Supporting Information for

Contribution of dietary uptake to PAH bioaccumulation in a simplified pelagic food chain: Modeling the influences of continuous vs intermittent feeding in zooplankton and fish

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Materials and Methods

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References

Materials

Deuterated phenanthrene (phenanthrene-d_{10}), deuterated anthracene (anthracene-d_{10}), deuterated fluoranthene (fluoranthene-d_{10}), and deuterated pyrene (pyrene-d_{10}) (98% atom D) were purchased from C/D/N Isotopes Inc. Surrogate standard 2-fluorobiphenyl was obtained from J&K Chemical Ltd. with purity > 97%. The internal standard substance *m*-terphenyl was purchased from AccuStandard with purity > 98%. High-performance chromatography grade methanol, *n*-hexane, dichloromethane, and acetone were purchased from J.T. Baker. All other analytical-grade reagents were from Xilong Chemical Co., Ltd. The poly- (dimethylsiloxane) (PDMS) elastomer was prepared from a Silastic MDX4-4210 BioMedical grade Elastomer kit (Dow Corning) purchased from Baili (Shanghai) Medicinal materials trade Inc. of China. Solid-phase microextraction (SPME) glass fibers coated with 30 μm thick poly- (dimethylsiloxane) at 13.188 μL/cm were purchased from Polymicro Tech. Artificial water (AFW) made from Milli-Q water was used as the exposure medium for *Daphnia magna* with the composition of 294 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 123 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 64.8 mg L^{-1} $NaHCO_3$, and 6.25 mg L^{-1} KCl (pH 7.8 ± 0.2, hardness 250 ± 25 mg L^{-1} as $CaCO_3$, less than 0.2 mg L^{-1} dissolved organic carbon). BG-11 medium was used for the culture of *Chlorella vulgaris* in the present study with the composition of 1.5 g/L $NaNO_3$, 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$, 6 mg/L Citric acid ($C_6H_8O_7 \cdot H_2O$), 1 mg/L $EDTANa_2$ ($C_{10}H_{16}N_2O_8$), 20 mg/L Na_2CO_3, 1 mL/L Trace metal mix A5 (2.86 g/L H_3BO_4, 1.81 g/L $MnCl_2 \cdot 4H_2O$, 0.222 g/L $ZnSO_4$, 0.39 g/L Na_2MoO_4, 0.079 g/L $CuSO_4 \cdot 5H_2O$, 0.049 g/L $Co(NO_3)_2 \cdot 6H_2O$), 40 mg/L K_2HPO_4, and 6 mg/L Ferric ammonium citrate ($Fe(NH_4)_3(C_6H_5O_7)_2$) in AFW.

Cultivation of *Chlorella vulgaris* (*C. vulgaris*)

The freshwater green algae *C. vulgaris* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. *C. vulgaris* were grown in the 3 L Erlenmeyer flasks connected with a glass tube for air flow-through (3 L/min) containing 2 L sterile BG-11 medium with the initial pH around 7.5. These flasks were placed in an incubator at a temperature of 25 ±0.5 ℃ under 16h: 8 h (light: dark) photoperiod with a light intensity of 2300 lx.

To explore the growth of *C. vulgaris*, a total of 20 mL medium with a density of 1×10^7 cells/mL was inoculated into 500 mL of sterilized BG-11 medium with or without air flow-through in a clean bench. The *C. vulgaris* in each treatment was sampled at 0, 0.5, 1, 2, 3, 4, 5, 9, and 11 d after inoculation. The cell numbers in each sample were counted using a Flow Cytometer (ACEA NovoCyte^{TM}). The growth of algae within limited space and resources can be described by a logistic function as follow:

$$N = \frac{K}{1+e^{a-rt}} \tag{1}$$

where N is the density of algae (cells/mL); K is the maximum capacity of algae (cells/mL); r is the intrinsic growth rate (d^{-1}); t is growth time (d); a is a dimensionless constant depending on initial density (N_0) and maximum capacity. The parameters K, r, and a were estimated by fitting equation (1) to the measured algal density using the software MATLAB (R2012b, The MathWorks, Inc., Natick, MA), and shown in Figure S1.

Based on the growth kinetics, the algae were inoculated approximately every 5 days. To determine the algae biomass, a suspension of algae at the exponential phase (5 days after inoculum with air flow-through) was collected and then diluted in gradient to approximately cover the range of algal density within 10^5 to 10^7 cells mL^{-1}. After the determination of cell numbers, these algal suspensions (10 mL) were concentrated by centrifugation at 2500 *g*/min for 3 min and then freeze-dried over 48 h to obtain the dry weights. According to the linear regression, the algae biomass was 7.89×10^{-9} mg/cell (Figure S2).

Cultivation and feeding behavior of *Daphnia magna* (*D. magna*)

Cultivation of *D. magna*

D. magna were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and they were cultured in our laboratory for 5 years. They were grown in 2 L glass beakers containing AFW. These beakers were placed in an incubator at a temperature of 23 ± 0.5 ℃ under 16h: 8 h (light: dark) photoperiod with a light intensity of 2300 lx. The density of *D. magna* was kept at approximately one individual per 10 mL AFW. *D. magna* were fed on the green algae *C. vulgaris* daily at 5×10^4 cells mL^{-1}. The AFW was refreshed every other day.

Ingestion rate of *D. magna*

To determine the ingestion rate of *D. magna* on *C. vulgaris*, a total of 5 mature *D. magna* (~14-day old) were put into a 20 mL glass tube containing a 15 mL suspension of algae harvested at exponential phase. Four algal densities were designed including 5×10^4, 5×10^5, 5×10^6, and 8×10^6 cells mL^{-1}. The control groups with the same algal density did not receive *D. magna*. The feeding test lasted for 2 h, and the cell numbers in each algal suspension were determined every 20 min using a Flow Cytometer. Each treatment was conducted in quintuplicate. The ingestion rate of *D. magna* on *C. vulgaris* (at exponential phase) at each algal density was calculated according to a previous study when there was no growth of the algae during the feeding test.[1]

$$I = \frac{V}{N} \cdot \frac{C_1 - C_2}{t_2 - t_1} \tag{2}$$

where I is the ingestion rate (cells/individual/h); V is the volume of algae (mL); N is the numbers of *D. magna* (individual); C_1 is the algal density at time point t_1 of the feeding test (cells/mL); C_2 is the algal density at the time point of t_2 of the feeding test (cells/mL). As shown in Figure S3, t_1 represents the start of the feeding test, i.e. 0 min, and t_2 represents the end of feeding test, i.e. 120 min.

