Supporting information

Enhanced Bioconcentration of Bisphenol A in the Presence of Nano-TiO_2 Can Lead to Adverse Reproductive Outcomes in Zebrafish

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Text S1: Characterization of n-TiO_2

The crystal structure was confirmed by powder X-ray diffraction (XRD) using an X'Pert PRO XRD instrument (PANalytical, Almelo, Netherlands). The diameter sizes were determined by transmission electron microscopy (TEM; JEOL2010F) at an acceleration voltage of 100 kV. The surface area of nano-TiO_2 was measured by the Brunauer-Emmett-Teller method using an ASAP 2020 physisorption analyzer (Micromeritics, Atlanta, US). The average diameter and ζ potential in water (100 μg/L) were determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern instruments, Worcestershire, UK) (1).

Text S2: Chemical analysis of BPA in water and tissues

For tissues, briefly, fish were randomly collected from each replicate tank, and liver, brain and gonads were weighed after freeze-drying, crushed with 5 mL dichloromethane/methanol (2:1 v/v)by homogenizer, and then ultrasonic extraction for 5 min. A 0.9% KCl solution was added to the mixture (20% of final volume). After centrifugation at 1000 × g for 10 min, the dichloromethane phase was removed and evaporated to dryness and redissolved in 1 ml of methanol: hexane (1:20). Samples were applied to Sep-Pak NH_2 500-mg cartridges, previously conditioned with 5ml methanol and 5 ml methanol: hexane (1:20), at a flow of 1 ml /min. Before desorption, the cartridges were washed with 5 ml of hexane. After drying, samples were desorbed by 4 ml of methanol. Finally, the extract was evaporated to dryness using a flow of N_2 and redissolved in 200 μL of methanol: H_2O (3:2). Aliquots of 20μL were used for analysis by UPLC-MS-MS (Waters Corp., Milford, MA, USA).

For water, the samples were percolated through a Sep-Pak C_{18}extraction tube at a flow of approximately 1 mL/min, previously conditioned with 5mL of methanol and 5mL of water, and subsequently washed with 2mL of water. After 5min of drying under vacuum, the compounds were desorbed by 4mL of methanol. Finally, the sample was evaporated to dryness using a flow of N_2 and redissolved in 1mL methanol: H_2O (3:2). Aliquots of 20 μL were used for analysis by UPLC-MS-MS (Waters, Massachusetts, USA). Quantitative analysis was carried out at a selected ion monitoring (SIM) in negative mode for the ions *m*/*z* 241 (BPA-d_{16}) and 227 (BPA).

Samples for recovery and analytical precision both in tissues and water were

spiked at three different levels of BPA and then treated as normal samples. A 30 μL of a 25 ng/μL BPA-d_{16} solution were added to all the samples as internal standard before pretreatment. Quantification was conducted by a calibration curve created with a BPA standard. Procedural blanks were analyzed simultaneously.

Text S3: Histological examination of gonad development

The ovaries and testes (n = 6, 2 from reach replicate tank) were fixed in Bouin's solution for 24 h. The samples were then dehydrated and paraffin-embedded, sectioned into 6 μm sections along the long axis of the gonad, and then the sections were stained with hematoxylin and eosin (H&E, Sigma–Aldrich, Shanghai, China). A total of nine tissue sections per sample were collected. Six samples from each sex and treatment group were randomly selected for histological and stereological analyses. The stage of oogenesis and spermatogenesis were identified and quantified using previously described methods (2). Sections from all treatment groups were examined under a light microscope (Olympus MVX10), equipped with an Olympus Camedia C-5050 camera. Ovarian follicles were staged as: primary oocyte, cortical alveolar oocyte, early vitellogenic oocyte, mature oocyte, or atretic oocyte. A total of three structural components of the testis were considered: spermatogonia, spermatocytes and spermatides (2). The percent of follicles at each stage of development was expressed as a percent of the total number of follicles, as well as the volume densities of each compartment in the testis estimated using Image Pro Plus 6.0 (Media Cybernetics Company, MD, USA), as previously described (2).

