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

Supplementary Information for

Influence of organic colloids on uptake, accumulation and effects of benzophenone-3 in aquatic animals

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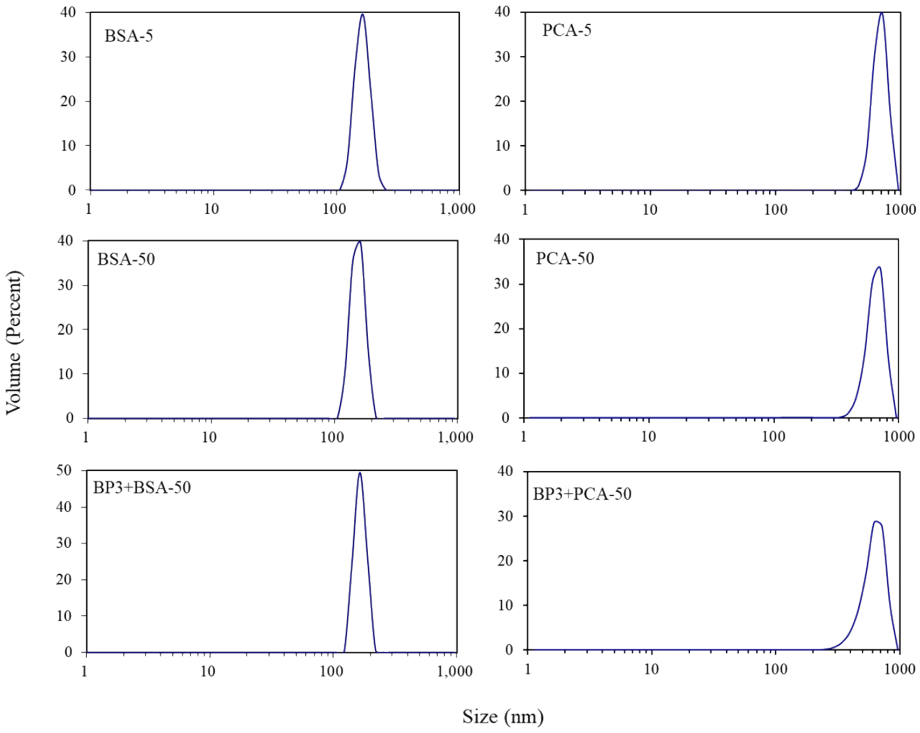

Fig. S1. Hydrodynamic diameter distribution of colloids in different groups.

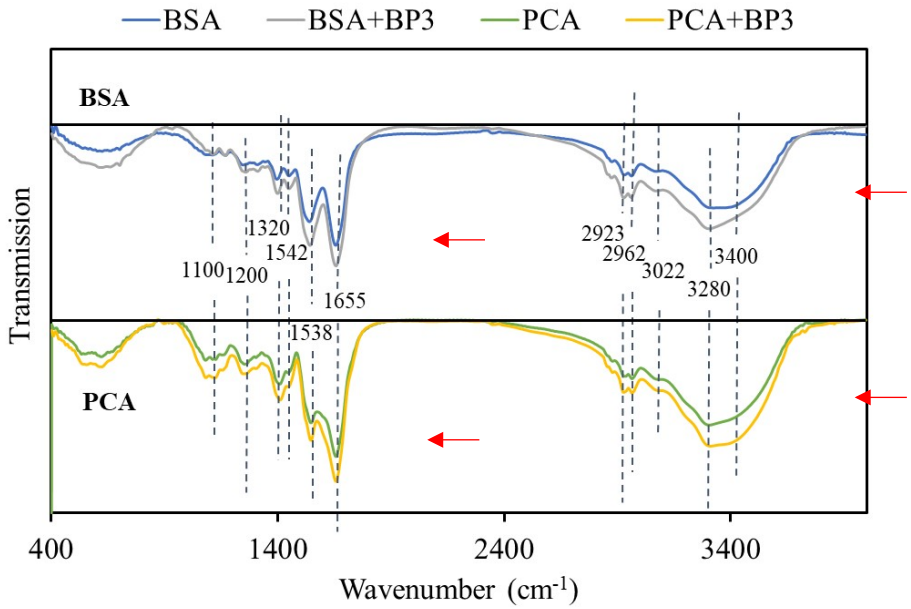

Fig. S2. Fourier transform infrared spectroscopy (FTIR) spectra (cm^{-1}). The red arrow indicated different intensity shift in two protein colloids groups.