The response of ingestion rate to the algal density was described by a sigmoid function:[2]

$$I = \frac{I_{\max}}{1 + e^{-k(C - C_m)}} \tag{3}$$

where I_{max} is the theoretical maximum ingestion rate (cells/individual/h); k is a dimensionless constant; C is algal density (cells/mL), and C_m is the half-saturation constant. The calculated ingestion rate at each algal density from Figure S3 was fitted into the equation (3) to estimate the I_{max}, k, and C_m. The estimated values were $I_{max} = 1.17\times10^6 \pm 3.54\times10^4$, $k = 4.27\times10^{-6} \pm 1.83\times10^{-6}$, and $C_m = 7.36\times10^5 \pm 1.02\times10^5$ (mean ± standard deviation, $n = 5$).

Feeding behavior

In order to investigate the feeding interval of *D. magna* on *C. vulgaris*, we took a video to observe the feeding behavior of *D. magna* under an anatomic microscope. One mature *D. magna* was placed onto a glass slide with one drop of algal suspension (~ 1 mL) at 5×10^5 or 5×10^6 cells mL^{-1}. The observations lasted for 1 hour with algal suspension refreshed every 5 min. The observations were repeated six times by using 6 different mature *D. magna*. The video lasted for 1 hour for each treatment (5×10^5 cells/mL or 5×10^6 cells/mL). Here, we only provided a segment of the video under 5×10^5 cells/mL algae.

As shown in the segment of video (Video S1). It could be observed that the algae were harvested from the suspension into the groove of *D. magna* and then carried forward to the anterior of the groove. Afterward, the ingested algae were pulsed into the gut every 3.33 ± 0.23s through oral cavity at 5×10^5 cells/mL and 3.43 ± 0.72s at 5×10^6 cells/mL (mean ± standard deviation, $n = 6$). The unassimilated algae (feces) were excreted in an irregular pattern. Moreover, the excreted algae (feces) were not distinguished with the algae in the suspension by *D. magna*, which were once again pulsed into the food groove.

Dissection of *Daphnia magna*

The sampled *D. magna* were dried by filter paper and put on a clean glass slide under a anatomic microscope. They were fixed by two dissecting pins into the food groove. Afterward, another dissecting pin was plunged into the first appendage, and then dragged towards the upper inclined place with a 45 degree of angle to separate the gut from the body of *D. magna* as shown in Figure S4.

Establishment of passive dosing systems

The details on the establishment of passive dosing were elaborated in our previous study.[3, 4] Briefly, PDMS pre-polymer and corresponding attached catalyst in Silastic MDX4-4210 BioMedical grade Elastomer kit were placed (*m:m*, 10:1) in a glass beaker, followed by intensive stir with a glass rod to obtain a homogeneous mixture and then transferred to a plastic valve bag. A total of 12 ± 0.1 g mixtures were casted into a 60 mm-diameter glass culture dish (~2 to 3 cm in depth) to obtain a passive dosing dish. The values of methanol loading solution (MeOH) and AFW ($K_{MeOH: AFW}$) for phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10} were 4.57×10^4, 5.13×10^4, 7.17×10^4, and 7.53×10^4, respectively. The passive dosing systems were established by placing 3 passive dosing dishes in a 2 L glass beaker. For PAHs, the equilibrium between passive dosing dishes and AFW in our passive dosing systems could be achieved after exposure for 24 h at static condition.

Analysis of the PAHs-d_{10}

Extraction of PAHs-d_{10} in water

The freely dissolved PAHs-d_{10} in the exposure medium were extracted by solid-phase microextraction (SPME), and details are elaborated in our previous studies.[5] Briefly, the SPME fibers were first cut into 1 cm length pieces (0.132 μL), and then they were soaked into methanol for 72 h to remove impurities. At each sampling point, a total of 100 mL exposure medium was transferred into a 100 mL glass bottle capped with PTFE (SHUNIU), and then one piece of 1cm SPME fiber was put into the bottle, before which it was rinsed with Milli-Q water for 1 min. Subsequently, the bottle was agitated at 120 rpm at 25 ℃ in the dark for 24 h, which was long enough for PAHs-d_{10} reaching equilibrium between exposure medium and fiber.[5] Afterward, the SPME fiber was taken out from the bottle, dried with filter paper, and placed into a 200 μL inner lining pipe built in a 2-mL Agilent sample vial. A total of 200 μL *n*-hexane containing 50 μg/L *m*-terphenyl was injected into the lining pipe. The vials were placed at 4 ℃ for at least 48 h for desorption of PAHs-d_{10} from the fibers to hexane before analysis.

The total PAHs-d_{10} in the exposure medium were extracted by liquid-liquid extraction. At each sampling point, a total of 25 mL exposure medium was transferred into a 125 mL glass separating funnel, and then a total of 8 mL dichloromethane was added. The funnel was violently shaken by hands for 1 min and then stand still for 3 min. After separation, the liquid in the bottom layer was collected, and a total of 8 mL dichloromethane was added for the second extraction. Finally, the two extracts were combined and concentrated to less than 0.5 mL under gentle nitrogen flow, and then added with 2 mL *n*-hexane, and again concentrated to less than 0.5 mL under gentle nitrogen flow. Finally, the concentrated eluents were transferred into a 2-mL sample vial provided by Agilent. Each vial was added with 50 μL of *m*-terphenyl (1 mg L^{-1}) as an internal standard and then diluted to 1 mL with *n*-hexane. The vial was sealed and kept at -4 ºC before analysis.

