Supporting Information

Quantifying Bioavailability of Pyrene Associated with Dissolved Organic Matter of Various Molecular Weights to *Daphnia magna*

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Supporting information includes:

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5 tables

9 figures

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Fractionation of Dissolved Organic Matter (DOM) by Ultrafiltration

Dissolved organic matter solutions were prepared as follows: The NaOH solution (1 mol L^{-1}) was added dropwise into the beaker containing a certain amount of the fulvic acid and humic acid respectively until the solid powder was dissolved completely. Subsequently, the solutions were transferred to ultrapure water and the pH value was adjusted to 7.00±0.10. After three days, the supernatant was filtered with 0.45 μm membrane (Millipore) to remove particulate matter, and the prepared solutions of fulvic acid and humic acid were mixed at the ratio of 1:1 in volume. Then the DOM solution was fractionated into five fractions with various molecular weights by a Millipore Mini Pellicon ultrafiltration (UF) system (Billerica, MA), which was equipped with UF membranes with nominal molecular weight cutoffs of 10 000, 5 000, 3 000 and 1 000 Da. Specifically, UF membranes should be rinsed with ultrapure water (at least 3 L) before use, the pressure of inlet (Pin) was 15 psi, and the pressure of out (Pout) was 5 psi. In order to eliminate the organic carbon on the membrane originated from pyrogen and the residue of before, the UF membrane was rinsed with 0.01 mol L^{-1} NaOH solution for 20 min (Pin: 15 psi, Pout: 5 psi). Then the UF membrane was rinsed with ultrapure water again until the PH value of the outflow was approximately 7.0. The UF experiment was conducted from high molecular weight (10 000 Da) to low molecular weight (1 000 Da) to reduce the influence of concentration polarization of macromolecular organic matter on the UF procedure. The different DOM fractions received from UF experiment mentioned above were F_1 (>10 000 Da), F_2 (5 000-10 000 Da), F_3 (3 000-5 000 Da), F_4 (1 000-3 000 Da) and F_5 (<1 000 Da), and the unfractionated DOM was expressed as F_0. After analysis of the DOC concentrations of F_0-F_5, the recovery rate of organic carbon was calculated to be 80.52% (Table S1). The photograph of F_0-F_5 solution was shown in Figure S1. The color of DOM solution with various molecular weights became darker with the increasing molecular weight of DOM, which was accordance with the other research.[1]

Characterization of DOM with Various Molecular Weights

The molecular weight of each fraction was characterized by gel permeation chromatography (GPC) (Agilent Technologies). And other instrumental conditions were as follows: TSK gel: G3000PWXL, Column No.: S0127, Detector: UV254 nm, Temperature: 30 °C.[1] As shown in Figure S2, the GPC graph of the DOM fractions (F_2-F_5) was depicted, whereas the graph of F_1 was not detected because the wide molecular weight and the weak ultraviolet absorption at 254 nm, the similar reports that the signal of compounds with higher molecular weight are weaker compared to compounds with lower molecular weight were found in other research.[2,3] According to the main peak of GPC graph and standard curves, the average apparent molecular weight was calculated which are summarized in Table S2. The measured apparent molecular weights were in accordance with the theoretical molecular weights (F_2-F_5).

Preparation of Passive Dosing Dishes

PDMS pre-polymer and the catalyst obtained together were placed (m:m, 10:1) in a plastic valve bag, and then was stirred for 10 min by glass rod to obtain homogeneous mixture. Then a total of 12 g ± 0.01 g of the mixtures were placed to a 60 mm-diameter glass culture dish, which could maintain 500 mL water.[4] The dishes were placed into a vacuum freeze drier to remove air. For the curing of these dishes, they were laid out on the middle layer of an oven at 110 °C for 48 h. In order to eliminate impurities of PDMS dishes, the methanol was added to these cured dishes, and then they were taken out after 72 h and rinsed with the ultrapure water three times.

Determination of $K_{MeOH:AFW}$ and $K_{PDMS:AFW}$

A series of pyrene solutions in methanol (0, 0.3, 2.0, 3.0, 4.0, and 6.0 g L^{-1}) were prepared. Then the PDMS dishes were placed into these methanol solutions containing pyrene (one dish per 100 mL methanol solutions), and the methanol solutions were refreshed every 24 h. After 72 h loading, these PDMS dishes were rinsed with the ultrapure water three times, and then were placed into artificial freshwater (AFW) in the glass beaker, meanwhile, a piece of 1 cm PDMS

fiber was placed into each glass beaker containing AFW. After 4 days, the pyrene concentrations in AFW were determined by methods of solid-phase extraction (Oasis HLB columns),[5] and the fiber was taken out and immersed into 200 μL n-hexane at least 24 h, then the pyrene in n-hexane solution was determined by GC-MS.

