Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Parental transfer of titanium dioxide nanoparticles aggravated MCLR-induced developmental toxicity in zebrafish offspring

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Table S1.

Sequences of primers for the tested genes related to the neurotransmitters and neurodevelopment.

Gene name	Sequence of the primer (5'- 3')	Gene bank accession No.
gapdh	Forward: ctggtgacccgtgctgctt Reverse: tttgccgccttctgcctta	NM_001115114
P53	Forward: gggcaatcagcgagcaaa Reverse: actgaccttcctgagtctcca	AF365873
Bax	Forward: ggctatttcaaccagggttcc Reverse: tgcgaatcaccaatgctgt	AF231015
Bcl-2	Forward: tcactcgttcagaccctcat Reverse: acgctttccacgcacat	NM001030253
Bid	Forward: atgggacagtggtgcagtttt Reverse: aatctttcacttctctaactgctcaaca	NM_001079826
Caspase-3	Forward: atgctggagaaacatgccatgcag Reverse: agggtgtttacttgggcctgaaga	NM131877
Caspase-8	Forward: tgctgcatcagaagaactaa Reverse: cctccggtttgattccatc	NM_131340
Caspase-9	Forward: gaagacggcgaaatcgatgc Reverse: ctggcggttctgacaacttcc	NM15288408
Puma	Forward: tggaaagcagagtggacgaa Reverse: gatggcagggctggatga	NM001045472
Apaf-1	Forward: ttctacagtaaacgcccacc Reverse: tatctagtatttccccatattcc	AF251502
Mdm2	Forward: aagcagtgatcctgagagtcc Reverse: atccgaagactcgctgttc	BC096975

Table S2.

The content of MCLR in exposure water (before and after water renewal).

samples	[a]exposure water (μg/L)		[b]exposure water (μg/L)	
μg/L	**MCLR**	**MCLR+ nTiO$_2$**	**MCLR**	**MCLR+ nTiO$_2$**
0	ND	ND	ND	ND
0.5	0.48±0.0099	0.49±0.013	0.39±0.0074	0.20±0.0052
4	3.9±0.011	3.9±0.08	2.87±0.049	1.9±0.16
32	30±0.59	30±0.80	23±0.32	15.7±0.12

[a] represents the content of MCLR after water renewal.

[b] represents the content of MCLR before water renewal.

ND, Not detected. WW, Wet weight.

Table S3

The expression of apoptosis relevant genes of F1 zebrafish larvae derived from parental exposure to MCLR and co-exposure of MCLR and n-TiO_2.

Concentrations	**0**		**0.5**		**4**		**32**	
Gene[a]	MCLR	MCLR+n-TiO_2	MCLR	MCLR+n-TiO_2	MCLR	MCLR+n-TiO_2	MCLR	MCLR+n-TiO_2
P53	1.03±0.28	1.25±0.20	1.13±0.10	1.00±0.38	1.13±0.10	1.51±0.08*	1.13±0.10	1.94±0.13*
Bax	1.01±0.14	1.02±0.05	1.04±0.08	1.24±0.42	1.18±0.22	1.95±0.24*	1.27±0.03	1.79±0.38*
Bcl-2	1.00±0.09	1.03±0.04	1.01±0.17	1.25±0.32	1.47±0.67	2.2±0.25*	1.41±0.07	1.71±0.19*
Bid	1.02±0.22	1.01±0.06	1.02±0.17	1.57±0.57*	1.37±0.58	2.17±0.13*	1.39±0.15	1.91±0.60*
Caspase-3	1.01±0.17	1.03±0.56	1.12±0.09	1.14±0.50	1.06±0.20	2.16±0.78*	1.37±0.15	2.54±0.37*
Caspase-8	1.01±0.19	1.34±0.17	1.02±0.90	1.04±0.30	1.40±0.35	3.54±0.25*	1.12±0.20	2.55±0.13*
Caspase-9	1.01±0.13	1.39±0.16	1.05±0.24	1.75±0.11*	1.41±0.02	3.69±0.22*	1.12±0.12	2.34±0.49*
Puma	1.04±0.33	1.02±0.14	1.03±0.16	1.19±0.23	1.25±0.18	1.95±0.16*	1.34±0.39	2.37±0.73*
Apaf-1	1.06±0.04	1.31±0.16	1.34±0.19	1.60±0.27*	1.84±0.49*	1.99±0.60*	1.31±0.13	2.14±0.33*
Mdm2	1.00±0.02	0.94±0.49	1.01±0.13	0.73±0.15	0.98±0.40	0.88±0.21	0.65±0.25	0.45±0.08*

[a] Values represent means ± SEM of 3 replicates of each treatment and are expressed as fold change.