Extraction of PAHs-d_{10} in *Daphnia magna*

The PAHs-d_{10} in the gut and whole body except gut of *D. magna* were extracted by organic solvent.[3] After dissection, the whole body except gut was put into 5 mL glass vials with 2 mL extraction agent, and the gut was put into 2 mL glass vials with 1 mL extraction agent. The extraction agent was the solution made up of *n*-hexane and dichloromethane (1: 1, *v*: *v*), and a total of 10 μL (10 mg L^{-1}) of surrogate standard 2-fluorobiphenyl was added into each glass vial. Each glass vial was sealed and placed overnight at room temperature. Afterward, they were vortexed for 30 s and then bathed in an ultrasonic machine at 30 ℃ for 30 min. The extract in each glass vial was transferred, and another 2 mL or 1 mL extraction agent was added for the second vortex (30 s) and

ultrasonic bath (30 ℃, 30 min). The two extracts were combined into a new 5 mL or 2 mL glass vial and then concentrated to about 0.5 mL under a gentle nitrogen blow. Subsequently, a total of 120 (or 20 mg) sodium sulfate was added into glass vials with the whole body except gut samples (or gut samples). The glass vials were vortexed for 1 min to ensure the elimination of water in the samples, and then another 2 mL (or 1 mL) *n*-hexane was added. After, the extract was filtered through a 0.45 μm Teflon membrane, and then continued to be concentrated with a gentle nitrogen blow to about 0.5 mL and finally transferred into a 2-mL sample vial provided by Agilent. Each vial was added with 50 μL of *m*-terphenyl (20 ppb) as an internal standard and then diluted to 1 mL with *n*-hexane. The vial was sealed and kept at -4ºC before the analysis of the PAHs-d_{10} concentrations.

Quality assurance and quality control

The concentrations of extracted PAHs-d_{10} were analyzed using a gas chromatograph mass spectrometer (Shimadzu GC-MS/MS TQ8040) equipped with a Rxi-5Sil MS column (length, 30 m; internal diameter, 0.25 mm; and film thickness, 0.25 μm). Details can be found in our previous study.[4] Briefly, the determined limits of quantification (LOQs) (S/N = 3) for GC-MS/MS analysis of the PAHs-d_{10} were in the range of 0.05-0.1 μg L^{-1}. The correlation coefficient of the internal standard calibration curve for each PAH-d_{10} was higher than 0.99.

The recoveries for extraction of PAHs-d_{10} in water by liquid-liquid extraction were 83.8 ± 4.26%, 84.8 ± 4.36%, 87.0 ± 3.79%, 87.4 ± 4.14% (mean ± standard deviation, n = 3) for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively. The recoveries for extraction of PAHs-d_{10} in *D. magna* by solvent extraction as indicated by 2-fluorobiphenyl was 77.4 ± 14.3% (mean ± standard deviation, n = 43). The background concentrations of PAHs-d_{10} in artificial water were 3.01 ± 1.42, 2.56 ± 1.83, 4.42 ± 1.71 and 4.04 ± 1.72 ng/L (mean ± standard deviation, n = 3) for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively. The background concentrations of PAHs-d_{10} in the body except gut of *D. magna* were 5.15 ± 3.67, 3.26 ± 3.07, 4.64 ± 4.81 and 5.69 ± 5.80 ng/g wet weight (mean ± standard deviation, n = 6) for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively. The background concentrations of PAHs-d_{10} in the gut of *D. magna* for the four PAHs-d_{10} were all under the detection limits (n = 6).

For the measurement of freely dissolved concentrations by SPME, the partition coefficient of PAHs-d_{10} between PDMS and AFW (K_{PDMS}) were $1.10 \times 10^4 \pm 1.62 \times 10^3$, $1.21 \times 10^4 \pm 1.10 \times 10^3$, $2.16 \times 10^4 \pm 3.20 \times 10^3$ and $2.39 \times 10^4 \pm 4.64 \times 10^3$ L/L (mean ± standard deviation, n = 6) for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively. The freely dissolved concentration basically maintained at a constant level during exposure when algal density at 5×10^5 cells/mL; but it dropped 22.5%, 5.21%, 12.2%, and 17.9% for phenanthrene-d_{10}, anthracene-d_{10},

fluorancene-d_{10} and pyrene-d_{10}, respectively, at the end of exposure when algal density at 5×10^6 cells/mL as shown in Table S2. According to the results shown in Figure S3, the *C. vulgaris* would aggregate especially at high algal density. Therefore, the exposure medium was stirred by a glass rod every 12 h during the exposure experiment. *D. magna* were all in well condition (no immobilization and mortality) throughout the exposure and depuration process in each treatment.

Calculation of BCF or BAF

The lipid normalized bioconcentration factor at steady-state (waterborne-only, $BCF_{ss\text{-}lip}$, L kg^{-1}) or bioaccumulation factor (waterborne and dietary uptake, $BAF_{ss\text{-}lip}$, L kg^{-1}) was calculated as:

$$BCF_{ss\text{-}lip}(BAF_{ss\text{-}lip}) = \frac{C_{b\text{-}ss}}{C_w \cdot f_{lip}} \quad (4)$$

where $C_{b\text{-}ss}$ is the steady-state concentration of the PAHs-d_{10} in aquatic organisms (ng g^{-1}); C_w is the freely dissolved concentration of PAHs-d_{10} in water at the initial of exposure (ng mL^{-1}); f_{lip} is the lipid content of aquatic organisms (%). BCF_{ss} and BAF_{ss} are the values only at steady-state without lipid normalization.

Partitioning PAHs-d_{10} into *C. vulgaris*

To allow partitioning of PAHs-d_{10} into algae, the algae at exponential phase (Figure S1) were harvested by centrifugation (2500 *g*/min for 3 min), and then re-suspended into a 500 L glass beaker containing 400 mL AFW and three passive dosing dishes where the algal density was finally at 5×10^6 cells mL^{-1}. The exposure lasted for three days. Before the addition of algae, the exposure medium was initially placed on an orbiting table at 60 rpm/min for one day to ensure the equilibrium between AFW and passive dosing dishes.[3] The exposure medium (126 mL) was sampled at time points of 24, 48, and 72 h. Among which, 100 mL of medium was used to determine freely dissolved concentrations by solid-phase microextraction (SPME), 25 mL of medium was used to extract total concentrations by liquid-liquid extraction, and 1 mL of medium was used to measure the algal density. The bioconcentration factor (BCF, mL/g dry weight) of PAHs-d_{10} in the algae was calculated as follow:

$$BCF_{algae} = \frac{C_t - C_w}{C_w[\text{algae}]} \quad (5)$$

where C_t is the total concentrations in the medium (ng/L); C_w is the freely dissolved concentrations (ng/L); [algae] was the mass concentration of *C. vulgaris* (g/mL). The results were shown in Table S3.