The partition coefficients ($K_{MeOH:AFW}$) between the MeOH loading solution and AFW could be calculated as follow equation:[6]

$$K_{\mathrm{MeOH:AFW}} = \frac{C_{\mathrm{MeOH}}}{C_{\mathrm{AFW}}} \quad \text{(S1)}$$

Where C_{MeOH} (μg L^{-1}) is the pyrene concentration in methanol, C_{AFW} (μg L^{-1}) is the pyrene concentration in AFW. The detailed derivation procedure of $K_{MeOH:AFW}$ was reported in previous research.[7] The value of partition coefficient of pyrene between methanol and AFW was 80664 according to the linear regression between C_{MeOH} and C_{AFW} (Figure S3).

The partition coefficient of pyrene between PDMS on 1 cm fiber and the AFW ($K_{PDMS:AFW}$) could be calculated according to equation (S2) and (S3).[5]

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

$$C_{\mathrm{PDMS}} = \frac{200 * C_{\mathrm{n-hexane}}}{13.55 * 0.01} \quad \text{(S3)}$$

Where C_{PDMS} (μg L^{-1}) is the pyrene concentration in PDMS fiber, C_{AFW} (μg L^{-1}) is the pyrene concentration in AFW, $C_{n\text{-hexane}}$ is the pyrene concentration in n-hexane, 200 is the volume of n-hexane (μL), 13.55 represents the density of PDMS coating on the fiber (μL m^{-1}) and 0.01 is the length of the fiber used for determination (m). Thus, the partition coefficient of pyrene between PDMS on 1 cm fiber and the AFW ($K_{PDMS:AFW}$) could be deduced from equation (S2) and (S3):

$$K_{\mathrm{PDMS:AFW}} = \frac{200 * C_{\mathrm{n-hexane}}}{13.55 * 0.01 * C_{\mathrm{AFW}}} = \frac{200}{13.55 * 0.01} * K_{\mathrm{n-hexane:AFW}} \quad \text{(S4)}$$

Where $K_{n\text{-hexane:AFW}}$ could be calculated by linear regression between the pyrene concentration in n-hexane and AFW (Figure S4). $K_{PDMS:AFW}$ was calculated to be 14790 according to equation (S4).

Bioaccumulation Kinetics Experiments of Pyrene in *D. magna*

The bioaccumulation experiments were conducted in a 1 L glass beaker. A total of 50 *D. magna* (14 days old) were placed in each beak with 500 mL AFW containing 10 μg L^{-1} pyrene, which was controlled by the PDMS passive dosing system. They were cultured at a RXZ-500B temperature-monitored artificial climate incubator (Beijing, China) at 23 ± 1 °C for 48 h with a 16:8 (light/dark) photoperiod (light intensity of 2300 lx). A total of 5 *D. magna* were sampled at 1, 3, 7, 11, 24, 36, 48 h and stored at -20 °C respectively until the determination of pyrene concentration. Each experimental treatment was performed in triplicate.

Analysis of Freely Dissolved Pyrene by Solid-Phase Microextraction

Briefly, a piece of 1 cm PDMS fiber (Polymicro Tech) was placed into each Erlenmeyer flask containing 120 mL exposure solution. The flask was kept in the dark and left shaking at 120 rpm at 24 °C for 24 h. Subsequently, the fiber was transferred to 200 μL n-hexane. The pyrene concentration in n-hexane was analyzed by gas chromatography-mass spectrometer (GC-MS) (Shimadzu, Japan) after 24 h.

Determination of the Total Pyrene Concentration by Liquid-Liquid Extraction

To determinate the total concentration of pyrene in exposure systems, the method of liquid-liquid extraction was used.[8] In brief, a total of 10 mL exposure solution was transferred to a separatory funnel, and then 100 μL 2-fluorobiphenyl (recovery standard, 2 mg L^{-1}) was added. Subsequently, a total of 10 mL ultrapure water and 15 mL dichloromethane were added. The mixture was shaken for 10 min. After still stratification, the bottom liquid was transfer to a glass funnel containing anhydrous sodium sulfate for elimination of water in the extraction liquid. The exposure solution was extracted with dichloromethane once more without adding ultrapure water and recovery standard. The combined dichloromethane extracts were concentrated to less than 2 mL with rotary evaporator. Then the concentrated liquid was blown to less than 0.5 mL with N_2. At last, a total of 100 μL m-terphenyl (internal standard, 600 μg L^{-1}) and n-hexane was added to

this concentrated liquid until reach 1 mL accurately for GC-MS determination.