Literature Cited

(1) Wang, Q. W.; Chen, Q.; Zhou, P.; Li, W. W.; Wang, J. X.; Huang, C. J.; Wang, X. F.; Lin, K. F.; Zhou, B. S. Bioconcentration and metabolism of BDE-209 in the presence of titanium dioxide nanoparticles and impact on the thyroid endocrine system and neuronal development in zebrafish larvae. *Nanotoxicology* **2014**, *8(S1)*, 196-207.

(2) Wang, Q. W.; Lai, N. L. S.; Wang, X. F.; Guo, Y. Y.; Lam, P. K. S.; Lam, J. C. W.; Zhou, B. S. Bioconcentration and transfer of the organophorous flame retardant 1,3-dichloro-2-propyl phosphate causes thyroid endocrine disruption and developmental neurotoxicity in zebrafish larvae. *Environ. Sci. Technol.* **2015**, *49*, 5123-5132.

Figure S1. TEM images of n-TiO_2 particles in water

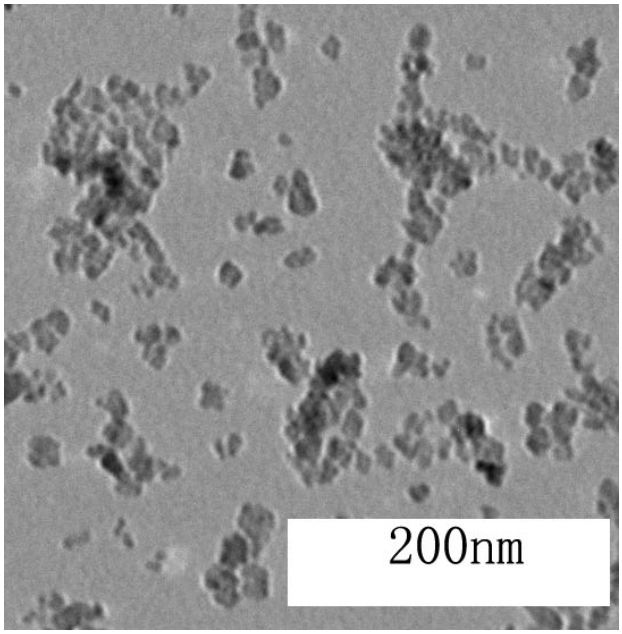

Figure S2. Time-course sorption kinetics of BPA (200 μg/L) onto n-TiO_2 (100 μg/L) in water. Values are mean ± SEM (n=3 replicates).

