Supporting Information for

Dietary Uptake Patterns Affect Bioaccumulation and Biomagnification of Hydrophobic Organic Compounds in Fish

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Methods and Materials

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%. 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. The commercial fish food (with ≥ 29% protein, ≥ 2% lipids, ≥ 8% total amino acid, ≤ 15% fiber, ≤ 15% ash, ≤ 12% water, and 0.5–2.0% total phosphorus) was purchased from Beijing Sanyou Chuang Mei feed Technology Co., Ltd. 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.

Cultivation of *Daphnia magna* (*D. magna*) and zebrafish

D. magna were raised in our laboratory for 1 year before they were used for the exposure experiments. They were cultured in artificial water (AFW) made from Milli-Q water 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).[1] The temperature was maintained at 23 ± 0.5 ℃ and the photoperiod was set under 16 h: 8 h (light: dark), and the density of *D. magna* was kept at one individual per 10 mL AFW. *D. magna* fed on the green algae *Chlorella vulgaris* on a daily basis at a density of approximately 5 × 10^5 cells mL^{-1}. The AFW was refreshed every other day.

Mature zebrafish (wild-type line AB) of the same generation and similar size were cultured in AFW for at least 2 weeks with the temperature maintained at 23 ± 0.5 ℃ and the photoperiod set under 16 h: 8 h (light: dark). They fed on commercial fish food on a daily basis (1.5% of the wet weight of zebrafish). The zebrafish were placed in AFW without feeding for 1 day to clear their guts before the exposure experiments.

Establishment of passive dosing systems

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 squeezed into each 60 mm-diameter glass culture dish to obtain a passive dosing dish. These dishes were vacuumed to eliminate trapped air, and then placed at room temperature for at least 24 h, and subsequently placed in an oven at 110ºC for 48 h to complete curing. Then passive dosing dishes were immersed in methanol for at least 72 h to remove impurities and oligomers. To obtain the partition coefficients between methanol loading solution (MeOH) and AFW ($K_{\mathrm{MeOH:\,AFW}}$), a series of MeOH with gradient concentrations of phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10} were prepared. Passive dosing dishes were immersed in these different MeOH for at least 72 h with the loading solution refreshed every 24 h. The density was kept at one dish per 100 mL loading solution. After loading, these dishes were rinsed with Milli-Q water and then placed into AFW for 24 h, which was long enough for the PAHs-d_{10} to reach equilibrium between passive dosing dishes and AFW. The partition coefficient ($K_{\mathrm{MeOH:\,AFW}}$) was calculated as follows:

$$K_{\mathrm{MeOH:\,AFW}} = \frac{C_{\mathrm{MeOH}}}{C_{\mathrm{AFW}}} \tag{1}$$

where C_{MeOH} is the PAH-d_{10} concentration in MeOH (μg L^{-1}); C_{AFW} is the freely dissolved PAH-d_{10} concentration in AFW (μg L^{-1}). The detail information on the derivation of $K_{\mathrm{MeOH:\,AFW}}$ was elaborated in our previous study.[2] The values of $K_{\mathrm{MeOH:AFW}}$ 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, through linear regression between C_{MeOH} and C_{AFW} in terms of the equation (1) (Figure S12). According to the values of $K_{\mathrm{MeOH:\,AFW}}$, the stock was prepared by dissolving 0.4571 g L^{-1} phenanthrene-d_{10}, 0.5125 g L^{-1} anthracene-d_{10}, 0.7168 g L^{-1} fluoranthene-d_{10}, and 0.7534 g L^{-1} pyrene-d_{10} in methanol. An aliquot of the stock was diluted at 100: 1 to obtain the loading solution. Passive dosing dishes were loaded in dilution solution for at least 72 h, for which the corresponding freely dissolved concentrations of the PAHs-d_{10} in AFW were at 100 ng L^{-1}. After loading, these dishes were rinsed with Milli-Q water. The passive dosing systems were established by placing 3 passive dosing dishes in a 2 L glass beaker.

Pre-exposure of the PAHs-d_{10} to *D. magna*

Mature *D. magna* (7 days old) without eggs on their back were sieved through 18-mesh (1.2 mm) sifter to guarantee the similar size and no loss of the PAHs--d_{10} due to reproduction during the exposure. A total of 80 mature *D. magna* were exposed for 48 h to 100 ng L^{-1} PAHs-d_{10} maintained by passive dosing dishes at 23 ± 0.5°C under a 16 h: 8 h (light: dark) photoperiod. *D. magna* were sampled at time points 24 and 48 h. At each time point, a total of 40 *D. magna* were sampled by a glass pipet to the culture dish and rinsed with Milli-Q water. Another 40 *D. magna* were sampled from AFW as a control. Then these *D. magna* were dried by filter paper and transferred to pre-weighed aluminum foils to obtain the wet weight. Sampled *D. magna* were stored at -20°C until subsequent procedures. During the exposure, nothing was fed to *D. magna*. The AFW was sampled at time points 0 h and 48 h to measure the concentrations of the PAHs-d_{10}. The experiment was conducted in triplicate.

Spiking commercial fish food with the PAHs-d_{10}

Commercial fish food was spiked with the PAHs-d_{10} by soaking them into methanol solution for 24 h with high PAHs-d_{10} concentrations (up to several hundred mg kg^{-1}) in an incubator at 60 rpm under 25°C and then dried at 65°C overnight.

Exposure experiments of zebrafish

Single dietary exposure to spiked fish food. The spiked fish food (3% of the wet weight of zebrafish) was fed to zebrafish in AFW without PAHs-d_{10}. After ingestion for 20 min, the water was changed completely to avoid the pollution of the PAHs-d_{10} desorbed from the spiked fish food. During the change, we did not observe obvious stressful responses to zebrafish. The changed water was filtered through a pre-weighed glass-fiber filter (pore size 0.45 μm; Whatman, GF/F) which has been dried at 65°C overnight to calculate the weight of uneaten spiked fish food. The actual intake of spiked fish food was about 1.5% of the wet weight of zebrafish.