Extraction and Determination of Pyrene in *D. magna*

The gut, tissues without gut, and the whole body of *D. magna* stored at -20 °C were freeze dried for 72 h, and then were placed into 10 mL glass scale test tube. A total of 100 μL 2-fluorobiphenyl (recovery standard, 2 mg L^{-1}) and 8 mL extraction liquid (dichloromethane:n-hexane = 1:1, v:v) were added into each 10 mL glass scale test tube. The tubes were covered by glass stopper and parafilm, and then were stored at −4 °C for 24 h, followed by a 30 min ultrasonic extraction using ultrasonic cleaners. The extraction liquid was taken out and added to each 15 mL glass tube, and repeat the ultrasonic extraction procedure with extraction liquid (8 mL) as mentioned above once again. All extracts in the 15 mL glass tubes were blown to less than 2 mL with N_2, and were filtered with 0.45 μm Teflon membrane (USA). Then the filtrates were blown to less than 0.5 mL with N_2 to obtain concentrated liquid. At last, a total of 100 μL m-terphenyl (internal standard, 600 μg L^{-1}) and n-hexane were added to this concentrated liquid until reach 1 mL accurately for GC-MS determination.

149 Table S1. Volume and dissolved organic carbon (DOC) concentration of DOM (F_0-F_5)

	F_5	F_4	F_3	F_2	F_1	F_0
	<1 000 Da	1 000-3 000 Da	3 000-5 000 Da	5 000-10 000 Da	>10 000 Da	HA:FA (v:v, 1:1)
Volume (L)	4.00	2.51	1.50	0.45	1.45	16.78
DOC (mg C L^{-1})	70.67	114.16	192.44	724.80	487.02	139.86

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Table S2. The measured molecular weight (*MW*) of DOM fractionated by ultrafiltration

DOM size fraction	F_5	F_4	F_3	F_2	F_1
	<1 000 Da	1 000-3 000 Da	3 000-5 000 Da	5 000-10 000 Da	>10 000 Da
Measured average *MW* (Da)	840	2410	3240	4790	nd

nd means not detected

Table S3. Expected and measured values of freely dissolved pyrene concentrations before (day 0) and after (day 2) the experiments of immobilization (n=6)

	Expected C_{free} ($\mu g\ L^{-1}$)	Measured C_{free} ($\mu g\ L^{-1}$)	
		Mean	SD
DAY 0	20	18.7	0.219
	40	38.5	0.447
	60	60.2	0.457
DAY 2	20	18.4	0.241
	40	38.0	0.346
	60	59.4	0.133

Table S4. Expected and measured values of freely dissolved pyrene concentrations before (day 0) and after (day 2) the experiments of pyrene determination in *D. magna* (n=6)

	Expected C_{free} ($\mu g\ L^{-1}$)	Measured C_{free} ($\mu g\ L^{-1}$)	
		Mean	SD
DAY 0	60	58.7	0.136
DAY 2	60	57.9	0.215

Table S5. Organic carbon-normalized distribution coefficient (K_{doc}) values of pyrene under different conditions (Average of three replicates, RSD < 10%)