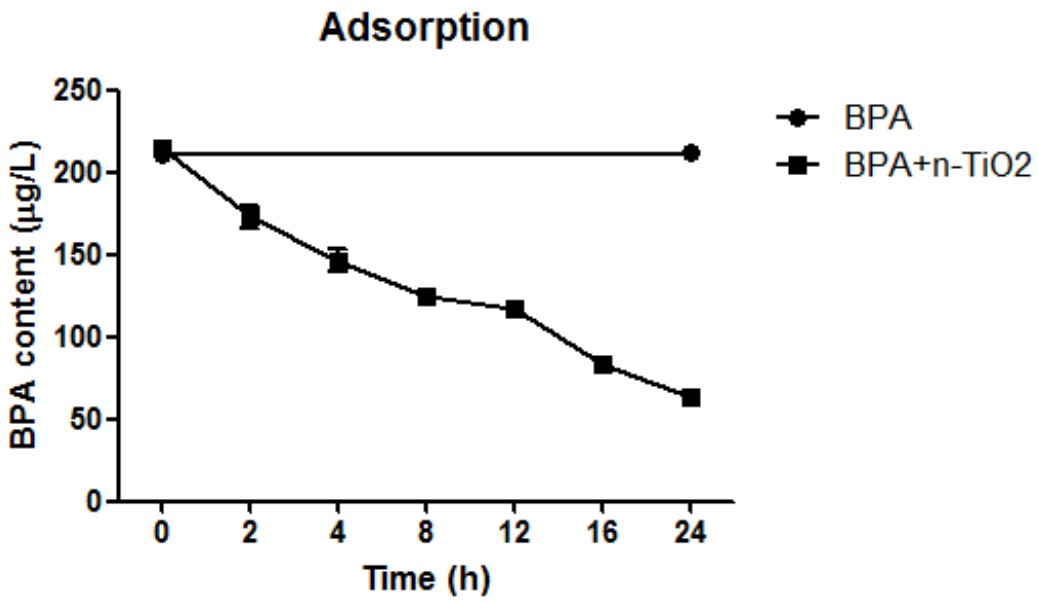

Figure S3. Concentrations of BPA measured after (A) and before (B) renewal of the exposure water. The zebrafish were exposed to BPAs (0, 2, 20 and 200 μg/L) and/or in the presence of 100 μg/L n-$TiO_2$for 24h. Data are expressed as means ± SEM of three replicate samples. Significance differences ($P < 0.05$) are indicated by * vs. the corresponding BPA groups without n-TiO_2; $P < 0.01$ are indicated by **; $P < 0.001$are indicated by ***.

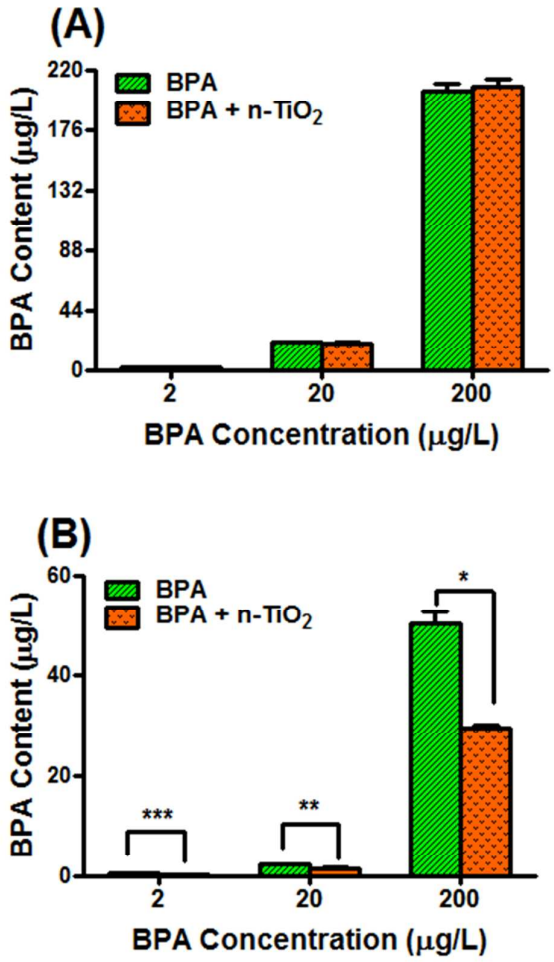

Figure S4. Histological examination of gonad development in zebrafish after exposure to BPA (0, 2, 20, 200 μg/L) and/or with n-TiO_2 (100 μg/L) for 21days. (A): The percentages of primary oocyte, corticalalveolar oocyte, early vitellogenic oocytes, mature oocyte and atretic oocyte in ovary of female zebrafish. (B): The percentages of spermatogonia, spermatocytes, and spermatides in testes of male zebrafish. Values represent the mean ± SEM of three replicate samples for six individual fish from 3 replicate tanks.