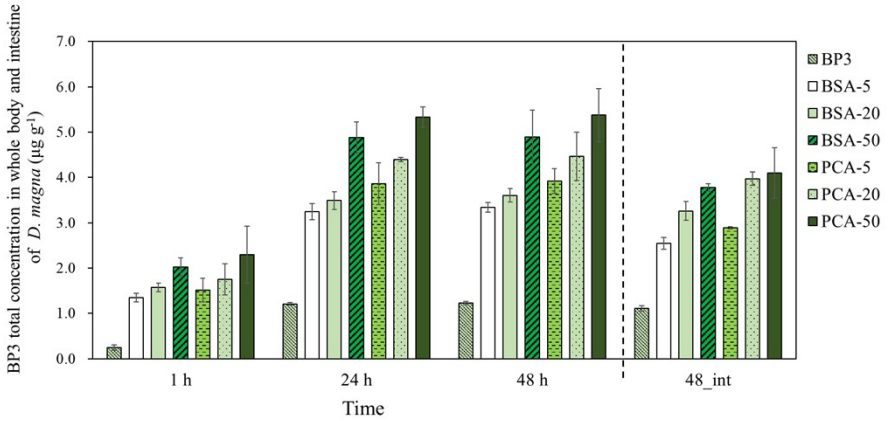

Fig. S3. BP3 concentrations in the intestine of zebrafish and the whole body of daphnids. The significant difference was analyzed among the groups exposed for 24 h and 48 h. 48_int represents the BP3 concentration in the intestine (C_{gut}) of daphnids after exposure of 48 h.

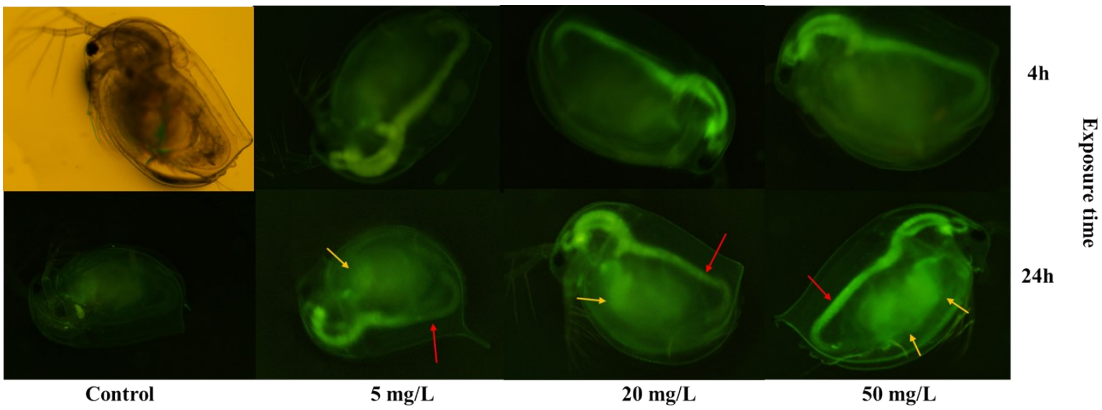

Fig. S4. The fluorescence image of daphnids in different concentrations of PCA matrix (The red and yellow arrows indicate the fluorescence intensity difference in intestine and other parts of the body, respectively). The brightness of all pictures has been increased by 50% to identify more clearly. The fluorescence of control may come from the residue of green algae, the food for daphnids.

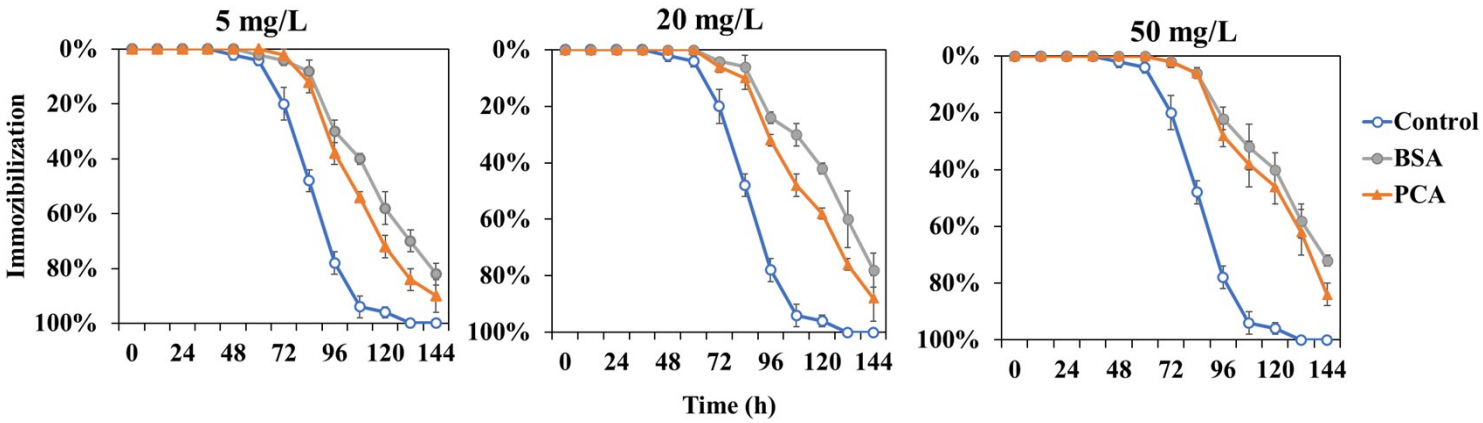

Fig. S5. The immobilization rate of daphnids in different concentrations of protein colloid matrix.