Details for model implementation for *D. magna*

We used Runge−Kutta integrator in the software Simulation Analysis and Modeling (SAAM II, version 2.3.1.1, University of Washington) to numerically solve the differential equations in the toxicokinetic model. The kinetic parameters including I, k_u, k_f, k_g, k_b, k_{eg}, and k_{eb} were estimated by simultaneously fitting the experimental data in toxicokinetic experiments to the model. Considering that food is not available for *D. magna* during bioconcentration and following depuration, the elimination in the gut and body may be different from the counterpart in the bioaccumulation and following depuratoin.[6] Therefore, the k_{eg} and k_{eb} were estimated separately in these two treatments instead of sharing the same values as the other kinetic parameters. The algal density in the exposure medium decreased during exposure, but the ingestion rate did not show large variations (Figure S5). Therefore, the ingestion rate was assumed to be constant during the exposure. The model inputs and initial starting values for each parameter to be estimated can be found in Table S4. The experimental data included the measured concentrations of the PAHs-d_{10} in the gut ($C_{g\_measured}$, ng g^{-1}) and whole body except gut of *D. magna* (C_b, ng g^{-1}). Due to the difficulty in separating the ingested algae and gut of *D. magna* (a very thin membrane), the $C_{g\_measured}$ was practically the quotient of the amount of PAHs-d_{10} in both ingested algae and gut to the measured wet weight of gut including ingested algae ($W_{g\_measured}$):

$$C_{g\_measured}(t) = \frac{C_g(t)W_g + m_f(t)}{W_{g\_measured}} \quad (6)$$

The best-fit estimations for kinetic parameters were obtained by minimizing the residual sum of squares between measured concentrations and modelled ones. The optimization used a modified Gauss-Newton method. A level of 0.05 was set for the convergence criterion for phenanthrene-d_{10}, anthracene-d_{10} and fluorancene-d_{10}. For pyrene-d_{10}, the convergence criterion was set at 0.15. Different model fits were also compared with Akaike's Information Criterion (AIC) as an indicator of goodness of fit.

Estimation of assimilation efficiency (AE) in the toxicokinetic model

$$\begin{cases} \dfrac{\mathrm{d}m_{\mathrm{g}}}{\mathrm{d}t} = IC_{\mathrm{f}} - k_{\mathrm{g}}m_{\mathrm{g}} - k_{\mathrm{eg}}m_{\mathrm{g}} + k_{\mathrm{b}}m_{\mathrm{b}} \\ \dfrac{\mathrm{d}m_{\mathrm{b}}}{\mathrm{d}t} = k_{\mathrm{u}}C_{\mathrm{w}}W_{\mathrm{b}} + k_{\mathrm{g}}m_{\mathrm{g}} - k_{\mathrm{b}}m_{\mathrm{b}} - k_{\mathrm{eb}}m_{\mathrm{b}} \end{cases} \tag{7}$$

$$\frac{\mathrm{d}\left(m_{\mathrm{b}} + m_{\mathrm{g}}\right)}{\mathrm{d}t} = k_{\mathrm{u}}C_{\mathrm{w}}W_{\mathrm{b}} + IC_{\mathrm{f}} - k_{\mathrm{eg}}m_{\mathrm{g}} - k_{\mathrm{eb}}m_{\mathrm{b}} \tag{8}$$

$$\frac{\mathrm{d}\left(\dfrac{m_{\mathrm{b}} + m_{\mathrm{g}}}{W_{\mathrm{tot}}}\right)}{\mathrm{d}t} = k_{\mathrm{u}}C_{\mathrm{w}}\frac{W_{\mathrm{b}}}{W_{\mathrm{tot}}} + \frac{IC_{\mathrm{f}}}{W_{\mathrm{tot}}} - \frac{k_{\mathrm{eg}}C_{\mathrm{g}}W_{\mathrm{g}}}{W_{\mathrm{tot}}} - \frac{k_{\mathrm{eb}}C_{\mathrm{b}}W_{\mathrm{b}}}{W_{\mathrm{tot}}} \tag{9}$$

Assuming W_{b} approximately equals to W_{tot}

$$\frac{\mathrm{d}C_{\mathrm{b}}}{\mathrm{d}t} = k_{\mathrm{u}}C_{\mathrm{w}} + IRC_{\mathrm{f}} - \frac{k_{\mathrm{eg}}C_{\mathrm{g}}W_{\mathrm{g}}}{W_{\mathrm{tot}}} - k_{\mathrm{eb}}C_{\mathrm{b}}, IR = I / W_{\mathrm{tot}} \tag{10}$$

$$\mathrm{AE} = 1 - \frac{k_{\mathrm{eg}}C_{\mathrm{g}}W_{\mathrm{g}}}{W_{\mathrm{tot}}} / \left(IR \times C_{\mathrm{f}}\right) \tag{11}$$

After fitting, C_{g} (t) in numerical format could be derived, and then we calculated the integration of C_{g} (t) in a period of time (for example, 24 h, i.e. the area under the curve). Afterward, the integration times estimated k_{eg} and W_{g} to derive the total elimination amount during this period of time. Then the product was divided by the total ingestion amount of toxicant during this period (calculated by $IR \times W_{\mathrm{tot}} \times 24\ \mathrm{h} \times C_{\mathrm{f}}$).

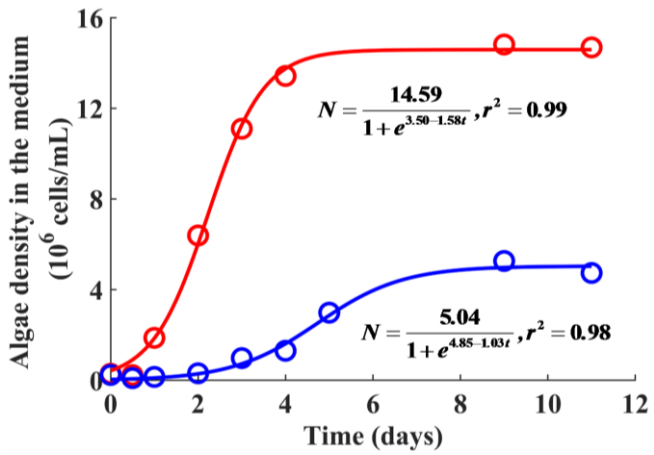

Figure S1. Growth curve of *C. vulgaris* with (red) and without (blue) air flow-through.