Analysis of the PAHs-d_{10}

The PAHs-d_{10} in AFW were extracted by solid phase extraction (SPE) cartridge.[3] Briefly, each SPE cartridge (Waters HLB, 500 mg, 6 cc) was conditioned successively with 3 mL dichloromethane for 3 times, 3 mL methanol for 3 times, and 3 mL Milli-Q water for 3 times. The sampled water (300

mL) was firstly filtered through a glass-fiber filter (pore size 0.45 μm; Whatman, GF/F), and then flowed through SPE cartridge at a rate of 15 mL/min. After that, the cartridge was freeze-dried for 48 h and then eluted with 6 mL dichloromethane for three times. The combined eluents were 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 internal standard and then diluted to 1 mL with *n*-hexane. The vial was sealed and kept at -4 ºC before analysis of the PAH-d_{10} concentrations.

The PAHs-d_{10} in zebrafish, *D. magna*, and spiked commercial fish food were extracted by organic solvent.[2, 4, 5] In details, the samples were freeze-dried for at least 72 h and then ground in a ceramic mortar to get homogenates. Subsequently, each homogenate was transferred into a 5 mL glass tube added with 4 mL extraction agent (*n*-hexane: dichloromethane = 1:1, v:v) and 10 μL (10 mg L^{-1}) of surrogate standard 2-fluorobiphenyl. The tube was sealed and vortexed for 30 s and then bathed in an ultrasonic machine for 30 min. The extract in the tube was transferred, and another 4 mL extraction agent was added for the second vortex (30 s) and ultrasonic bath (30 min). The two extracts were combined and transferred into a 10 mL glass tube. Subsequently, the mixture was concentrated to less than 2 mL under a gentle nitrogen blow and then filtered through a 0.45 μm Teflon membrane. After, the filtrate was concentrated with gentle nitrogen blow to less than 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 (1 mg L^{-1}) as internal standard and then diluted to 1 mL with *n*-hexane. The vial was sealed and kept at -4ºC before analysis of the PAH-d_{10} concentrations.

The concentrations of the 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). The carrier gas was high-purity helium with a constant flow rate of 1 mL/min. The oven temperature program started from 80ºC initially for 3 min, and then increased to 250ºC at a rate of 10ºC/min, and finally fixed at 250ºC for 2 min. The injector temperature was 280ºC. The sample size was 1 μL in the way of splitless injection. Multi-reaction monitoring (MRM) transition mode was applied to determine PAHs-d_{10} by monitoring two precursor ion/product ion transitions for quantification and confirmation. The source temperature was set at 230ºC with EI ion source. The detail setups were shown in Table S9.

Lipid analysis

The lipid contents of zebrafish, *D. magna*, and commercial fish food were analyzed by solvent extraction method, and the dosage of solvent was scaled back to adapt for the present study without changing the solvent ratios proposed in the original literature.[6] The samples were freeze-dried for at least 72 h and then ground in a ceramic mortar to get homogenates. Subsequently, each homogenate was transferred into a 10 mL centrifuge tube added with 1 mL chloroform, 2 mL methanol, and 0.8 mL distilled water. The tube was vortexed for 30 s and then bathed in an ultrasonic machine for 5 min. Then another 1 mL chloroform was added followed by being vortexed for 30 s and bathed in an ultrasonic machine for 5 min. After, another 1 mL distilled water was added followed by being vortexed for 30 s and bathed in an ultrasonic machine for 5 min. After stratification for 10 min, the bottom layer containing lipid was transferred to a pre-weighed 5 mL centrifuge tube. The mixture was evaporated to dryness under gentle nitrogen flow and then the centrifuge tube was dried at 65°C overnight. The lipid content of each sample was obtained by the difference in the weight of dried centrifuge and original centrifuge.

Quality assurance and quality control

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 coefficients of internal standard calibration curves for each PAH-d_{10} were all higher than 0.99 (Figure S13). The recoveries of SPE for phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10} were 87.6 ± 10.6%, 76.7 ± 8.6%, 87.6 ± 10.1%, and 85.4 ± 9.9% (n = 5), respectively. The recoveries of extraction for phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10} in zebrafish were 98.6 ± 0.12%, 98.4 ± 0.14%, 99.2 ± 0.05%, and 99.1 ± 0.06% (n = 6), respectively.

The freely dissolved concentrations of the PAHs-d_{10} were maintained constant through passive dosing dishes in the first 25 days (bioconcentration and bioaccumulation) during the 34-day exposure (one-way ANOVA with a Tukey *posthoc* test, $p > 0.067$ for the four PAHs-d_{10}, Table S2). It should be noted that for only single dietary uptake exposure to spiked fish food, the concentrations of the PAHs-d_{10} in AFW at time point 20 min (i.e. the end of ingestion) were up to 156 ± 7 ng L^{-1}, 128 ± 8 ng L^{-1}, 126 ± 11 ng L^{-1}, and 118 ± 10 ng L^{-1} for phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10}, respectively, indicating the occurrence of desorption of PAHs-d_{10} from commercial

fish food. Nonetheless, the AFW was changed immediately after the 20 min-ingestion and the concentrations of the PAHs-d_{10} at time point 24 h in AFW were below the detection limits, suggesting that the accumulation of the PAHs-d_{10} can be considered only from dietary uptake. For only dietary uptake from *D. magna* during a 13-day exposure, the concentrations of the PAHs-d_{10} in AFW were extremely low (Table S2). Due to the ferocious predation, the *D. magna* were ingested very rapidly by zebrafish once they were put into AFW, which made the release of the PAHs-d_{10} into AFW from *D. magna* (depuration) very limited.

No significant differences in the dry weight of the whole body (except GI tract) were found among the zebrafish of different groups, indicating that the PAHs-d_{10} and food intake did not have obvious effects on zebrafish growth. No mortality was observed in any groups throughout the experiment.

According to the results shown in Table S2 and S10, *D. magna* were exposed to basically the same freely dissolved PAH-d_{10} concentrations with zebrafish. Moreover, the steady-state concentrations of the PAHs-d_{10} in *D. magna* were basically achieved after exposure for 24 h, as indicated there was no significant difference observed in the concentrations between 24 h and 48 h (p = 0.542, 0.858, 0.619, and 0.408 for phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10}, respectively, Figure S14).

Estimation of the parameters in the kinetic model.