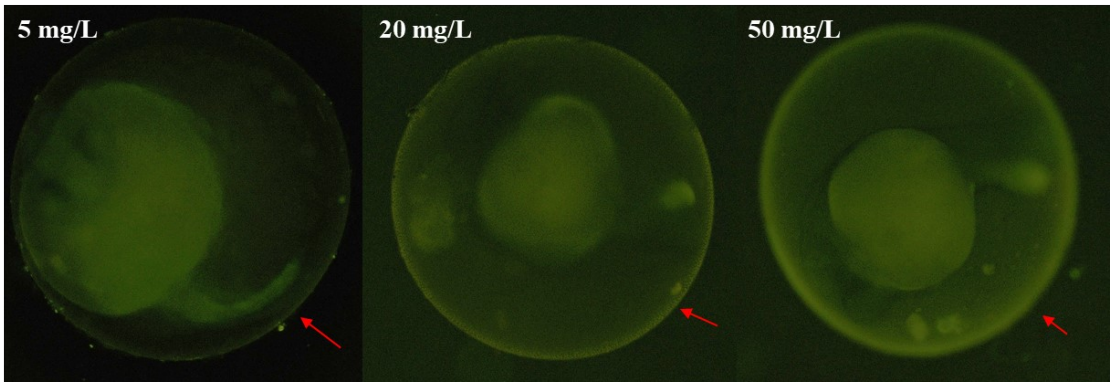

Fig. S6. The fluorescent image of 24 hpf embryos exposed for 4 h in different concentration PCA matrix. To identify more clearly, the brightness of figures has been increased by 200%. No picture of PCA attached to the surface of daphnids due to the interference of high fluorescence in intestine.

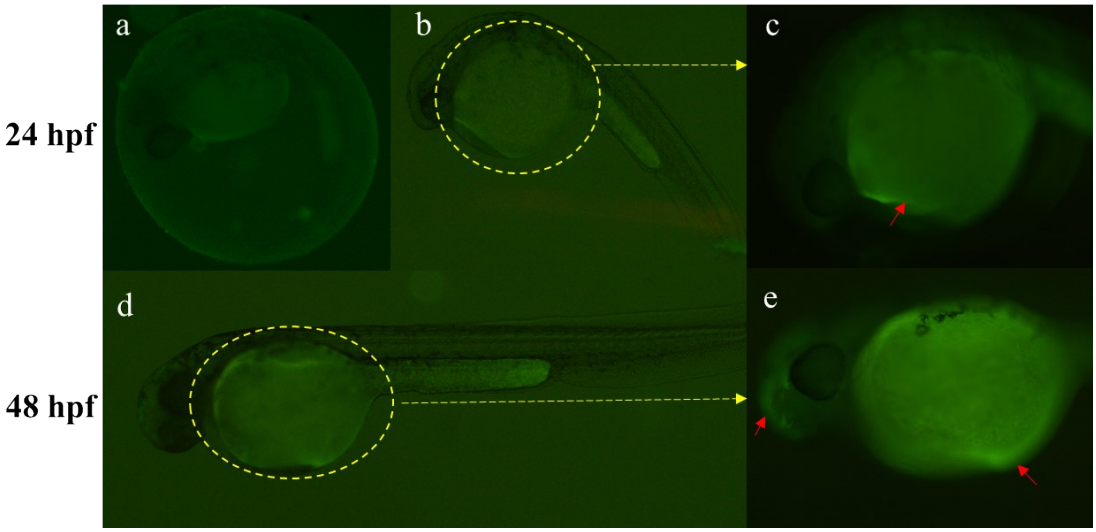

Fig. S7. The fluorescent image of by zebrafish embryos exposed for 4 h in PCA matrix at a concentration of 50 mg L^{-1}. The red arrow indicated the main accumulation site, yolk sac and brain. To identify more clearly, the brightness of figure a has been increased by 200%, b and d have been increased by 300%, c and e have been increased by 100%.