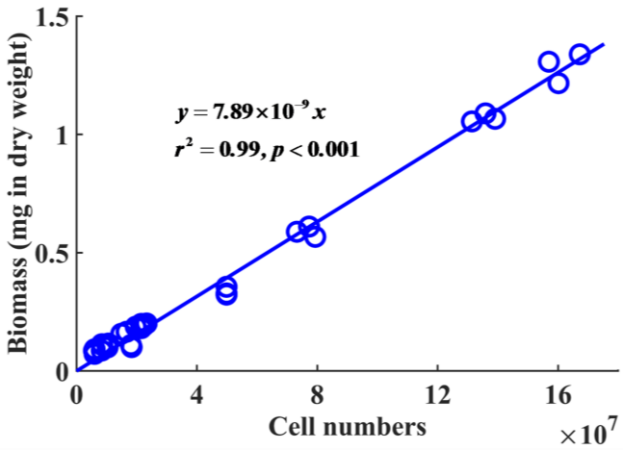

Figure S2. Relationship between biomass in dry weight and algal density.

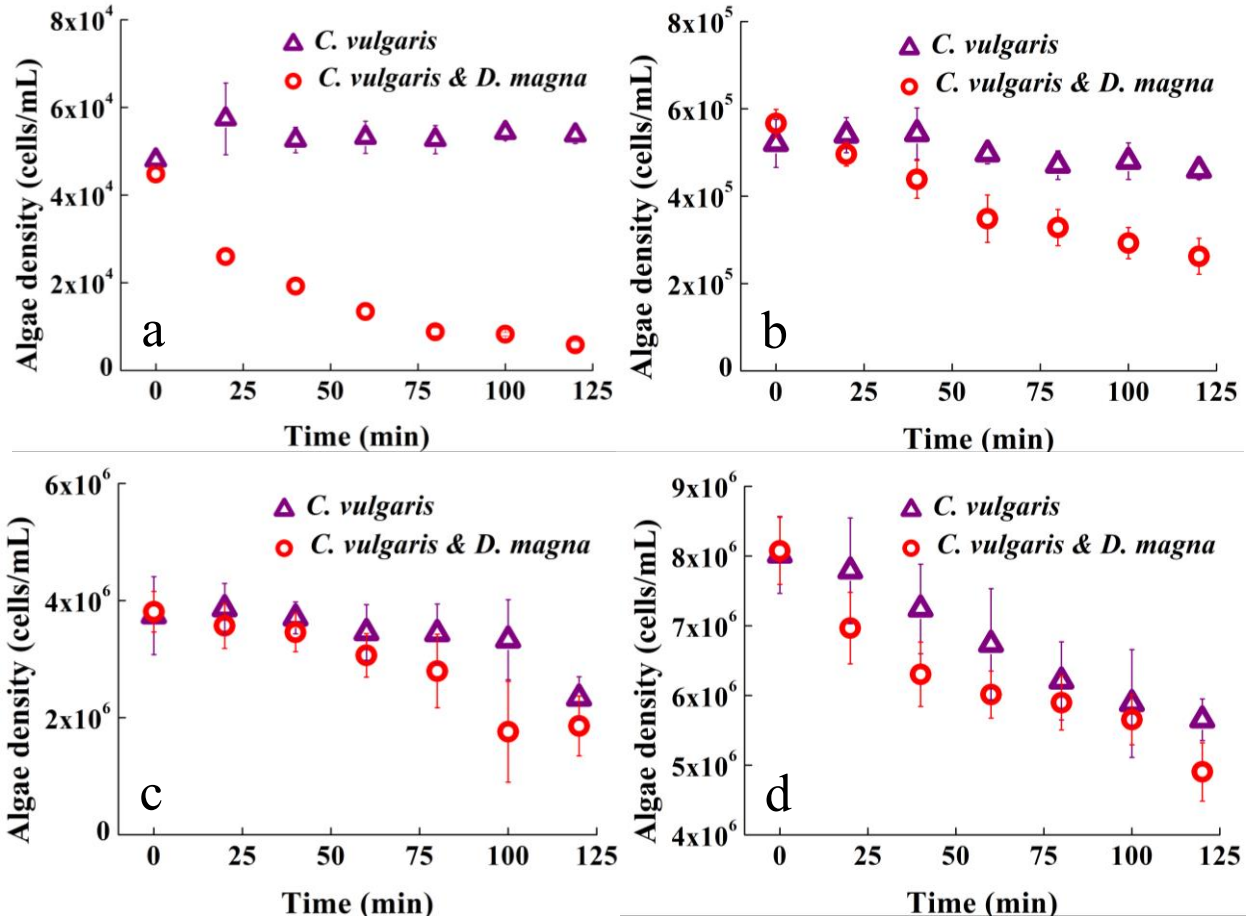

Figure S3. Variation of algal density during a 2 hour feeding test with algal density at 5×10^4 cells/mL (a); 5×10^5 cells/mL (b); 5×10^6 cells/mL (c); 8×10^6 cells/mL (d) (mean ± standard deviation, $n = 5$).

Figure S4. Dissection diagram for *D. magna* under the microscope.

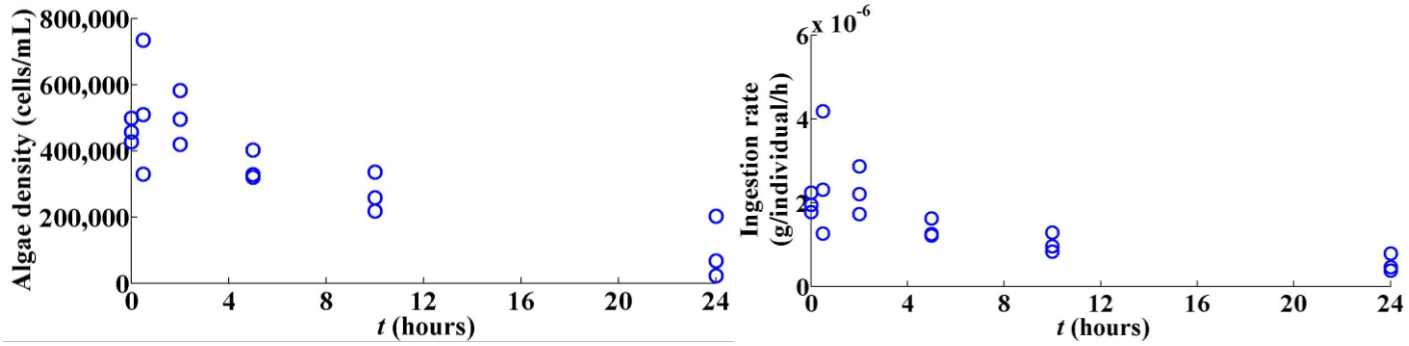

Figure S5. Variation of algal density and ingestion rate of *D. magna* with exposure time in the bioaccumulation experiment with initial cell density at 5×10^5 cells/mL; the ingestion rate was estimated from the corresponding algal density in terms of the equation (3), and the average ingestion rate was 1.63×10^{-6} g dry weight *C. vulgaris*/individual/h.