According to the differential equation set (1) in the main text, the function of $m_f(t)$, $C_i(t)$, and $C_b(t)$ can be obtained through integration. The corresponding $C_f(t)$ can be obtained (C_f is the PAH-d_{10} concentration in the ingested food, ng g^{-1}, defined as m_f / W_f, where W_f is the dry weight of the ingested food, g). The W_f is the function of t which can be regarded as first-order kinetics (Figure S6), and the $W_f(t)$ for *D. magna* and spiked fish food was regarded as the same in the present study. The measured data were the PAH-d_{10} concentrations in the gastrointestinal (GI) tract including ingested food (C_g, ng g^{-1}) and the whole body (except GI tract) of zebrafish (C_b, ng g^{-1}). The function of C_g can be obtained as follows:

$$C_g(t) = \frac{C_i(t)W_i + C_f(t)W_f(t)}{W_i + W_f(t)} \quad (2)$$

The parameters were estimated by fitting the data in each experimental treatment except the control

group (Figures 2–5 and Figures S1–4) all into the C_g (t) and C_b (t). Considering the heteroscedasticity of the data, the method of weighted least squares was applied. The FSD weighting scheme was applied which assigned the reciprocal of each datum as the weight. The objective function (OF) for optimization of fitting was obtained:

$$OF = \frac{1}{M}\sum_{j=1}^{j}\sum_{i=1}^{N_j}\left(\log\left[v_j \cdot y_{i,j}{}^2 \right] + \frac{\left(y_{i,j} - y_{\text{model}} \right)^2}{v_j \cdot y_{i,j}{}^2} \right) \quad (3)$$

where M is the total number of the data; j is the number of data sets; N_j is the number of data points in the j-th data set; v_j is the estimated variance parameter in the j-th data set; $y_{i,j}$ is the measured i-th datum in the j-th data set; y_{model} is the model value corresponding to $y_{i,j}$ at time point $t_{i,j}$. The differential equation set in the main text was solved using the Runge−Kutta integrator. A level of 0.05 was set for the convergence criterion. The fitting process was conducted by Simulation Analysis and Modeling (SAAM II modeling software version 2.3.1, University of Washington, Seattle, WA, USA). The model inputs can be found in Table S3.

Monte Carlo simulation

In natural aquatic environments, the average frequency of dietary uptake will be affected by the food web. Different aquatic ecosystems will lead to different average frequencies of dietary uptake. However, dietary uptake is a random process at any moment. Therefore, in the present study, a Monte Carlo simulation based on the kinetic model and estimated parameters was run to study the effect of dietary uptake pattern on bioaccumulation of the PAHs-d_{10} in zebrafish, considering the randomness of dietary uptake from *D. magna*. We assumed that the number of encountering *D. magna* at time point t for zebrafish (N (t)) was a Poisson point process with parameter λ reflecting the average frequencies of dietary uptake. The Poisson point process has been widely used to describe the numbers of a random event occurred during a period of time.[7] The weight of *D. magna* at each time encounter, which represented the amount of food, was treated as a random variable conforming to a normal distribution N (μ, σ^2). Moreover, the freely dissolved concentrations of the PAHs-d_{10} in water and the concentrations of the PAHs-d_{10} in *D. magna* were set as the same as in the experimental section (Table S3 and S5). The maximum intake of *D. magna* (calculated in dry weight) was set to be no more than 2% of the wet weight of zebrafish. The food weight variation in the GI tract of zebrafish is shown in Figure S6. If the amount of food at time point t_i when zebrafish encounters *D. magna* is

more than the difference between the maximum intake and $W_f(t_i)$ (the weight of *Daphnia manga* in the GI tract of zebrafish at time point t_i), zebrafish will consume *Daphnia manga* until the maximum intake is achieved; if the amount of food at time point t_i is less than the difference between the maximum intake and $W_f(t_i)$, zebrafish will consume all the *D. magna* at time point t_i.

For each PAH-d_{10}, we set two hundred different conditions with the average number of encountering *D. magna* per day (n) ranging from 1 to 20 per day ($n = 24 / \lambda$) and μ ranging from 10% to 100% relative to the maximum intake. The σ was fixed at 10% of μ. For each condition, we made use of the result that the interval between successive events of $N(t)$ were independent exponential random variables. Based on the parameter λ, we firstly generated these interarrival times by random code in MATLAB, thus obtaining the moment of dietary uptake. Then we generated the number of encountering *D. magna* based on the normal distribution $N(\mu, \sigma^2)$. Finally, each initial value of ingestion amount of the PAHs-d_{10} was obtained considering the concentrations of the PAHs-d_{10} in *D. magna* were constant during simulation (Table S5). The simulation curve was obtained through consecutive integration between these interarrival times according to each initial value of ingestion amount of the PAHs-d_{10} and estimated parameters of the kinetic model. The simulation of each condition was run 100 times for 500 hours each. We abandoned the first 200 hours' data for each run to assure that the concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish reach the steady-state. The time points that the PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than the steady-state with waterborne-only uptake, and the ones higher than that in *D. magna* after lipid normalization (Table S6) were recorded to calculate the frequencies reaching these two situations, respectively, which can represent the corresponding probabilities. The simulation was conducted using MATLAB (R2012b, The MathWorks, Inc., USA).

In order to confirm to what extent additional dietary uptake from *D. magna* will significantly increase the steady-state concentrations of the PAHs-d_{10} with waterborne-only uptake, the reverse process of the *t*-test was applied to calculate the steady-state concentrations of the PAHs-d_{10} with additional dietary uptake from *D. magna*. Theoretically, we assumed that the standard deviation was fixed either for the steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish with waterborne-only uptake or for ones with both waterborne uptake and dietary uptake. If the sample sizes remained the same, the values can be calculated by the following equations:

$$\begin{cases} l = \left(\frac{s_x^2}{n} + \frac{s_y^2}{m} \right)^2 / \left(\frac{s_x^4}{n^2(n-1)} + \frac{s_y^4}{m^2(m-1)} \right) \\ t^* = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{s_x^2}{n} + \frac{s_y^2}{m}}} \\ t^* \geq t_{1-\alpha}(l) \end{cases} \quad (4)$$

where l is the calculated statistic; s_x is the standard deviation of the steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish with both waterborne uptake and dietary uptake; n is the corresponding sample size; s_y is the standard deviation of the steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish with waterborne-only uptake; m is the corresponding sample size; t^* is the calculated statistic; $\bar{x}$ is the average steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish with both waterborne uptake and dietary uptake; $\bar{y}$ is the average steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish with waterborne-only uptake. The statistic t^* conforms to t distribution. The results were shown in Table S11.