* Significant differences at $P<0.05$ between MCLR treated groups and the control group.

Test S1

The examination of the swimming behavior in F1 larvae

The quantification of larval locomotor activity at 120 hpf was according to Huang et al. (2010) with a Video-Track system (ViewPoint LifeSciences, Montreal, Canada). Larvae that were dead or deformity could not be elected in this assay. The larvae were plated into 24-well plate. Each larva was put into one well. To eliminate the difference of every 24-well, we used 4 plates to measure locomotor behavior. Before monitoring the swimming speed, the larvae were acclimated for 10 min and the 24-wells were kept at 28℃. The swimming speed was monitored under continuous visible light and response to dark-to-light transition (5 min light, 5 min dark, 5 min light, 5 min dark). The data (frequency of movements, distance traveled, and total duration of movements) was collected every 30 s and each experiment was repeated 4 times. Further analysis of the data was using custom Open Office Org 2.4 software.

Test S2

RNA extraction and cDNA synthesis

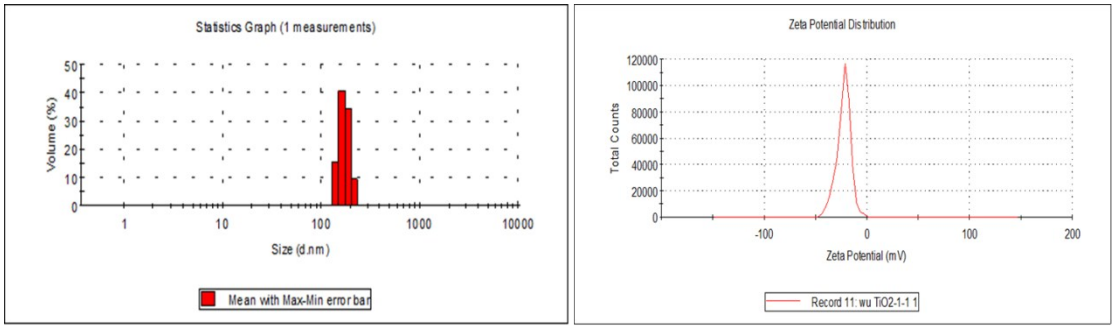
Briefly, total RNA was isolated by use of TRIzol regent, and digested by RNase-free DNase I (Promega, WI, USA) following the manufacturer's instructions. Concentrations of total RNA were estimated at 260 nm and the quality was verified by measuring the 260/280 nm ratio. One percent agarose-formaldehyde gel electrophoresis with ethidium bromide staining was used to further verify the quality of total RNA. The cDNA was synthesized using 2 μg of RNA from each sample mixed with 500 ng of random primer (Takara, Kyoto, Japan), 10 mmol/L of dNTPs and diethylpyrocarbamate-(DEPC-) treated water to a final volume of 20 μL. The reaction was

initiated using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) following the manufacturer's protocols.

Test S3

Western blot analysis

One hundred zebrafish larvae were collected and washed twice with PBS (pH 7.4) and then homogenized in ice-cold protein extraction buffer to extract proteins (Wuhan Boster Biological Technology, China). Each set of 150 larvae was pooled for protein preparation, such that n = 1 refers to protein from these 150 embryos. The homogenates were centrifuged for 10 min at 12,000 × g and supernatants were collected. Protein concentrations were determined by the Bradford method[33]; western blot analysis was performed with minor modifications following the previous method[22]. About 20 mg of protein from each sample was denatured, electrophoresed, and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked and blots were incubated in specific antibody against p53, Bax, Bcl-2, and GAPDH (Abcam, UK), and then secondary antibodies following the manufacturer's instructions. The NBT/BCIP system was used to evaluate the protein signal. The results of the western blots were quantified with Gene Snap software (Syngene, America).



(A) **(B)**

Fig. S1. The particle size of n-TiO_2 suspensions (A) and te distribution of particle size (n-TiO_2) (B) in water were shown.