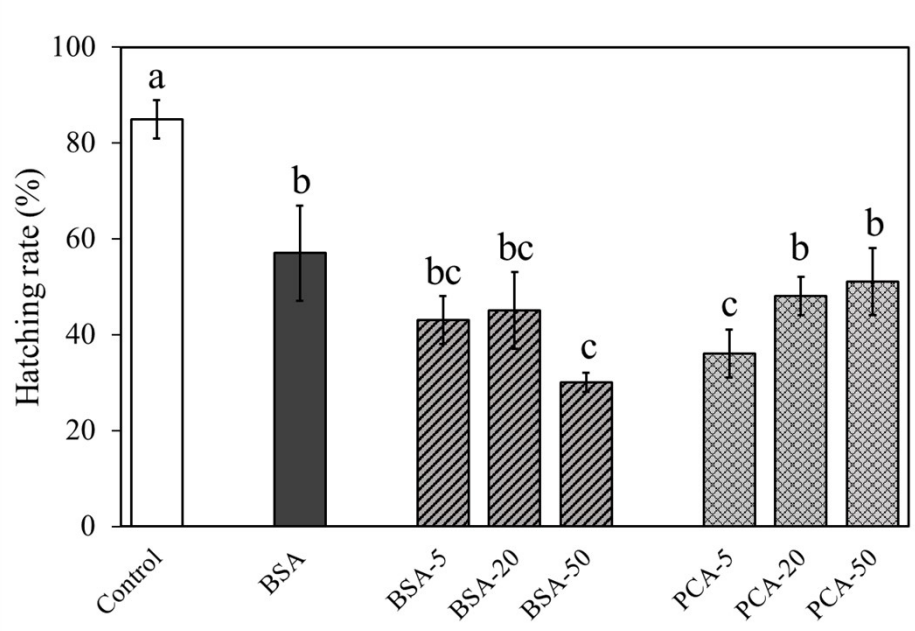

Fig. S8. The hatching rates of 24 hpf zebrafish embryo at 48 hpf in different matrix. Different letters express significant differences ($p < 0.05$). The numbers after protein presents the concentration of protein matrix (mg L^{-1}).

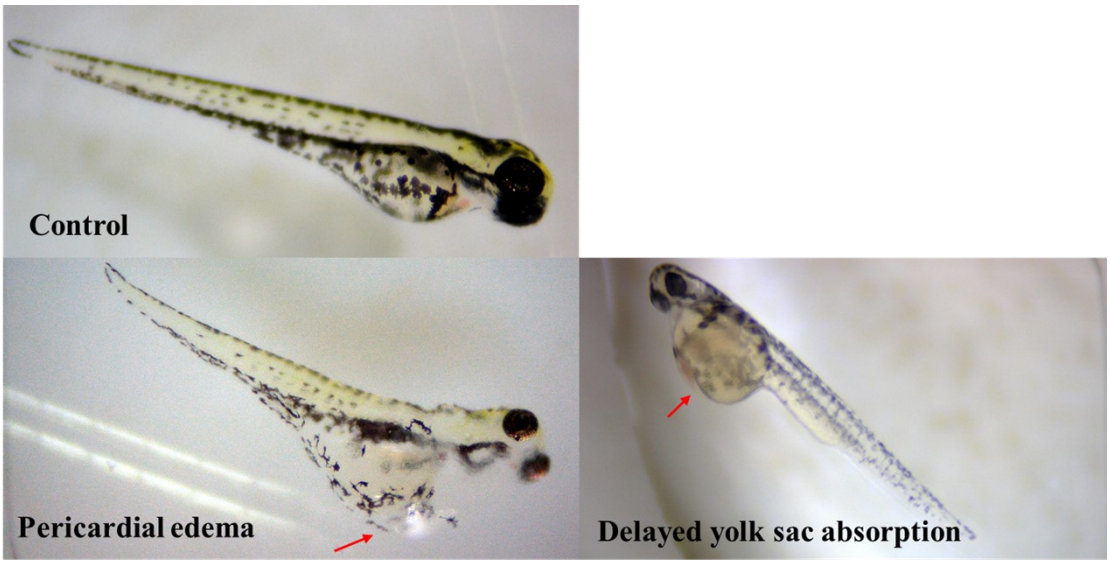

Fig. S9. Malformation image of juvenile zebrafish at 72 hpf in the exposure groups.