The calculation of BCF, BAF, and BMF

The lipid normalized bioconcentration factor (waterborne-only, $BCF_{ss\text{-}lip}$, L kg^{-1}) was calculated as:

$$BCF_{ss\text{-}lip} = \frac{C_{b\text{-}ss}}{C_w \cdot f_{lip\text{-}zebrafish}} \quad (5)$$

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

$$BAF_{ss\text{-}lip} = \frac{C_{b\text{-}ss}}{C_w \cdot f_{lip\text{-}zebrafish}} \quad (6)$$

The lipid normalized biomagnification factor was calculated as:

$$BMF_{ss\text{-}lip} = \frac{C_{b\text{-}ss}}{f_{lip\text{-}zebrafish}} / \frac{C_{f\text{-}ss}}{f_{lip\text{-}food}} \quad (7)$$

where $C_{b\text{-}ss}$ is the steady-state concentrations of the PAHs-d_{10} in the whole body (except GI tract) of zebrafish (ng g^{-1}, in dry weight); C_w is the freely dissolved concentrations of the PAHs-d_{10} in water (ng mL^{-1}); $C_{f\text{-}ss}$ is the concentrations of the PAHs-d_{10} in food (i.e. *D. magna* or spiked fish food, ng

g^{-1}, Table S4 and S5); $f_{lip\text{-}zebrafish}$ is the lipid content of zebrafish in dry weight (%, Table S6); $f_{lip\text{-}food}$ is the lipid content of food (%, i.e. *D. magna* or spiked fish food, Table S6).

The frequently used bioaccumulation kinetic model

The frequently used bioaccumulation (both waterborne uptake and dietary uptake) kinetic model is as follows:[8, 9]

$$\frac{dC_b}{dt} = k_u C_w + k_D C_D - C_b k_e \quad (8)$$

C_b is the HOC concentration in fish (ng g^{-1}); t is the exposure time (h); k_u is the HOC waterborne uptake rate constant (mL g^{-1} h^{-1}); C_w is the freely dissolved HOC concentration (ng mL^{-1}); k_D is the HOC dietary uptake rate constant (g g^{-1} h^{-1}), and k_D is a product of the food ingestion rate (I; g g^{-1} h^{-1}) and the HOC assimilation efficiency from the diet (dimensionless); C_D is the HOC concentration in diet (ng g^{-1}); k_e is the HOC elimination rate constant including depuration rate constant, fecal egestion rate constant, biotransformation rate constant, and growth dilution rate constant (h^{-1}).

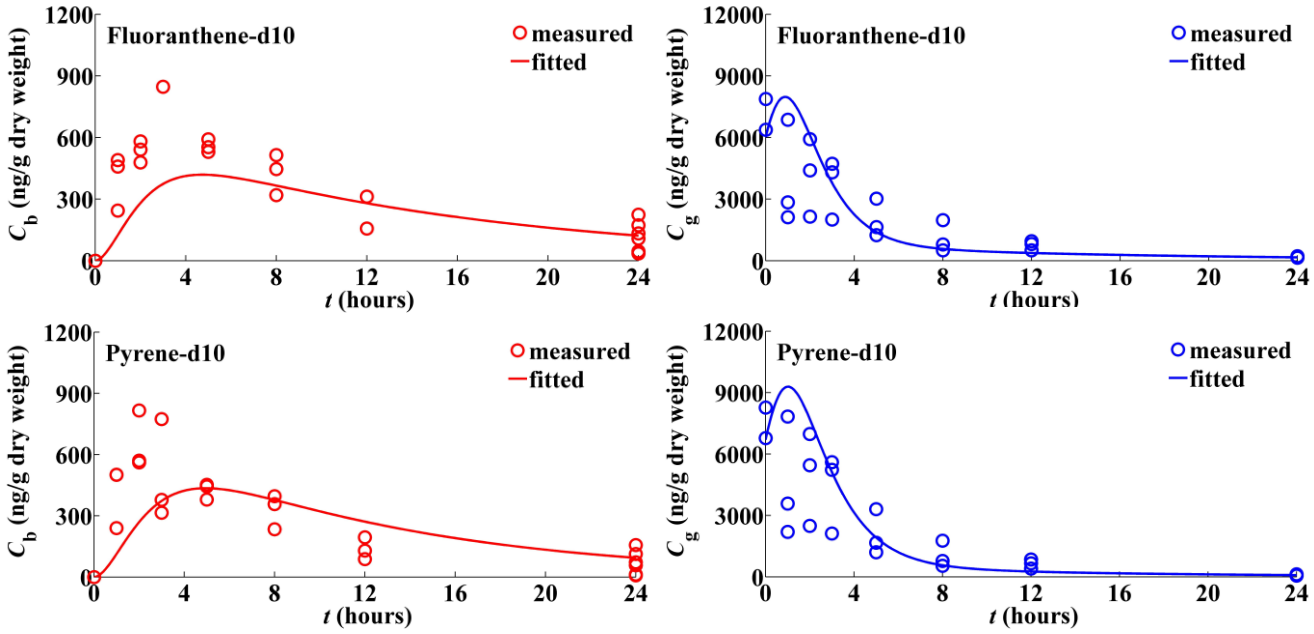

Figure S1. Variation in the PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish (C_b) and in the GI tract of zebrafish (C_g) after single dietary exposure to spiked fish food (μg g^{-1} PAHs-d_{10} concentrations).

