receptor; [e] Gonadosomatic index; [f] three generations.

Table S5

K_{oc} values of seven target compounds

Compound [a]	K_{oc} [b]
4-t-OP	18200
4-NP	38900
BPA	778
E1	4882
DES	569200
E2	4360
EE2	4840

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; E1: estrone; E2: estradiol; DES: diethylstilbestrol; EE2: 17α-Ethinylestradiol. [b] K_{oc}: organic carbon partitioning coefficient to E2, data from Reference 28 and Reference 29.

Table S6

Estrogenic equivalent factor of seven target compounds

Compound [a]	EEF [b]
4-t-OP	0.00093
4-NP	0.00063
BPA	0.00011
E1	0.3
DES	0.83
E2	1.0
EE2	2.2

[a] 4-t-OP: 4-*tert*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; E1: estrone; E2: estradiol; DES: diethylstilbestrol; EE2: 17α-Ethinylestradiol. [b] EEF: estrogenic equivalent factor relative to E2.

Table S7

Flow rates of effluents and different river reaches in the Pearl River system, river reaches information and dilution factors of effluents, the Liuxi River and Shijing River relative to the Zhujiang River.

Site		Inhabitants serviced	Location	Flow (m^3/s)		Dilution factor [a]	
				Dry season	Wet season	Dry season	Wet season
WWTP	Datansha (W1)	1500000	West channel	6.4 [b]	6.4	27.5	154
	Liede (W2)	2150000	Front channel	7.4	7.4	23.6	132
	Xilang (W3)	600000	Back channel	4.6	4.6	17.3	86.4
	Lijiao (W4)	1350000	Back channel	2.3	2.3	43.5	244
River	Liuxi River (S1)	-	-	17.3 [c]	156 [c]	16.2	5.8
	Shijing River (S14)	-	-	3.5 [d]	5.7 [d]	80.0	158
	Zhujiang River (West channel, S4, S5)	-	-	280 [c]	900 [c]	-	-
	Zhujiang River (Front channel, S7)	-	-	175 [c]	980 [c]	-	-
	Zhujiang River (Back channel, S8)	-	-	200 [c]	1120 [c]	-	-

[a] Dilution factor is defined as the ratio of the flow of effluent or river versus the flow of Zhujiang River.

[b] All of the STP effluent flow data were obtained from Guangzhou Sewage Treatment Co., Ltd.

[c] Data were provided by Guangdong Hydrology Bureau.