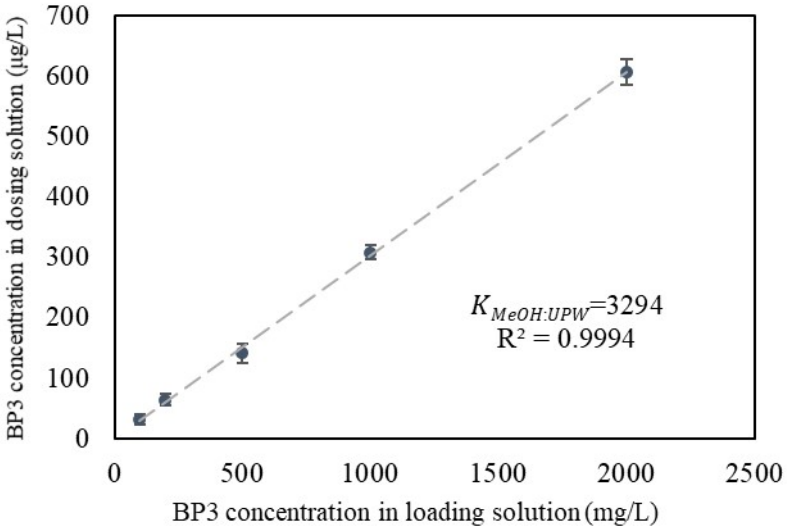

Fig. S10. The partition coefficient ($K_{MeOH:UPW}$) and the linear regression between the BP3 concentration in methanol loading solution and ultrapure water dosing solution.

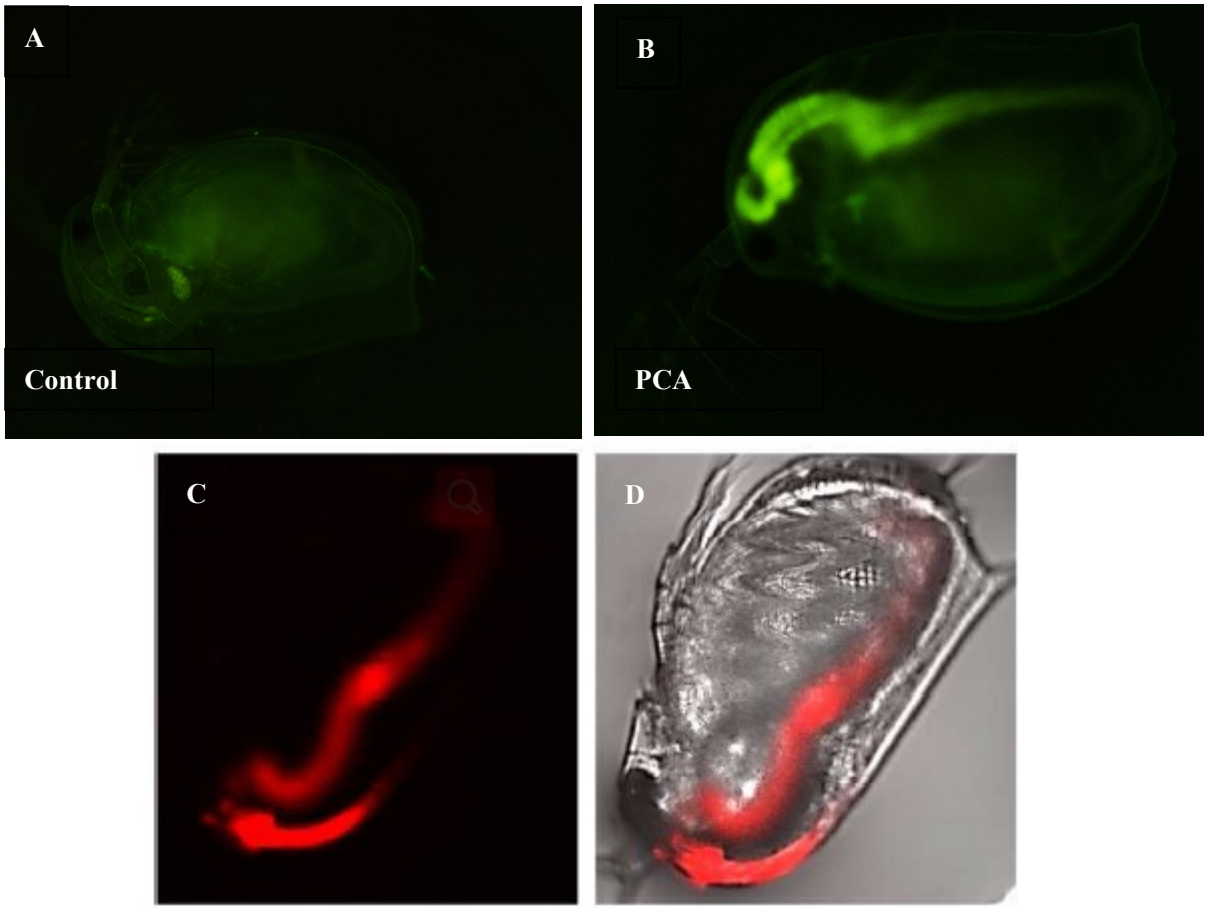

Fig. S11. The image of fluorescence microscope of control (A) and PCA (B) in this study; the labeled BSA (C and D) under laser scanning confocal microscopy (cited from Xia et al[1]).

