**Supporting Information**

**Distinct toxic interactions of TiO2 nanoparticles with four co-existing organochlorine contaminants on algae**

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**Compositions of the OECD medium**

Algal cell culture medium recommended by OECD is composed of ultrapure water and the following ingredients (mg/L): MgCl2·6H2O 12, NH4Cl 15, CaCl2·2H2O 18, KH2PO4 1.6, MgSO4·7H2O 15, FeCl3·6H2O 0.08, Na2EDTA·2H2O 0.1, H3BO3 0.185, MnCl2·4H2O 0.415, ZnCl2 3×10-3, CuCl2·2H2O 10-5, CoCl2·6H2O 1.5×10-3, Na2MoO4·2H2O 7×10-3, NaHCO3 50.

**Methods to determine concentrations of aqueous and algal accumulated OCs**

Twenty mL of algal suspensions were centrifuged at 4000 *g* for 15 min to separate the bioaccumulated OCs in the precipitated algal cells and the residual OCs in the supernatants. For atrazine, 0.5 mL of the supernatants and 0.5 mL of methanol were mixed for the analysis using high efficiency liquid chromatography (HPLC) (LC-20A, Shimadzu, Japan) after filtration through 0.22 μm polytetrafluoroethylene (PTFE) filter. The harvested cells were mixed with 3 mL of dichloromethane and sonicated (600 W, 40 kHz, 25 °C) for 15 min. The extracts were separated from leavings by centrifugation (4000 *g*, 15 min); the leavings were eluted twice using 1 mL of dichloromethane, and the washing solutions were added to the initial extract. The final extract was evaporated to dryness in water bath (40°C), and then 2 mL of methanol was added, sealed, and shaken in dark for 3 min (150 rpm, 25 °C); 0.5 mL of the obtained methanol solution and 0.5 mL of deionized water were mixed for the HPLC analysis after the filtration. The HPLC was fitted with a hypersil BDS C18 column (150 mm length, 4.6 mm i.d., and 5 μm phase thickness). The mobile phase was methanol:water (70:30, v/v) with a flow-rate of 1 mL/min. UV detection was carried out at a wavelength of 225 nm.

For PeCB, HCB or PCB-77, the supernatants were extracted twice using 2.5 mL of n-hexane and the harvested cells were blended with 5 mL of n-hexane and sonicated (600 W, 40 kHz, 25 °C) for 15 min. The extracts of the supernatants and cells were dewatered using anhydrous sodium sulfate (after baking at 450 °C for 4 h). The anhydrous sodium sulfate was washed twice using 2 mL n-hexane, and the wash solutions were mixed into the initial extract. The final volumes of extracts were adjusted to 10 mL with n-hexane and 1 mL was taken for analysis using a gas chromatograph (GC, 7693, Agilent, USA). The GC was fitted with an HP-5 capillary column (30 m length, 0.32 mm i.d., and 0.25 μm phase thickness, 5% phenyl - 95% methyl siloxane). The detector was an electron capture detector (ECD), and nitrogen was used as the carrier (flow rate: 2.0 mL/min) and as the make-up gas (flow rate: 25.0 mL/min). The injection port was held at 300 °C and used in the split-less mode with split-less time of 0.50 min. The oven temperature was held at 120 °C for 1 min following injection and was then raised at the rate of 10 °C/min to 200 °C, from 200 °C to 240 °C at the rate of 2 °C/min, from 240 °C to 290 °C at the rate of 10 °C/min, and held there for 2 min.

**Methods to separate and quantify the bioaccumulated TiO2NPs in algae and free TiO2NPs in the culture medium**

After the exposure, algae-TiO2NPs complexes were separated from free TiO2NPs using the density equilibrium method (Perreault et al., 2012; Eroglu et al., 2009) with minor modifications. TiO2NPs in the complexes were considered to be bioaccumulated (cell surface bound or internalized) by the algal cells. Different mass percent concentrations (20%, 40%, 60%, 80%, 100% and 120%) of sucrose solutions were prepared in the OECD medium. Three mL of each sucrose solutions were slowly put in inclined 22 mL vials according to the gradient descent of density from bottom to top. After settling for 6 h, the algae-TiO2NPs mixtures collected after the centrifugation of the exposure suspensions were slowly placed on top of the sucrose gradient. The algae-TiO2NPs complexes and the free TiO2NPs were clearly separated by centrifugation (1000 *g*, 20 min). The algae-TiO2NPs complexes accumulated at the downside of the vial, while the free TiO2NPs remained at the upside layers.