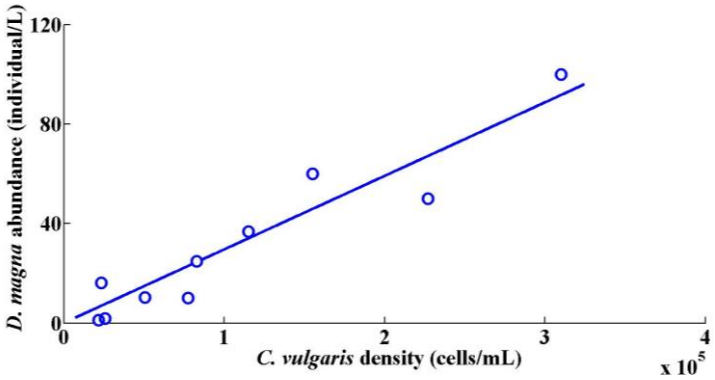

Figure S6. The relationship between *D. magna* abundance and algal density was roughly estimated from the field data.[7-14] $y = 0.0002955x$, the 95% confidence bounds of the slope is (0.0002449, 0.000346), $r^2 = 0.8977$, and the limit is set at 100 individuals/L.

Table S1. Physiological values for *D. magna*.

	Description	Values
Length (mm)	Adult *D. magna* (14 days, mm)	2.27 ± 0.12 (n = 10)
Whole-body weight (mg)	Dry weight; without dissection	0.078 ± 0.02 (n = 13)
	Wet weight; without dissection	0.927 ± 0.071 (n = 9)
Water content (%)	Without dissection	92 ± 1.9 (n = 9)
Gut (wet weight, mg)	When food is not available (bioconcentration)	0.0135 ± 0.0062 (n = 28)
	When food is available with 5×10^5 cells mL^{-1} algae	0.0155 ± 0.0077 (n = 30)
Body except gut (wet weight, mg)	When food is not available (bioconcentration)	0.518 ± 0.162 (n = 28)
	When food is available with 5×10^5 cells mL^{-1} algae	0.842 ±0.143 (n = 30)

The weight of gut when food is available with 5×10^5 cells mL^{-1} algae includes the weight of ingested algae.

Table S2. The measured total concentrations (C_t) and freely dissolved concentrations (C_w) of PAHs-d_{10} in the exposure medium (mean ± standard deviation, n = 3).

PAHs-d_{10}	Treatment	0 h		24 h	
		C_t (ng/L)	C_w (ng/L)	C_t (ng/L)	C_w (ng/L)
Phenanthrene-d_{10}	Bioconcentration	535 ± 69.7	549 ± 29.3	510 ± 34.9	530 ± 4.27
	Bioaccumulation with 5×10^5 cells/mL	576 ± 59.2	445 ± 5.79	594 ± 35.2	458 ± 5.79
	Bioaccumulation with 5×10^6 cells/mL	-	426 ± 59.9	-	330 ± 31.7
Anthracene-d_{10}	Bioconcentration	522 ± 71.4	487 ± 38.3	515 ± 18.9	518 ± 12.8
	Bioaccumulation with 5×10^5 cells/mL	596 ± 62.7	434 ± 12.3	641 ± 45.7	453 ± 12.3
	Bioaccumulation with 5×10^6 cells/mL	-	403 ± 19.8	-	382 ± 59.2
Fluoranthene-d_{10}	Bioconcentration	450 ± 52.3	493 ± 41.0	464 ± 29.5	474 ± 27.7
	Bioaccumulation with 5×10^5 cells/mL	455 ± 34.7	353 ± 17.4	450 ± 10.1	352 ± 17.4
	Bioaccumulation with 5×10^6 cells/mL	-	436 ± 33.4	-	383 ± 12.6
Pyrene-d_{10}	Bioconcentration	443 ± 39.9	475 ± 37.7	452 ± 38.2	464 ± 32.9
	Bioaccumulation with 5×10^5 cells/mL	475 ± 18.3	363 ± 28.0	448 ± 14.0	346 ± 28.0
	Bioaccumulation with 5×10^6 cells/mL	-	458± 34.9	-	376 ± 16.8

Table S3. Measured total concentrations (C_t, ng/L) and freely dissolved concentrations (C_w, ng/L) of PAHs-d_{10} as well as measured algal density (cells/mL) in the exposure medium, and calculated bioconcentration factor (BCF, mL/g dry weight) of PAHs-d_{10} in *C. vulgaris* over exposure time (mean ± standard deviation, n = 3).

Exposure time		1 day	2 day	3 day
Algal density		$5.35 \times 10^5 \pm 6.82 \times 10^4$	$5.31 \times 10^5 \pm 6.56 \times 10^4$	$5.25 \times 10^5 \pm 8.84 \times 10^4$
phenanthrene-d_{10}	C_t	781 ± 60.1	728 ± 117	759 ± 20.9
	C_w	519 ± 37.7	463 ± 16.8	496 ± 8.04
	BCF	$1.22 \times 10^5 \pm 2.71 \times 10^4$	$1.36 \times 10^5 \pm 5.55 \times 10^4$	$1.30 \times 10^5 \pm 1.95 \times 10^4$
Anthracene-d_{10}	C_t	770 ± 72.3	763 ± 135	758 ± 17.5
	C_w	550 ± 33.1	505 ± 19.1	536 ± 14.4
	BCF	$9.59 \times 10^4 \pm 2.38 \times 10^4$	$1.21 \times 10^5 \pm 5.70 \times 10^4$	$1.00 \times 10^5 \pm 5.61 \times 10^3$
Fluoranthene-d_{10}	C_t	600 ± 45.6	572 ± 74.9	552 ± 15.7
	C_w	449 ± 27	442 ± 28.9	443 ± 17.3
	BCF	$8.13 \times 10^4 \pm 3.95 \times 10^4$	$7.21 \times 10^4 \pm 5.50 \times 10^4$	$5.91 \times 10^4 \pm 2.50 \times 10^3$
Pyrene-d_{10}	C_t	576 ± 42.7	559 ± 72.7	537 ± 15.5
	C_w	440 ± 39.0	432 ± 33.8	431 ± 21.5
	BCF	$7.56 \times 10^4 \pm 4.75 \times 10^4$	$7.26 \times 10^4 \pm 5.44 \times 10^4$	$5.91 \times 10^4 \pm 1.72 \times 10^3$

There were no significant statistical differences over exposure time (one-way ANOVA with a Tukey *posthoc* test, p = 0.900, 0.679, 0.793, 0877 for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively), indicating that the PAH-d_{10} concentrations in *C. vulgaris* reached the steady-state within 24 h.