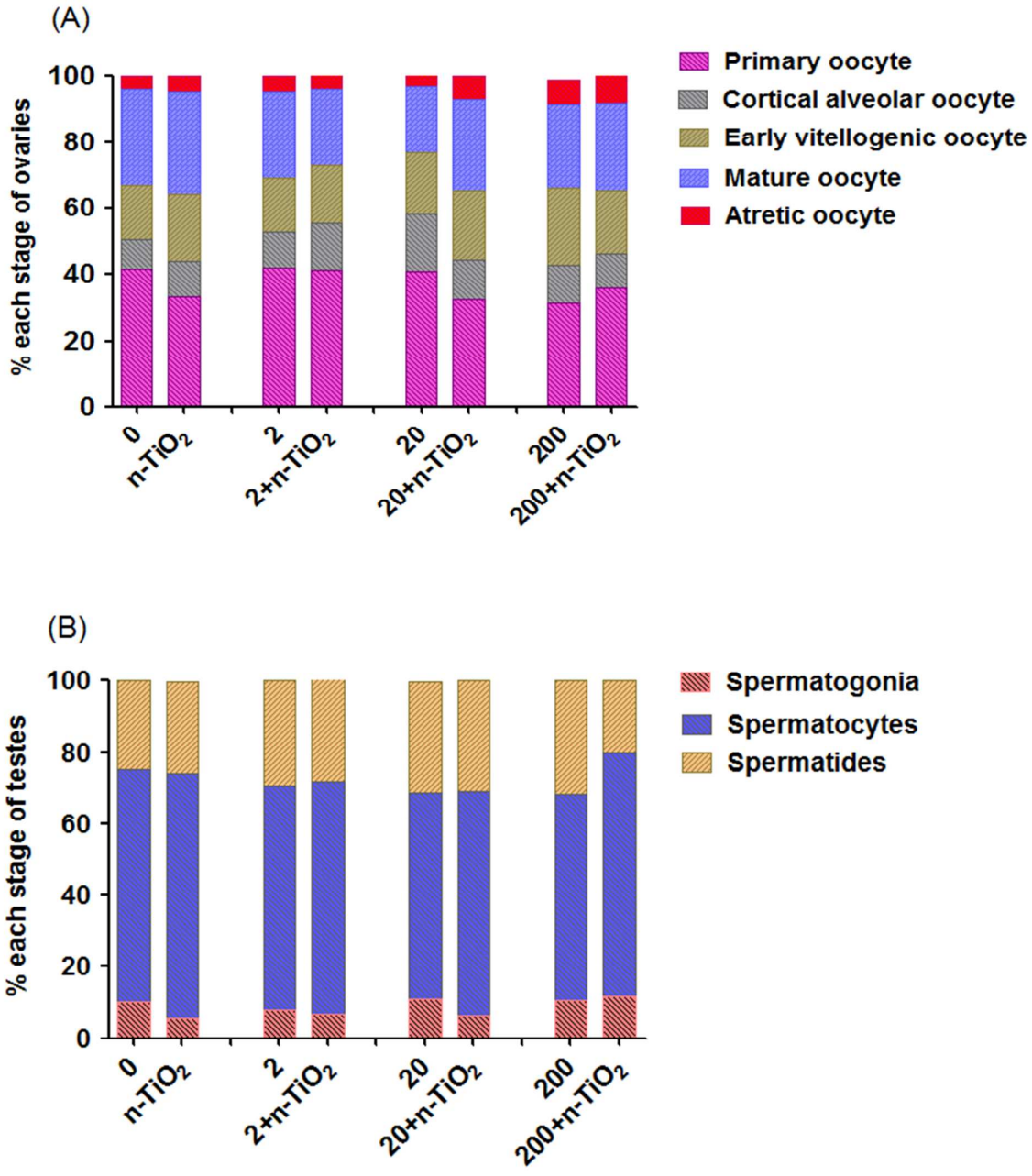

Figure S5. Cumulative number of eggs spawned per female zebrafish during 7-dayspre-exposure period in different concentrations of BPA (0, 2, 20, and 200 µg/L) with and without 100 µg/L n-TiO_2. Data are the mean values (mean ± SEM) per female in each treatment group (n = 3 replicate tanks). There was no significant difference among all groups.