DOM molecular weight and concentration	C_{free} of pyrene ($\mu g\ L^{-1}$)	Concentration of pyrene associated with DOM ($C_{DOM\text{-}pyrene}$, $\mu g\ kg^{-1}$)	K_{doc} ($L\ Kg^{-1}$)	log K_{doc}
<1 000 Da (10 mg C L^{-1})	20.0	9.88×10^{5}	4.94×10^{4}	4.69
1 000-3 000 Da (10 mg C L^{-1})	20.0	1.02×10^{6}	5.09×10^{4}	4.71
3 000-5 000 Da (10 mg C L^{-1})	20.0	1.36×10^{6}	6.82×10^{4}	4.83
5 000-10 000 Da (10 mg C L^{-1})	20.0	2.44×10^{6}	1.22×10^{5}	5.09
>10 000 Da (10 mg C L^{-1})	20.0	3.13×10^{6}	1.56×10^{5}	5.19
<1 000 Da (10 mg C L^{-1})	40.0	2.21×10^{6}	5.53×10^{4}	4.74
1 000-3 000 Da (10 mg C L^{-1})	40.0	2.37×10^{6}	5.93×10^{4}	4.77
3 000-5 000 Da (10 mg C L^{-1})	40.0	2.77×10^{6}	6.93×10^{4}	4.84
5 000-10 000 Da (10 mg C L^{-1})	40.0	4.05×10^{6}	1.01×10^{5}	5.01
>10 000 Da (10 mg C L^{-1})	40.0	7.71×10^{6}	1.93×10^{5}	5.28
<1 000 Da (10 mg C L^{-1})	60.0	3.04×10^{6}	5.06×10^{4}	4.70
1 000-3 000 Da (10 mg C L^{-1})	60.0	3.16×10^{6}	5.27×10^{4}	4.72
3 000-5 000 Da (10 mg C L^{-1})	60.0	3.57×10^{6}	5.94×10^{4}	4.77
5 000-10 000 Da (10 mg C L^{-1})	60.0	5.90×10^{6}	9.84×10^{4}	4.99
>10 000 Da (10 mg C L^{-1})	60.0	1.10×10^{7}	1.84×10^{5}	5.26
<1 000 Da (30 mg C L^{-1})	60.0	3.62×10^{6}	6.58×10^{4}	4.82
1 000-3 000 Da (30 mg C L^{-1})	60.0	4.01×10^{6}	7.29×10^{5}	4.86
3 000-5 000 Da (30 mg C L^{-1})	60.0	4.24×10^{6}	7.70×10^{5}	4.89
5 000-10 000 Da (30 mg C L^{-1})	20.0	2.38×10^{6}	1.19×10^{5}	5.08
>10 000 Da (30 mg C L^{-1})	20.0	3.63×10^{6}	1.82×10^{5}	5.26

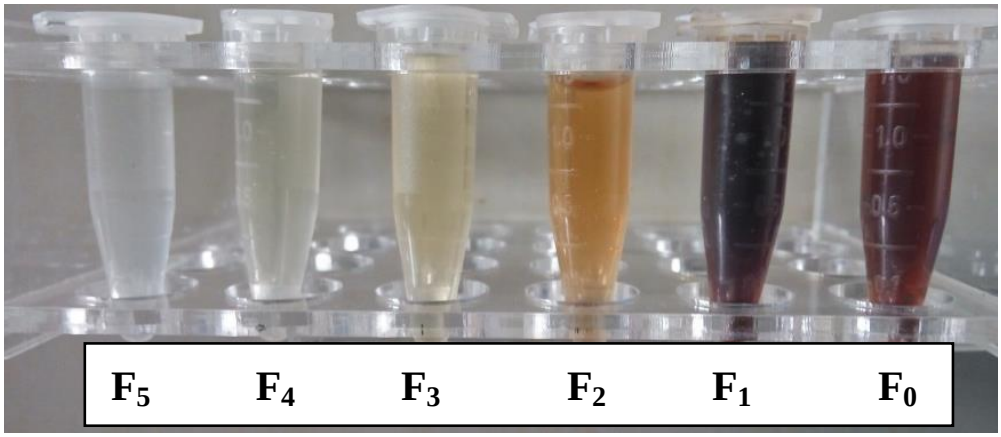


Figure S1. Samples of unfractionated DOM and five fractions of DOM through the ultrafilter (F_0: unfractionated DOM, F_1: >10 000 Da, F_2: 5 000-10 000 Da, F_3: 3 000-5 000 Da, F_4: 1 000-3 000 Da, F_5: <1 000 Da).