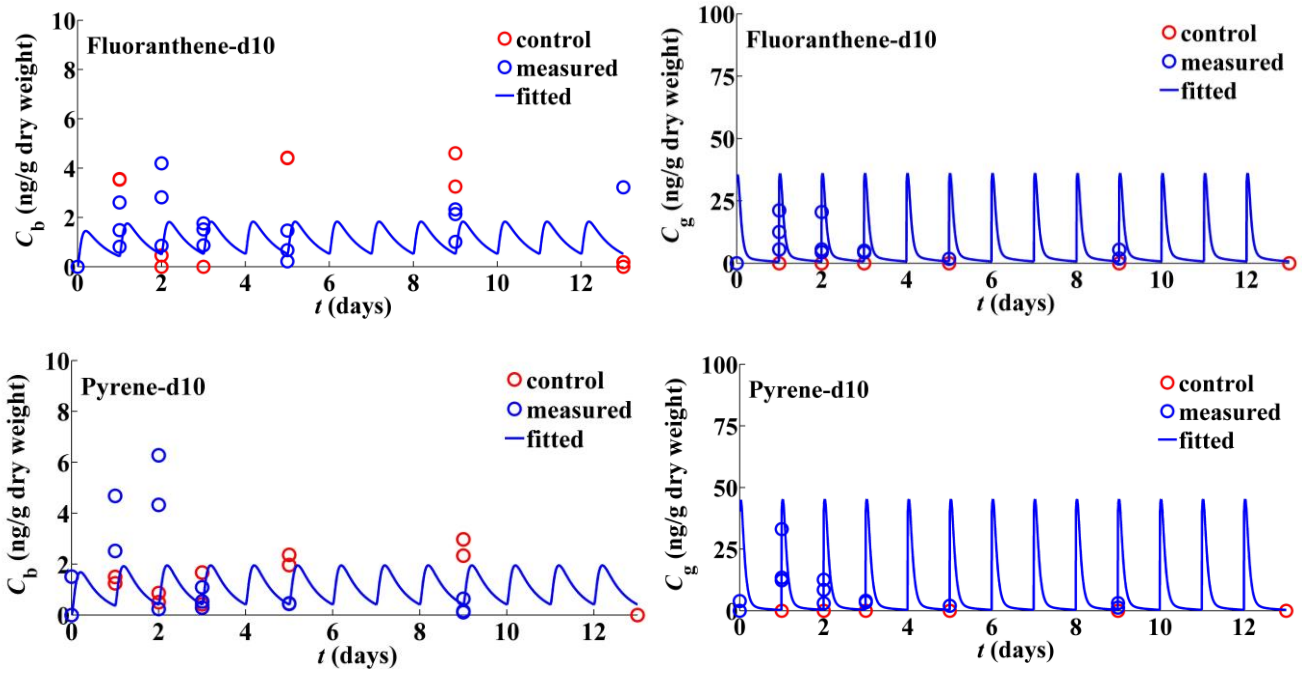

Figure S2. Variation in the PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish (C_b) and in the GI tract of zebrafish (C_g) with only dietary uptake from *D. magna* for 13 days (single dietary uptake per day, ng g^{-1} PAHs-d_{10} concentrations). The control group was conducted in artificial water (AFW) in the absence of the PAHs-d_{10} with non-spiked fish food.

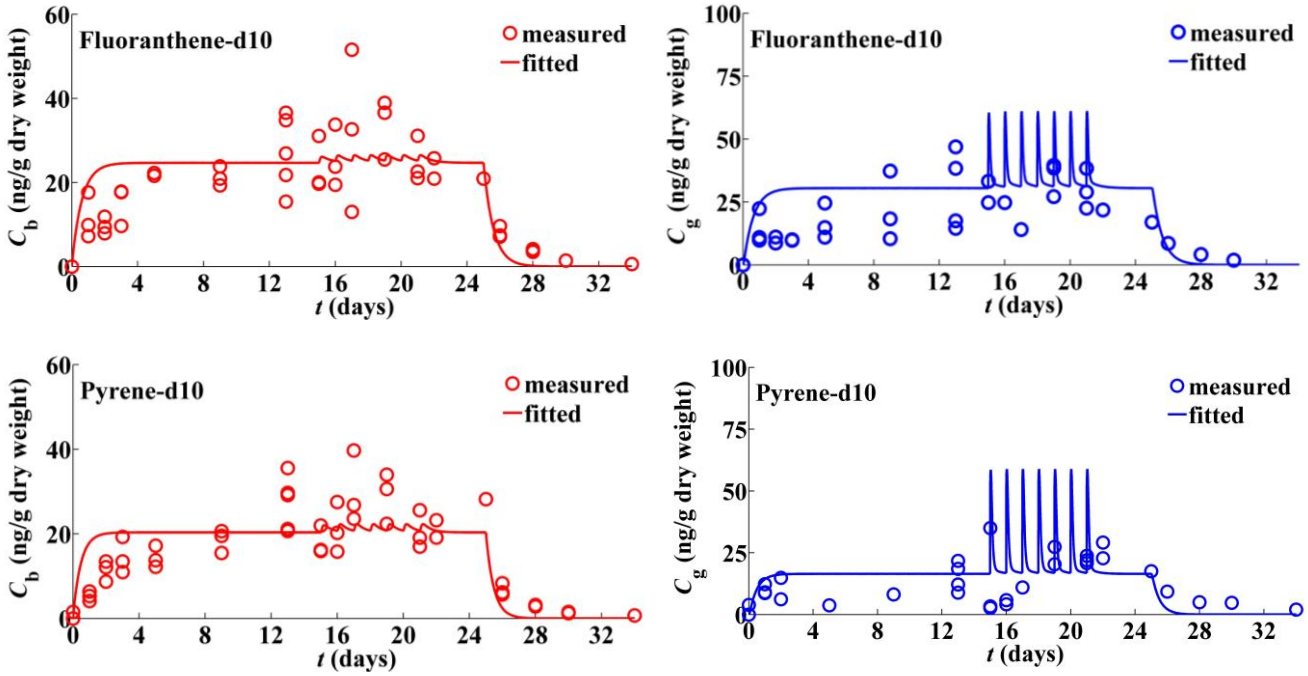

Figure S3. Variation in the PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish (C_b) and in the GI tract of zebrafish (C_g) during a 34-day exposure including a 15-day bioconcentration (0–15th day, waterborne-only uptake), a 10-day bioaccumulation (15–25th day, both waterborne uptake and dietary uptake from *D. magna* (ng g^{-1} PAHs-d_{10} concentrations), single dietary uptake per day started from the 15th day and ended at the 21th day), and a 9-day depuration (25–34th day).