Table S1 Physico-chemical characteristics of BSA and PCA

Physico-chemical parameters	BSA[a]	PCA[b]
Average molecular weight （kDa）	66	120
pH (25 ℃, 2% in H_2O)	7	7
Origin	Bovine serum	Blue-green algae
Fatty acid content	< 0.05%	
Assay	≥ 98%	> 97%

[a] data provided by SIGMA, USA.

[b] data provided by Taizhou Binmei Biotechnology Co., Ltd, China.

Table S2 Primer sequences used for the quantitative reverse transcription-polymerase chain reaction

Function	Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Accession number
Reference	*18s rRNA*	ttgttggtgttgttgctggt	ggatgctcaacaggggttcat	NM_200713
HPT axis related genes	*tshβ*	gcagatcctcacttcacctacc	gcacaggtttggagcatctca	AY135147
	tshr	gctccttgatgtgtccgaat	cgggcagtcaggttacaaat	NM_001145763
	tg	ccagccgaaaggatagagttg	atgctgccgtggaatagga	XM_001335283
	dio1	gttcaaacagcttgtcaaggact	agcaagcctctcctccaagtt	BC076008
	dio2	gcataggcagtcgctcattt	tgtggtctctcatccaacca	NM_212789
	ttr	cgggtggagtttgacacttt	gctcagaaggagagccagta	BC081488
	thrα	ctatgaacagcacatccgacaagag	cacaccacacacggctcatc	NM_131396
	thrβ	tgggagatgatacgggttgt	ataggtgccgatccaatgtc	NM_131340
	nis	ggtggcatgaaggctgtaat	gcctgattggctccatacat	NM_001089391
	trh	cacacagatggaggagcaga	agcagcatcaggtagcgttt	NM_001012365
	trhr	ctggtggtggtcaactcctt	gctttccaccgttgatgttt	NM_001114688

Table S3 The actual detection concentration (μg L^{-1}) of BP3 in treatments

Treatments	Normal concentration (μg L^{-1})	Actual concentration (μg L^{-1})
BP3	100	106.58±1.12
BSA-5		96.52±2.19
BSA-20		97.83±2.38
BSA-50		104.26±0.12
PCA-5		106.33±1.11
PCA-20		103.15±3.01
PCA-50		102.21±4.23

Table S4 Symbols, meaning, units and source of parameter values.

Symbol	Meaning
$C_{B,t}$	BP3 concentration accumulated in biota (μg g^{-1} wet weight).
C_{free}	BP3 concentration in water (μg g^{-1}).
C_{matrix}	BP3 concentration in matrix (μg g^{-1}). Shown in Eq. 3.
C_{matrix}	BP3 concentration in the matrix in intestine (μg g^{-1}). Shown in Eq. 3.
$C_{lip,t}$	Lipid normalized concentration in biota (μg g^{-1} wet weight). Calculated by M_{lip} and $C_{B,t}$.
C_{B,t_0}	BP3 concentration in biota at the beginning (μg g^{-1} wet weight).
C_B^{eq}	BP3 concentration in biota at bioaccumulation equilibrium (μg g^{-1} wet weight)
C_{lip}^{eq}	Lipid normalized BP3 concentration in biota at bioaccumulation equilibrium (μg g^{-1} lipid). Calculated by M_{lip} and C_B^{eq}.
f_{lip}	Lipid fraction in biota (-)
GRT	Gut residence time (h). Obtained from Bakir et al.[2]
IR	Ingestion rate (μg g^{-1} h^{-1}). Obtained by Eq. S6.
K_P	Protein colloid-water partition coefficient (L kg^{-1}). See Table 1.
k_u	Rate constant for uptake from water (h^{-1}). Fitted by Eq. S5.
k_e	Elimination rate constant (h^{-1}). Fitted by Eq. S5.
k_1	Forward rate constant for the matrix to lipid transport (h^{-1}). Fitted by Eq. 6.
k_2	Backward rate constant for lipid to the matrix transport (h^{-1}). Fitted by Eq. 6.
m_{matri}	The intestinal uptake amount of matrix in daphnids (μg g^{-1} wet weight). Obtained by Eq. S7.
M_{matri}	The matrix mass in biota (μg g^{-1} wet weight). Obtained in model localization $\boldsymbol{M_{matrix}}$.
M_{lip}	The lipid mass in biota, measured as 6×10^3 μg g^{-1} wet weight by a traditional extraction method.
n_B^{eq}	The amount of BP3 accumulated in biota at bioaccumulation equilibrium (μg). Shown in Eq. S7.
n_{gut}^{eq}	The amount of BP3 stranded in the gut in a bound state at bioaccumulation equilibrium(μg). Measured by a comparison between daphnids exposure in the suspensions with and without complex matrix for a certain time. Shown in Eq. S7.
n_{total}^{eq}	Total amount of BP3 extracted from daphnids at bioaccumulation equilibrium (μg). Shown in Eq. S7.