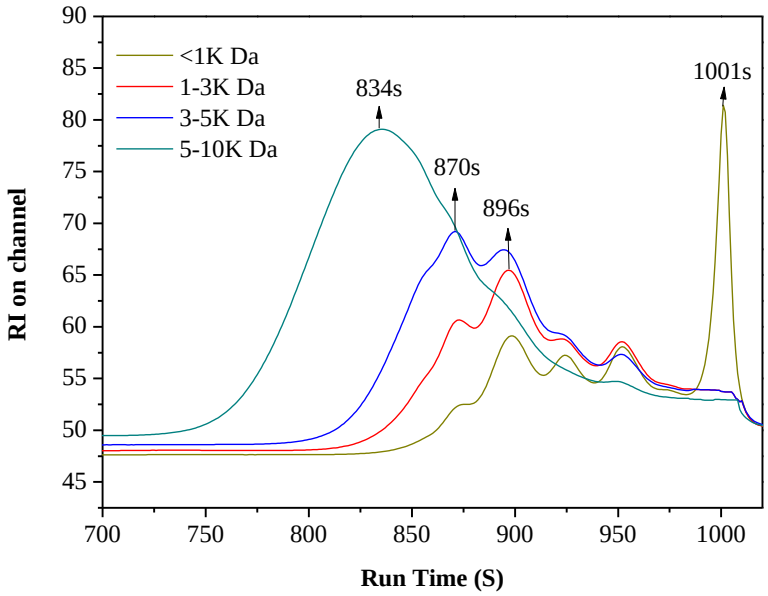
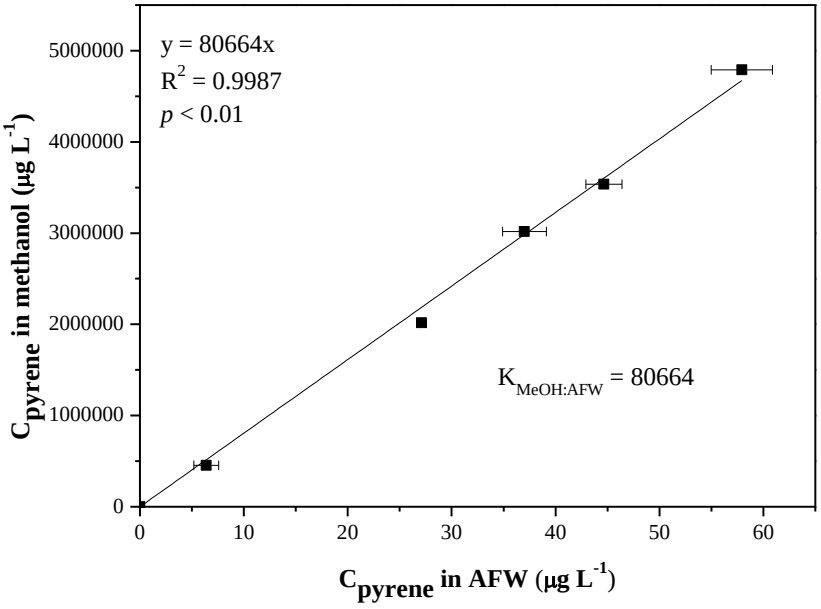


Figure S2. Gel permeation chromatography graph of the DOM with various molecular weights (15 mg C L^{-1}).



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238 Figure S3. Relationship between pyrene concentrations in the loading solution (methanol) and