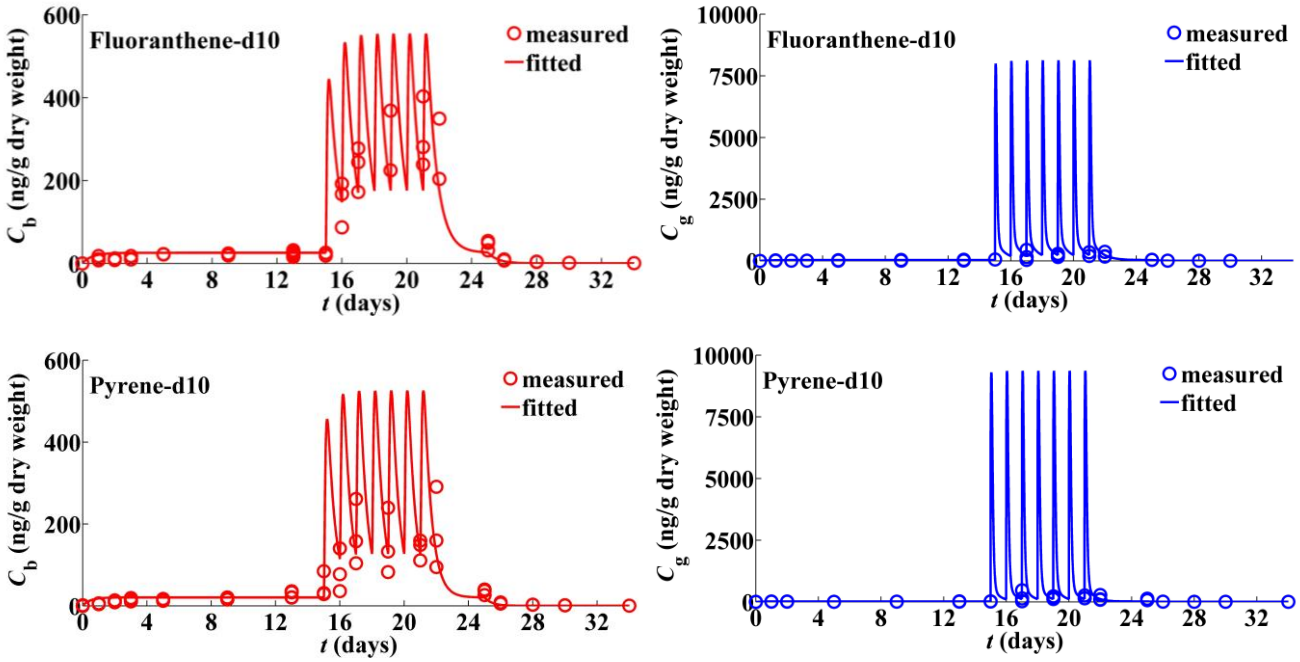

Figure S4. Variation in the PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish (C_b) and in the GI tract of zebrafish (C_g) during a 34-day exposure including a 15-day bioconcentration (0–15th day, waterborne-only uptake), a 10-day bioaccumulation (15–25th day, both waterborne uptake and dietary uptake from spiked fish food (μg g^{-1} PAHs-d_{10} concentrations), single dietary uptake per day started from the 15th day and ended at the 21th day), and a 9-day depuration (25–34th day).

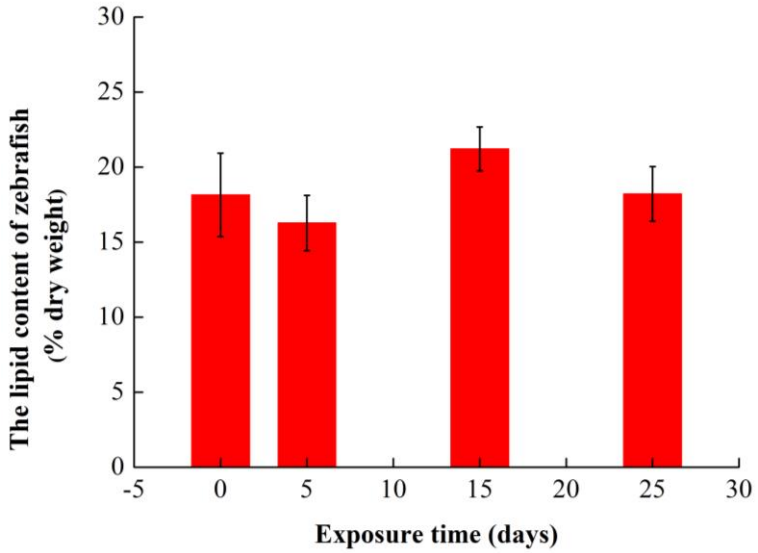

Figure S5. Lipid content of zebrafish (% dry weight) during a 25-day exposure (mean ± standard deviation, n = 3). During this period, the zebrafish fed on non-spiked fish food within 1.5% of the wet weight of itself in order to be in accordance with the food intake conducted in the dietary uptake experiments. No significant difference was observed in lipid content over exposure time (one-way ANOVA with a Tukey *posthoc* test, p = 0.700).

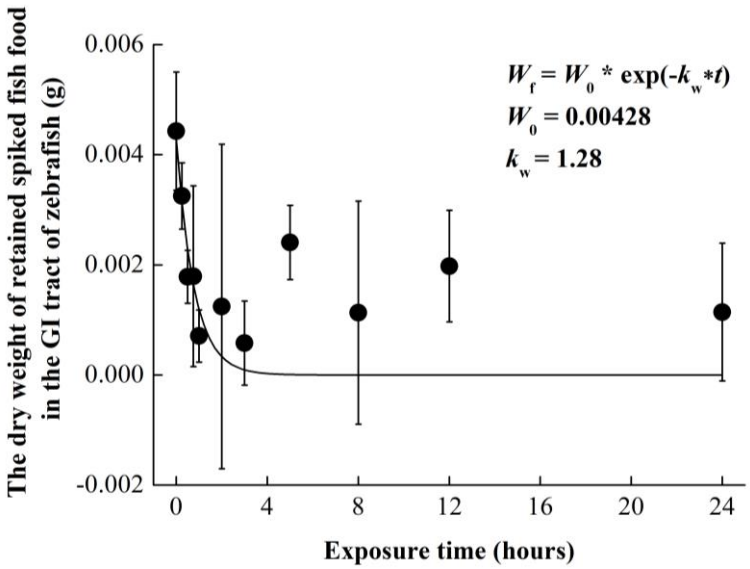

Figure S6. The kinetics of the retained spiked fish food weight in the GI tract of zebrafish, mean ± standard deviation, n = 3. The feeding amount of the spiked fish food (1.5 % of the wet weight of zebrafish) was consistent with that in dietary exposure to spiked fish food. The GI tract of zebrafish was sampled at different time points to measure the actual ingestion amount of the spiked fish food and obtain the kinetics of retained spiked fish food.