Text S1

Dialysis bag experiment was conducted following the methods of Lin et al. [3] to investigate the binding of BP3 with protein. Before the experiment, the dialysis bag was cut into 8 cm length and placed in the boiling water for 5 min; then it was washed by hot deionized water (60 − 80 °C) and room temperature deionized water twice. A total of 20 mL of BSA and PCA stock solution with the concentrations of 5, 20, and 50 mg L^{-1} was filled in the dialysis bags, respectively. The bags were sealed and placed into 200 mL deionized water in 500 mL polypropylene beakers, respectively; they were shaken at 90 rpm in darkness for 72 h to remove the component with molecular weight less than 7000 Da. A preliminary experiment results showed that no substantial BSA or PCA could pass through the dialysis bag. The pretreated dialysis bags containing the protein solution were placed into beakers with 200 mL deionized water, and they were opened and added with certain amounts of BP3 solution, with the nominal BP3 concentrations of 100 μg L^{-1} in these dialysis bags, respectively. Then the dialysis bags were sealed and the beakers were shaken at 120 rpm in darkness at 21 °C for 72 h; a preliminary experiment showed that 48 h was enough for BP3 to reach the equilibrium between the protein inside of the dialysis bag and the water outside of the dialysis bag. A control experiment was conducted with dialysis bag but without protein. Each of the treatment had three replicates. After equilibrium, water samples were collected from the inside and outside of the dialysis bags, respectively for the determination of BP3. And the amount of BP3 adsorbed per unit mass of protein colloid and the partition between protein and water (K_p) could be calculated.

$$K_p = \frac{C_{matrix}}{C_w} \tag{S1}$$

C_w (μg L^{-1}) was the free dissolved concentration of each treatment, which was equal to the concentration of BP3 outside the dialysis bag. C_{matrix} (μg g^{-1}) was the matrix-bound concentration of BP3, which was calculated based on the difference between inside and outside the dialysis bag.

For desorption, the dialysis bag experiment was also conducted. After the adsorption equilibrium, saturated solution was taken in dialysis bag, which was placed in ultrapure water. After 24 hours, the desorption rate (%) could be calculated based on the difference between inside and outside the dialysis bag.

Text S2

The establishment of passive dosing dishes was based on the method of Xia et al[4]. PDMS pre-polymer and the catalyst (m:m, 10:1) were put in a plastic thick bag, and then were mixed homogeneous enough (12 min) with a glass rod. The corner of this plastic bag was sheared to obtain a small hole, and then a total of 12 g ±0.01 g of the mixture was squeezed out from the bag and put in a glass culture dish (diameter: 60 mm), which could hold 500 mL BP3 solution stable. These PDMS dishes were put into the vacuum freeze drier to eliminate air, and then were placed in an oven at 110 ℃ for 48 h to solidify the PDMS. After cooling to room temperature, the methanol was put to the PDMS dishes to remove their impurities. After 72 h, they were transferred to the ultrapure water and were rinsed for three times. Then the PDMS disk were put into different concentration loading solution for 72 h, and then place it in ultrapure water for 72 h to detect the BP3 concentration of the dosing solution.

For the loading procedure, the partition coefficient of BP3 between PDMS and methanol (MeOH) ($K_{PDMS:MeOH}$, L kg^{-1}) were calculated as follows:

$$K_{PDMS:MeOH} = \frac{C_{PDMS}}{C_{MeOH}} \quad \text{(S2)}$$

For the dosing procedure, the partition coefficient of BP3 between ultrapure water and PDMS ($K_{UPW:PDMS}$, L kg^{-1}) were calculated as follows:

$$K_{UPW:PDMS} = \frac{C_{UPW}}{C_{PDMS}} \quad \text{(S3)}$$

where C_{MeOH} is the BP3 concentration in the methanol loading solution (μg L^{-1}); C_{PDMS} is the BP3 concentration in PDMS (μg kg^{-1}); C_{UPW} is the BP3 concentration in ultrapure water (μg L^{-1}). Thus, the partition coefficients of BP3 between methanol and ultrapure water ($K_{MeOH:UPW}$) could be calculated with the following equation:

$$K_{MeOH:UPW} = \frac{C_{MeOH}}{C_{UPW}} = \frac{1}{K_{PDMS:MeOH} \cdot K_{UPW:PDMS}} \quad \text{(S4)}$$

The partition coefficient ($K_{MeOH:UPW}$) were obtained by linear regression between the BP3 concentration in methanol loading solution and ultrapure water were shown in Fig. S10.