239 AFW (n =5).

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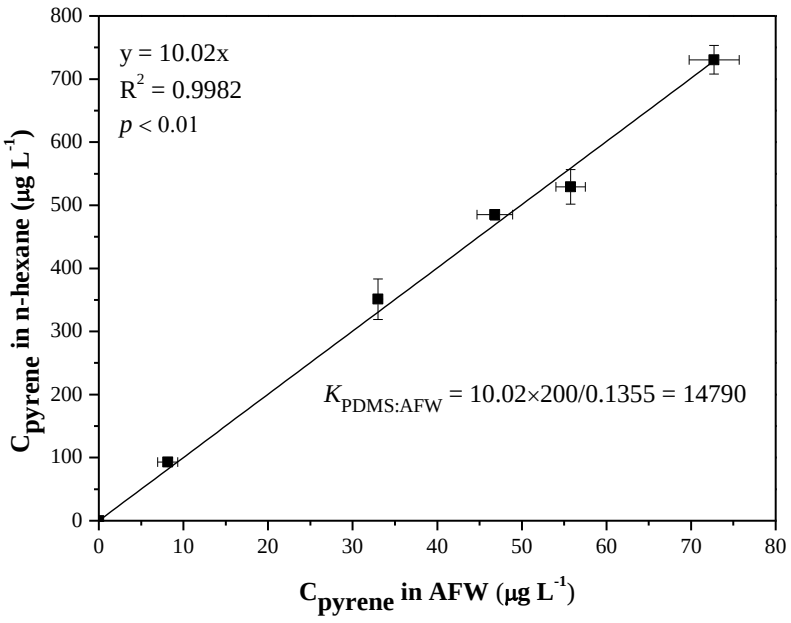
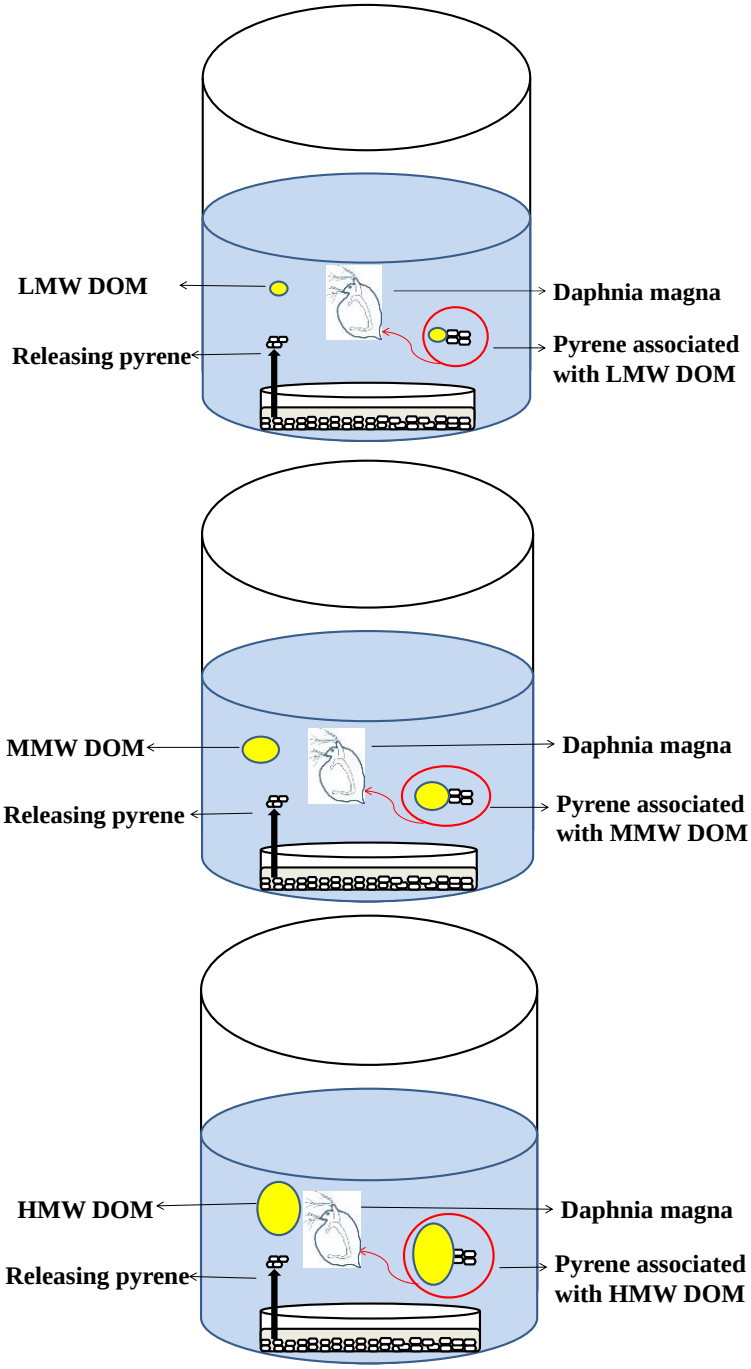


Figure S4. Relationship between pyrene concentrations in n-hexane containing 1 cm PDMS fiber (μg L^{-1}) and AFW (n =5).



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268 Figure S5. Schematic of pyrene associated with different molecular weight DOM taken up by *D.*

269 *magna*.

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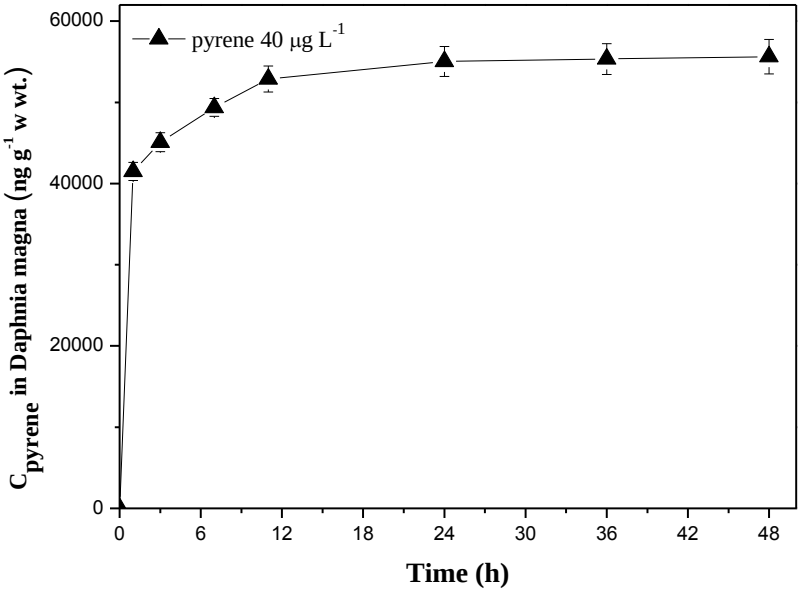