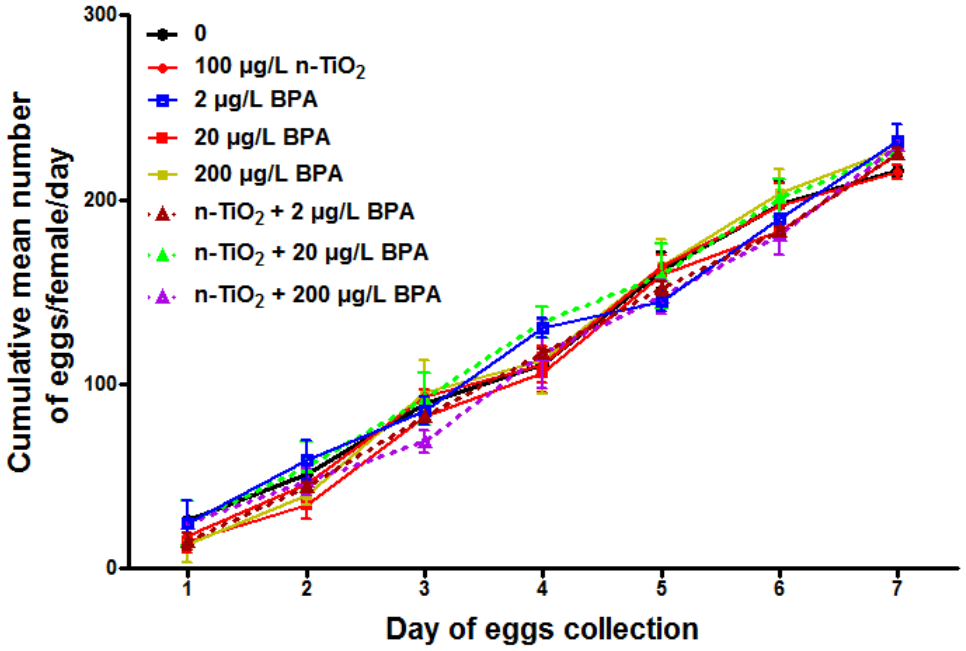

Table S1. Somatic index (HSI, BSI and GSI) of zebrafish after exposure to BPA (0, 2, 20, and 200 μg/L) or/and presence of 100 μg/L n-TiO_2 for 21d. The values represent mean ± standard error (SEM) of 3 replicates tanks (each replicate contained 6 individual fish). Significance differences ($P < 0.05$) are indicated by: * vs DMSO control; # vs the corresponding BPA groups without n-TiO_2.

Group (μg/L)	Female			Male		
	HSI	BSI	GSI	HSI	BSI	GSI
SC	3.5±0.3	1.1±0.1	20.3±3.2	1.3±0.1	1.4±0.1	1.3±0.1
BPA-2	3.3±0.3	1.1±0.2	20.2±2.3	1.2±0.1	1.3±0.1	1.3±0.1
BPA-20	3.1±0.4	1.1±0.2	16.1±1.0	1.3±0.1	1.3±0.1	1.1±0.1
BPA-200	3.4±0.5	1.0±0.1	13.2±0.5*	1.3±0.2	1.3±0.3	1.2±0.2
n-TiO_2-100	3.4±0.2	1.1±0.1	18.1±1.8	1.3±0.1	1.3±0.1	1.4±0.2
Comb-1	3.2±0.3	1.1±0.2	17.9±1.8	1.1±0.2	1.3±0.2	1.4±0.1
Comb-2	2.9±0.1	0.9±0.1	15.6±2.0	1.1±0.2	1.1±0.1	1.3±0.0
Comb-3	2.5±0.2*	0.7±0.1*#	12.5±2.1*	1.0±0.0	1.1±0.0	1.3±0.1

SC: Solvent control; BPA-2: 2 μg/L BPA; BPA-20: 20 μg/L BPA; BPA-200: 200 μg/L BPA; n-TiO_2-100: 100 μg/Ln-TiO_2; Comb-1: 2 μg/L BPA+ 100 μg/Ln-TiO_2; Comb-2: 20 μg/L BPA+ 100 μg/Ln-TiO_2; Comb-3: 200 μg/L BPA+ 100 μg/Ln-TiO_2.

Hepatosomatic index (HSI) = liver weight x100/body weight

Brainosomastic index (BSI) = brain weight x100/body weight

Gonadal somatic index (GSI) = gonad weight × 100/body weight.