Based on the value of $K_{MeOH:UPW}$, the concentration of BP3 in water can be deduced from methanol and vice versa[3]. Therefore, the freely dissolved BP3 concentration in the exposure system was detected by a piece of 1 cm PDMS fiber was put in each 120 mL exposure solution in conical flask. The flask, protected from light, was shaken at 120 rpm at 24 ℃ for 24 h. The fibers were taken out and dried with ashless paper, and then was immersed in 200 μL methanol at least 24 h. After that, the BP3 on PDMS was extracted by methanol, and the BP3 concentration in methanol was detected by UPLC-MS. And the accurate concentrations of BP3 in treatments could be calculated and shown in Table S3.

The rates (h^{-1}) of uptake (k_u) from water and loss (k_e)of the BP3 for daphnids were obtained by fitting the uptake phase data with this equation:

$$C_{lip,t} = \frac{k_u C_w}{k_u}(1 - e^{-k_e t}) \quad \text{(S5)}$$

In which, $$k_u = k_e \times \frac{C_B^{eq}}{C_w}$$

For the ingestion rate IR_t (μ g^{-1} h^{-1}) based on the method of Koelmans et al.[5]:

$$IR_t = 0.001 \times 0.435 \times (1000 \times W_t)^{0.771} \times f_{OM}^{-0.92} / W_t \quad \text{(S6)}$$

W_t is the weight (WW, g) of the organism as a function of time. f_{OM} indicates the organic matter content of ingested matrix, which is 1 in this study.

Since the protein colloids ingested by daphnids perform both the functions of food and carriers in intestine, it is necessary to figure out the protein colloids ingested by daphnids that play two functions in intestinal

According to Lin et al.[6], the total uptake amount of matrix in organisms can be calculated by:

$$C_M = \frac{C_{sol,i} - C_{sol,0}}{C_{matrix}} = \frac{n_{dap,i} - n_{dap,0}}{m_{matrix}} \quad \text{(S7)}$$

where C_M is the BP3 concentration in matrix; C_{matrix} is the concentration of matrix in a specific exposure suspension; $C_{sol,i}$ and $C_{sol,0}$ are the total concentrations of BP3 in exposure suspensions with and without matrix, respectively. The left side of the Eq. S3 equals to the concentration of BP3s accumulated on matrix. $n_{dap,i}$ and $n_{dap,0}$ are the amounts of BP3 accumulated in daphnids in exposure suspensions with and without matrix, respectively; m_{matrix} is the intestinal uptake amount of matrix in daphnids. Using the passive dosing method, the freely dissolved concentration of BP3 congener in each exposure vial was constant, so $(n_{dap,i} - n_{dap,0})$ accounts for the uptake of matrix-bound BP3. Therefore, the right side of Eq. S3 equals to the concentration of BP3 accumulated on the uptake matrix. Using this equation, the intestinal uptake amount of matrix (m_{matrix}) can be quantified.

Because PCA itself is fluorescent, from the fluorescence microscope (Fig. S11 A and B), the amount of matrix in intestine is far greater than that in other parts of the body. In the study of Xia et al.[1], the labeled BSA was observed under laser scanning confocal microscopy (Fig. S11 C and D), and also only a small part of new small molecule protein fragments may directly enter the other parts of daphnids except intestine, that is to say the matrix ingestion and matrix uptake pathway (Fig. 1), the amount of matrix effectively absorbed by daphnids is far less than the total amount of matrix in intestine. Therefore, the calculated total matrix amount can be regarded as the total amount of matrix in intestine.

References

1. X. Xia, A. H. Rabearisoa, X. Jiang and Z. Dai, Bioaccumulation of Perfluoroalkyl Substances by Daphnia magna in Water with Different Types and Concentrations of Protein, *Environmental Science & Technology*, 2013, **47**, 10955-10963.
2. A. Bakir, I. A. O'Connor, S. J. Rowland, A. J. Hendriks and R. C. Thompson, Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life, *Environmental Pollution*, 2016, **219**, 56-65.
3. H. Lin, X. Xia, X. Jiang, S. Bi, H. Wang, Y. Zhai, W. Wen and X. Guo, Bioavailability of pyrene associated with different types of protein compounds: Direct evidence for its uptake by Daphnia magna, *Environmental science & technology*, 2018, **52**, 9851-9860.
4. X. Xinghui, L. Husheng, Y. Zhifeng, Z. Xiaotian and W. Haotian, How does predation affect the bioaccumulation of hydrophobic organic compounds in aquatic organisms?, *Environmental Science & Technology*, 2015, **49**, 4911-4920.
5. A. A. Koelmans, E. Besseling, A. Wegner and E. M. Foekema, Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis, *Environmental Science & Technology*, 2013, **47**, 7812-7820.
6. W. Lin, R. Jiang, X. Xiao, J. Wu, S. Wei, Y. Liu, D. C. G. Muir and G. Ouyang, Joint effect of nanoplastics and humic acid on the uptake of PAHs for Daphnia magna: A model study, *Journal of Hazardous Materials*, 2020, **391**.