Table S4. Model inputs for estimation of kinetic parameters in *Daphnia magna*.

Parameter	Description / transfer direction	Value/ Initial value with estimation bound			
		Phenanthrene-d_{10}	Anthracene-d_{10}	Fluoranthene-d_{10}	Pyrene-d_{10}
Constant					
C_f (ng g^{-1})	Concentration in algae	49196	59745	35843	37884
C_w (ng mL^{-1})	Freely dissolved Concentration for bioconcentration	0.549	0.487	0.493	0.475
	Freely dissolved Concentration for bioaccumulation	0.458	0.453	0.352	0.346
W_{aq_gut} (g)	Wet weight of gut when food is not available	1.35×10^{-5}			
W_{aq_body} (g)	Wet weight of body except gut when food is not available	5.18×10^{-4}			
W_{aq+D_gut} (g)	Wet weight of gut when food is available	1.55×10^{-5}			
W_{aq+D_body} (g)	Wet weight of body except gut when food is available	8.42×10^{-4}			
Parameter					
I (g individual^{-1} h^{-1})	Ingestion rate	1.63×10^{-6}, [0, 1] (Figure S5)			
k_u (mL g^{-1} h^{-1})	Waterborne uptake rate	115[15], [0,1000]	115, [0,1000]	52[15], [0,1000]	115, [0,1000]
k_f (h^{-1})	Ingested algae to gut	1.95[4], [0, 1000]	1.87[4], [0, 1000]	0.79[4], [0, 1000]	0.68[4], [0, 1000]
k_g (h^{-1})	Gut to body except gut	3.75[4], [0, 10]	3.56[4], [0, 10]	3, [0, 10]	3, [0, 10]
k_b (h^{-1})	Body except gut to gut	0.169[4], [0, 10]	0.112[4], [0, 10]	0.1, [0, 10]	0.1, [0, 10]
k_{eg_Aq} (h^{-1})	Elimination rate in the gut for bioconcentration	0.01, [0, 10]	0.01, [0, 10]	0.01, [0, 10]	0.01, [0, 10]
k_{eg_Aq+D} (h^{-1})	Elimination rate in the gut for bioaccumulation	0.01, [0, 10]	0.01, [0, 10]	0.01, [0, 10]	0.01, [0, 10]
k_{eb_Aq} (h^{-1})	Elimination rate in body except gut for bioconcentration	0.1, [0,10]	0.1, [0,10]	0.1, [0,10]	0.1, [0,10]
k_{eb_Aq+D} (h^{-1})	Elimination rate in body except gut for bioaccumulation	0.1, [0,10]	0.1, [0,10]	0.1, [0,10]	0.1, [0,10]

Table S5. Estimated kinetic parameters of PAHs-d_{10} in *D. magna* based on original TK model[a] (mean ± standard deviation).

Parameters	Description / transfer direction	Phenanthrene-d_{10}	Anthracene-d_{10}	Fluoranthene-d_{10}	Pyrene-d_{10}
k_u (mL g^{-1} h^{-1})	Waterborne uptake rate	123 ± 14.6	260 ± 55.6	104 ± 20.2	180 ± 35.4
I (10^{-6} g individual^{-1} h^{-1})	Ingestion rate	1.99 ± 0.544	1.54 ± 0.784	1.44 ± 0.473	1.47 ± 1.17
k_f (h^{-1})	Ingested algae to gut	1000 (upper limit)	1000 (upper limit)	1000 (upper limit)	1000 (upper limit)
k_g (h^{-1})	Gut to body except gut	6.23 ± 2.08	2.79 ± 1.47	11.1 ± 2.26	3.01 ± 3.65
k_b (h^{-1})	Body except gut to gut	0.353 ± 0.120	0.249 ± 0.132	1.46 ± 0.305	0.330 ± 0.0312
k_{eg_Aq} (h^{-1})	Elimination rate in the gut for bioconcentration	0 (lower limit)	0 (lower limit)	0 (lower limit)	0 (lower limit)
$k_{eg\_Aq\_D}$ (h^{-1})	Elimination rate in the gut for bioaccumulation	2.95 ± 2.07	3.72 ± 2.34	3.09 ± 0.794	1.21 ± 0.589
k_{eb_Aq} (h^{-1})	Elimination rate in body except gut for bioconcentration	0.269 ± 0.0132	0.441 ± 0.0565	0.412 ± 0.0652	0.345 ± 0.0647
$k_{eb\_Aq\_D}$ (h^{-1})	Elimination rate in body except gut for bioaccumulation	0.138 ± 0.0789	0.106 ± 0.096	0.178[b]	0.168 ± 0.201
AIC	Akaike's Information Criterion	5.19	5.54	4.73	6.63

[a] fitting with equation (1), (3) and (4) in the main text.
[b] hitting the lower limit.

Table S6. PAH-d_{10} concentrations in *D. magna* with 5 × 10^6 cells/mL algae over exposure time (ng/g wet weight, mean ± standard deviation, n = 3).

	Measured from experiments		Estimated from TK model		
	24 h	48 h	Body except gut	Gut	Whole body
Phenanthrene-d_{10}	807 ± 73.7	972 ± 89.2	1136	4700	1200
Anthracene-d_{10}	966 ± 101	1117 ± 336	1286	6958	1388
Fluoranthene-d_{10}	1020 ± 100	1101 ± 194	916	8410	1051
Pyrene-d_{10}	1302 ± 272	1356 ± 331	1376	11148	1552

There are no significant statistical differences in the measured PAH-d_{10} concentrations in the whole body of *D. magna* between 24 h and 48 h (independent t-test, p = 0.069, 0.497, 0.557, and 0.839 for phenanthrene-d_{10}, anthracene-d_{10}, fluorancene-d_{10} and pyrene-d_{10}, respectively).

The estimated PAH-d_{10} concentrations in *D. magna* at steady-state was from the TK model based on estimated parameters. The ingestion rate was estimated from the sigmoid function equation (3).