Figure S6. Bioaccumulation curve of pyrene in *D. magna* in the absence of DOM during 48 h of exposure (mean ± standard deviation, n = 3).

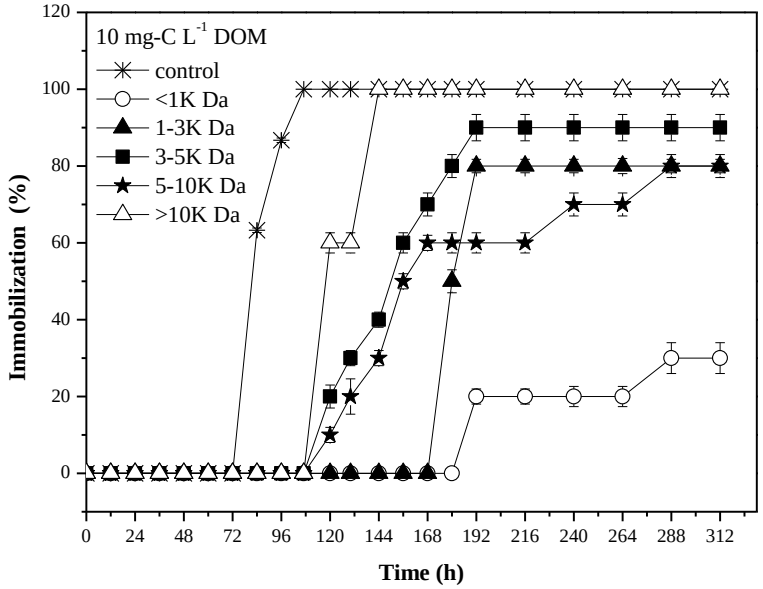

Figure S7. Effects of DOM (10 mg C L^{-1}) with different molecular weights on the immobilization of *D. magna* during 312 h of exposure (mean ± standard deviation, n = 3). Control means without DOM.