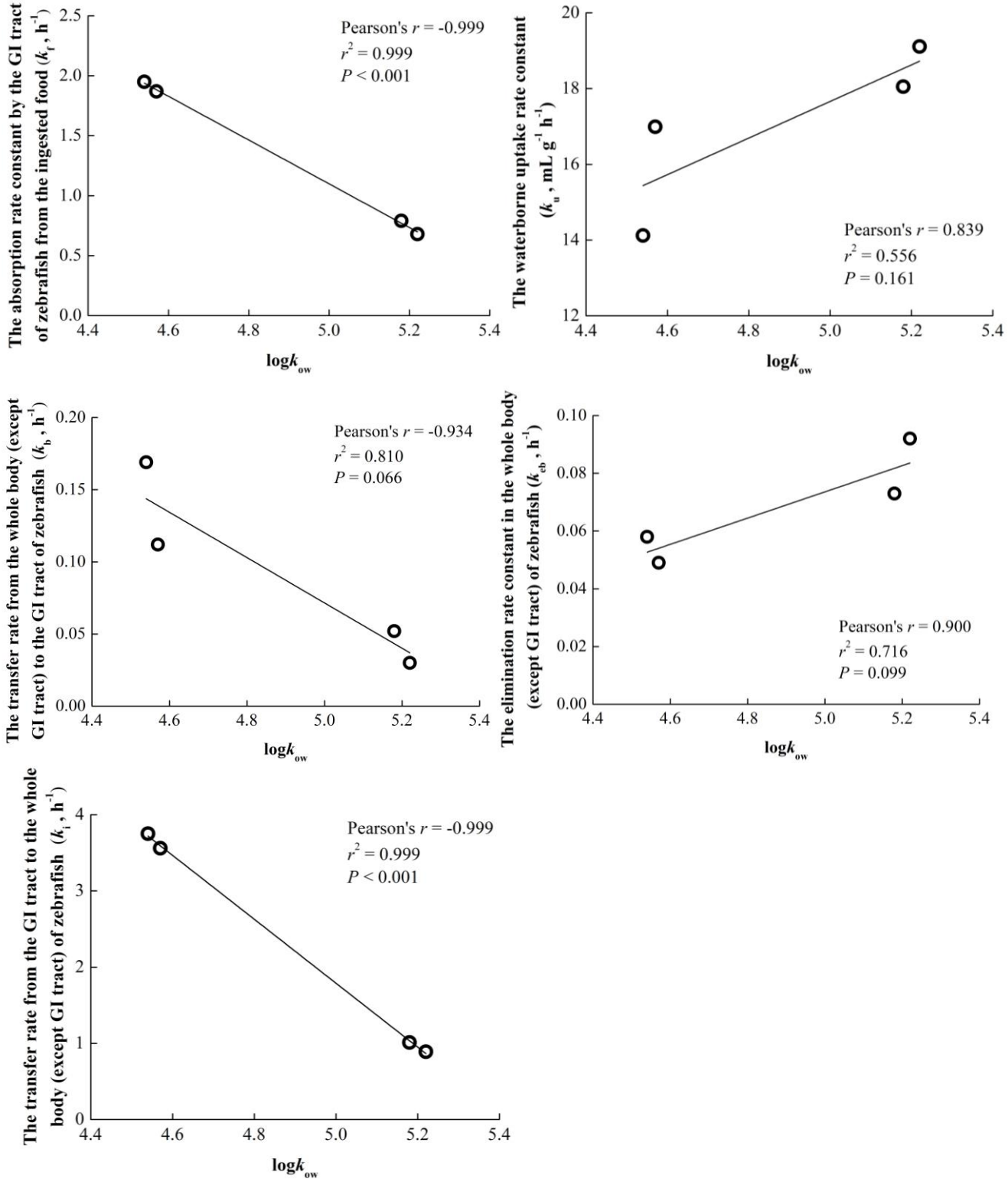

Figure S7. The correlation analysis between logarithmic octanol-water partitioning coefficients (logk_{ow}) and the estimated bioaccumulation kinetic parameters of the PAHs-d_{10} in zebrafish.