The internal body burden here is obtained without dissection, which is different from the internal body burden without the gut. We calculated the internal body burden as C_{total} in the bioconcentration and bioaccumulation with 5 × 10^5 cells/mL algae treatments. C_{total} is calculated as follows:

$$C_{total}(t) = \frac{C_g(t)W_g + C_b(t)W_b}{W_g + W_b}$$

where C_g is the measured concentrations in the gut (ng g^{-1} wet weight); C_b is measured concentrations in the whole body except gut of *D. magna* (ng g^{-1} wet weight); W_g and W_b are the wet weight of gut and the whole body, respectively (g, Table S1). The error was calculated as ((C_{total} – C_b) / C_b × 100%) ranged from -2.47% to 8.12% and from -2.42% to 11.9% for bioconcentration and bioaccumulation with 5 × 10^5cells/mL, respectively.

Table S7. The logarithmic lipid normalized bioconcentration factor (log$BCF_{ss\text{-}lip}$ L/kg) and bioaccumulation factor (log$BAF_{ss\text{-}lip}$ L/kg) at steady-state of PAHs-d_{10} in *D. magna* and zebrafish (mean ± standard deviation, n = 3), and corresponding assimilation efficiency (AE, %).

	D. magna				Zebrafish[a]	
	log$BCF_{ss\text{-}lip}$	log$BAF_{ss\text{-}lip}$ (5 × 10^5 cells mL^{-1} algae)	$BAF_{ss\text{-}lip}$ (5 × 10^6 cells mL^{-1} algae)	AE	log$BCF_{ss\text{-}lip}$	AE
Phenanthrene-d_{10}	4.38 ± 0.061	4.80 ± 0.056	5.08 ± 0.057	33.7	3.12 ± 0.103	85.7
Anthracene-d_{10}	4.55 ± 0.104	4.98 ± 0.081	5.17 ± 0.091	39.7	3.32 ± 0.125	87.6
Fluoranthene-d_{10}	4.24 ± 0.036	4.66 ± 0.102	5.18 ± 0.058	40.3	3.13 ± 0.134	72.3
Pyrene-d_{10}	4.56 ± 0.041	4.82 ± 0.061	5.22 ± 0.092	32.6	3.16 ± 0.098	67.5

[a]referred from our previous study.[4]

Table S8. Calculation of biotransformation rate constant of PAHs-d_{10} in *D. magna* and zebrafish.

PAHs-d_{10}	logK_{ow}	*D. magna*					
		k_u (mL g^{-1} ww h^{-1})	k_d (h^{-1})	k_{eb_Aq} (h^{-1})	$k_{eb\_Aq\_D}$ (h^{-1})	k_{M_Aq} (h^{-1})[a]	$k_{M\_Aq\_D}$ (h^{-1})[b]
Phenanthrene-d_{10}	4.57	125	0.236	0.270	0.147	3.35×10^{-2}	-8.95×10^{-2}
Anthracene-d_{10}	4.54	260	0.648	0.441	0.106	−0.207	−0.542
Fluoranthene-d_{10}	5.22	104	0.044	0.344	0.218	0.300	0.174
Pyrene-d_{10}	5.18	180	0.084	0.345	0.168	0.261	8.43×10^{-2}
		Zebrafish					
		k_u (mL g^{-1} ww h^{-1})	k_d (h^{-1})	k_{eb} (h^{-1})		k_M (h^{-1})	
Phenanthrene-d_{10}	4.57	3.06	2.08×10^{-3}	0.058		5.59×10^{-2}	
Anthracene-d_{10}	4.54	3.69	2.69×10^{-3}	0.049		4.63×10^{-2}	
Fluoranthene-d_{10}	5.22	3.93	5.99×10^{-4}	0.073		7.24×10^{-2}	
Pyrene-d_{10}	5.18	4.14	6.93×10^{-4}	0.092		9.13×10^{-2}	

For bioconcentration, the elimination rate constant in the whole body except GI tract or gut in zebrafish or *D. magna* (k_{eb}) includes depuration rate constant (k_d), biotransformation rate constant (k_M), and growth dilution rate constant. In our studies, there was no growth during the exposure, thus the k_M can be calculated by ($k_{eb} - k_d$). The depuration rate constant (k_d) can be calculated from waterborne uptake rate constant (k_u) according to Fick's first law and two resistance film model.[16, 17] $k_d = k_u / ((1 - f_{lip}) + f_{lip} \times k_{ow})$, where the dimension of k_u is (mL g^{-1} in wet weight h^{-1}); and f_{lip} is the lipid content based on wet weight; k_{ow} is the octanol-water partitioning coefficient and referred from our previous study.[5] The k_u and k_{eb} for zebrafish were referred from our previous study, and k_u was transferred to mL g^{-1} in wet weight h^{-1} by the conversion ratio of 0.217 (dry weight/wet weight of zebrafish).[4] The lipid content in dry weight is 40% in *C. vulgaris*,[18, 19] 20.5% in *D. magna*,[4] and 18.2% in zebrafish.[4] The lipid content in wet weight was 1.42% and 3.95% for *D. magna* and zebrafish, respectively.[4]

Considering there are two k_{eb} values obtained for *D. magna* including elimination rate constant under waterborne-only uptake (k_{eb_Aq}), and elimination rate constant under both waterborne and dietary uptakes ($k_{eb\_Aq\_D}$). k_{M_Aq} was derived by $k_{eb\_Aq} - k_d$, whereas $k_{M\_Aq\_D}$ was derived by $k_{eb\_Aq\_D} - k_d$. As discussed in the main text, the higher k_{eb_Aq} compared with $k_{eb\_Aq\_D}$ might be due to lipid loss under starvation when food was not available. Nevertheless, the biotransformation would be expected to be higher when food is available. Therefore, the $k_{M\_Aq\_D}$ might be more accurate for representing biotransformation rate constant of PAHs-d_{10} in *D. magna*. The negative value suggested that there would be no biotransformation.

Table S9. Bioconcentration factor of PCBs at steady-state (log$BCF_{ss\text{-}lip}$) in aquatic organisms after lipid normalization collected from literature.

log$BCF_{ss\text{-}lip}$ (L/kg)	Algae[20] (*Thalassiosira weissflogii*)	Copepod[20] (*Acartia clause*)	Adult zebrafish[21] (*Brachydanio rerio*)
PCB 31	6.97	6.29	6.19
PCB 101	7.78	6.87	7.00
PCB 153	8.58	7.23	7.18

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