The algae-TiO2NPs complexes were carefully collected into 15 mL glass tubes, and treated with a modified method (Zhu et al., 2010; Zhang et al., 2007). The samples were digested using pure HNO3 at 120°C for 2 h, and then digested by heating with 5 mL digestion solution (400 g ammonium sulphate were added into 700 mL pure H2SO4 and heated to completely dissolved). After the treatment, the TiO2NPs accumulated by algae were dissolved to titanium (IV) ions and were then determined using a graphite furnace atomic absorption spectrophotometer (AA-700, PerkinElmer, USA) with hollow cathode lamp for Ti. The graphite tube used was pyrolytic coated graphite tube, argon was used as the carrier gas, the wavelength was 364.3 nm, the slit width was 0.2 nm, the electric current of lamp was 40 mA, and the injection volume was 20 μL. Each batch of samples had parallel recovery experiments of standard addition, and the recovery rates are shown in Table S1.

**References cited:**

Perreault F, Oukarroum A, Melegari SP, Matias WG, Popovic R.2012. Polymer coating of copper oxide nanoparticles increases nanoparticles uptake and toxicity in the green alga *Chlamydomonas reinhardtii*. Chemosphere 87(11): 1388-1394.

Eroglu E, Melis A. 2009. "Density equilibrium" method for the quantitative and rapid in situ determination of lipid, hydrocarbon, or biopolymer content in microorganisms. Biotechnol Bioeng102(5): 1406-15.

Zhu XS, Chang Y, Chen YS. 2010. Toxicity and bioaccumulation of TiO2 nanoparticle aggregates in *Daphnia magna*. Chemosphere78(3): 209-215.

Zhang XZ, Sun HW, Zhang ZY, Niu Q, Chen YS, Crittenden JC. 2007. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. Chemosphere 67(1): 160-166.

**Table S1. Selected physicochemical properties and recovery rates of TiO2NPs and OCs**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Purity (%) | lg*K*owa | Recovery rate (%) | Providers |
|  | In medium | In algae |
| TiO2NPs | 98% | NDb | ND | 97.3 ± 1.1 | Zhejiang Hongsheng Material Technology Co. (China) |
| atrazine  | 97% | 2.65 | 95.7 ± 1.3 | 96.4 ± 6.6 | J&K scientific |
| PeCB | 99.9 | 5.18 | 93.2 ± 4.1 | 99.3 ± 6.8 | Sigma-Aldrich |
| HCB | 99.5 | 5.80 | 97.4 ± 2.3 | 101 ± 3 | Dr. Ehrenstorfer GmbH (Germany) |
| PCB-77 | 99% | 6.50 | 95.3 ± 3.7 | 101 ± 6 | Accustandard (USA) |

a *K*owstands foroctanol-water partition constant, which are from Hansch et al. (1991) and Schwarzenbach et al. (2002). b ND means not determined.

**References cited:**

Hansch C, Leo A, Taft RW. 1991. [A survey of Hammett substituent constants and resonance and field parameters. Chem Rev 91: 165-195.](http://pubs.acs.org/doi/abs/10.1021/cr00002a004)

Schwarzenbach RP, Gschwend PM, Imboden DM. 2002. Environmental organic chemistry, second edition. John Wiley & Sons Inc.

**Table S2. The predicted and observed 96 h-IC50 (normalized concentrations) of binary mixtures**

|  |  |  |  |
| --- | --- | --- | --- |
| Mixtures | Predicted by CA | Predicted by IA | Observed |
| Atrazine+TiO2NPs | 0.601±0.016b | 0.783±0.027a | 0.322±0.012c |
| PeCB+TiO2NPs | 0.772±0.016b | 0.880±0.048a | 0.793±0.063ab |
| HCB+TiO2NPs | 0.840±0.031c | 0.943±0.057b | 1.07±0.05a |
| PCB-77+TiO2NPs | 0.923±0.027c | 0.992±0.043b | 1.12±0.02a |

Note: Values with different letters (a–c) differ significantly (*p*<0.05).

 

**(A)**

**(B)**

**Fig. S1. Algal accumulation amounts of OCs with and without TiO2NPs (A) and of TiO2NPs with and without OCs (B) after culturing for 24 h. The initial concentrations of TiO2NPs, atrazine, PeCB, HCB, and PCB-77 were 16, 0.208, 0.528, 0.006, and 0.008 mg/L, respectively. The control in panel B stands for the treatment of TiO2NPs in the absence of OCs. The error bars represent standard deviations (n=3). Values with different letters (a**–**g) differ significantly (*p*<0.05).**