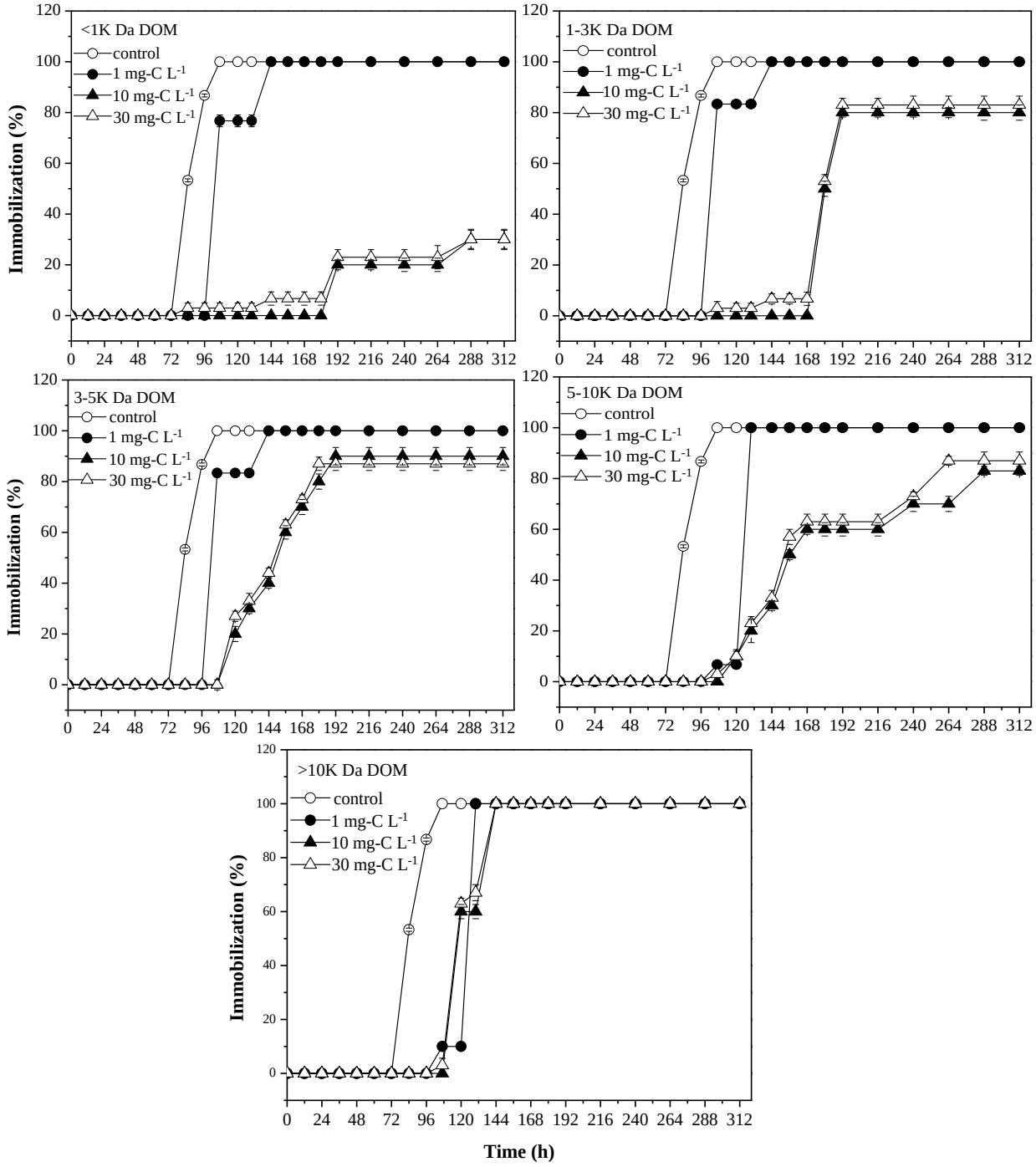

Figure S8. Effects of DOM with different concentrations on the immobilization of *D. magna* in the systems of DOM with various molecular weights during 312 h of exposure (mean ± standard deviation, n = 3). Control means without DOM.

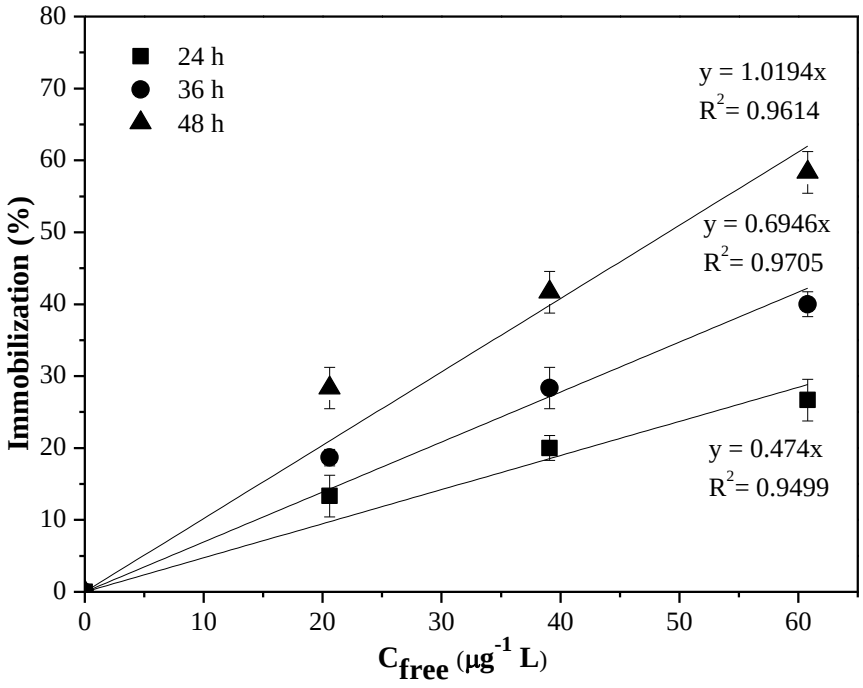

Figure S9. Relationship between the immobilization of *D. magna* in control group without DOM and the freely dissolved pyrene concentration (mean ± standard deviation, n = 3).

Reference

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