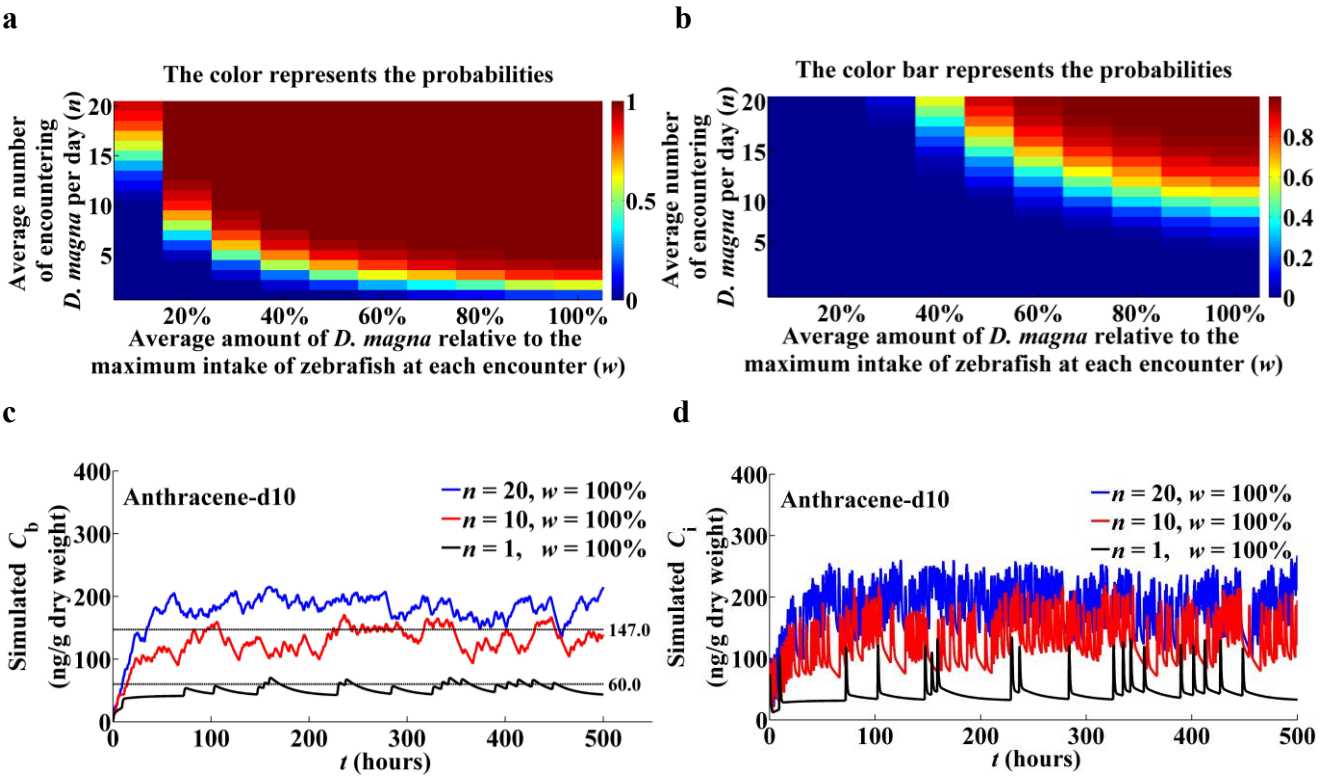

Figure S8. Probability distributions (a, b) and simulated concentrations (c, d) of anthracene-d_{10} in zebrafish under different dietary uptake patterns. (a) The probability distribution of the situation that additional dietary uptake from *D. magna* significantly increases the steady-state concentration of anthracene-d_{10} in the whole body (except GI tract) of zebrafish compared with waterborne-only uptake, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than the steady-state with waterborne-only uptake (i.e., 60.0, Table S11). (b) The probability distribution of biomagnification, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than that in *D. magna* (i.e. 147.0, Table S5) after lipid normalization (Table S6). (c) The simulation of anthracene-d_{10} concentrations in the whole body (except GI tract) (C_b). (d) The simulation of anthracene-d_{10} concentrations in the GI tract of zebrafish (C_i). Subsets c and d are the examples from repeated runs in Monte Carlo simulation.

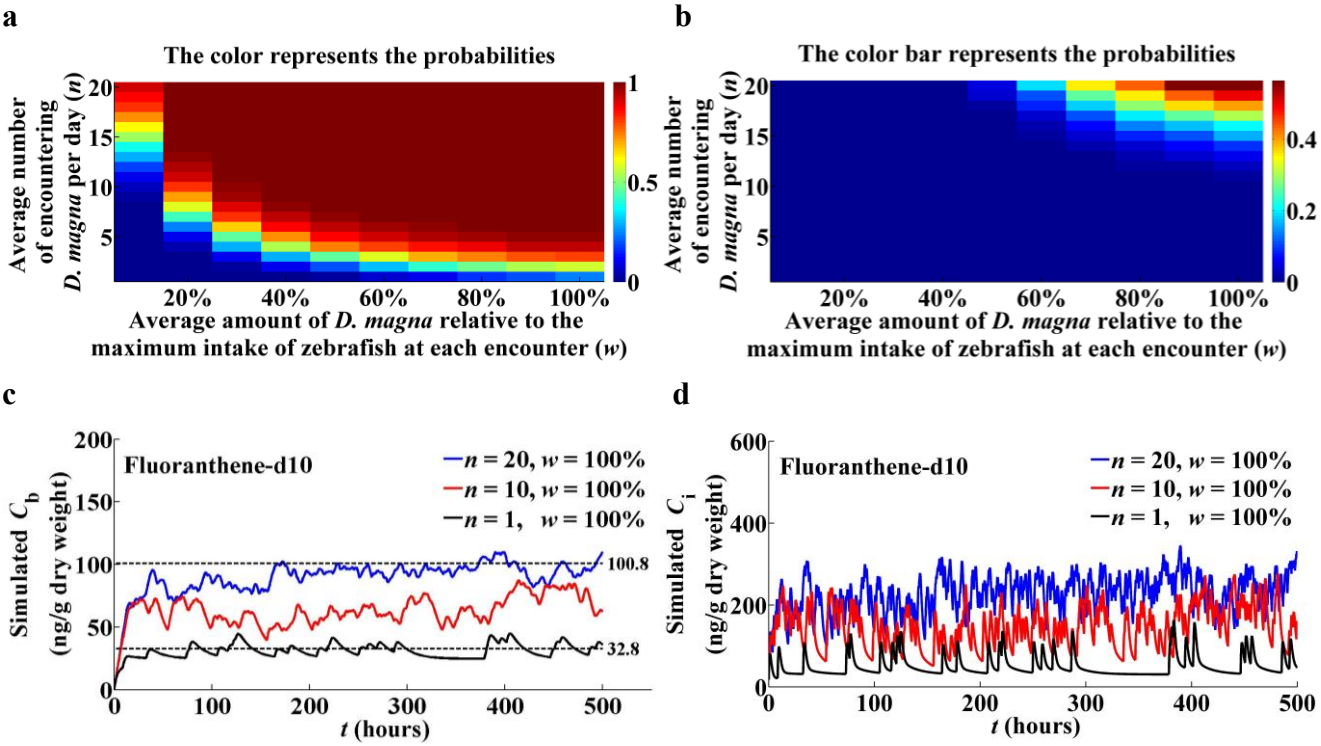

Figure S9. Probability distributions (a, b) and simulated concentrations (c, d) of fluoranthene-d_{10} in zebrafish under different dietary uptake patterns. (a) The probability distribution of the situation that additional dietary uptake from *D. magna* significantly increases the steady-state concentration of fluoranthene-d_{10} in the whole body (except GI tract) of zebrafish compared with waterborne-only uptake, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than the steady-state with waterborne-only uptake (i.e., 32.8, Table S11). (b) The probability distribution of biomagnification, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than that in *D. magna* (i.e. 100.8, Table S5) after lipid normalization (Table S6). (c) The simulation of fluoranthene-d_{10} concentrations in the whole body (except GI tract) (C_b). (d) The simulation of fluoranthene-d_{10} concentrations in the GI tract of zebrafish (C_i). Subsets c and d are the examples from repeated runs in Monte Carlo simulation.

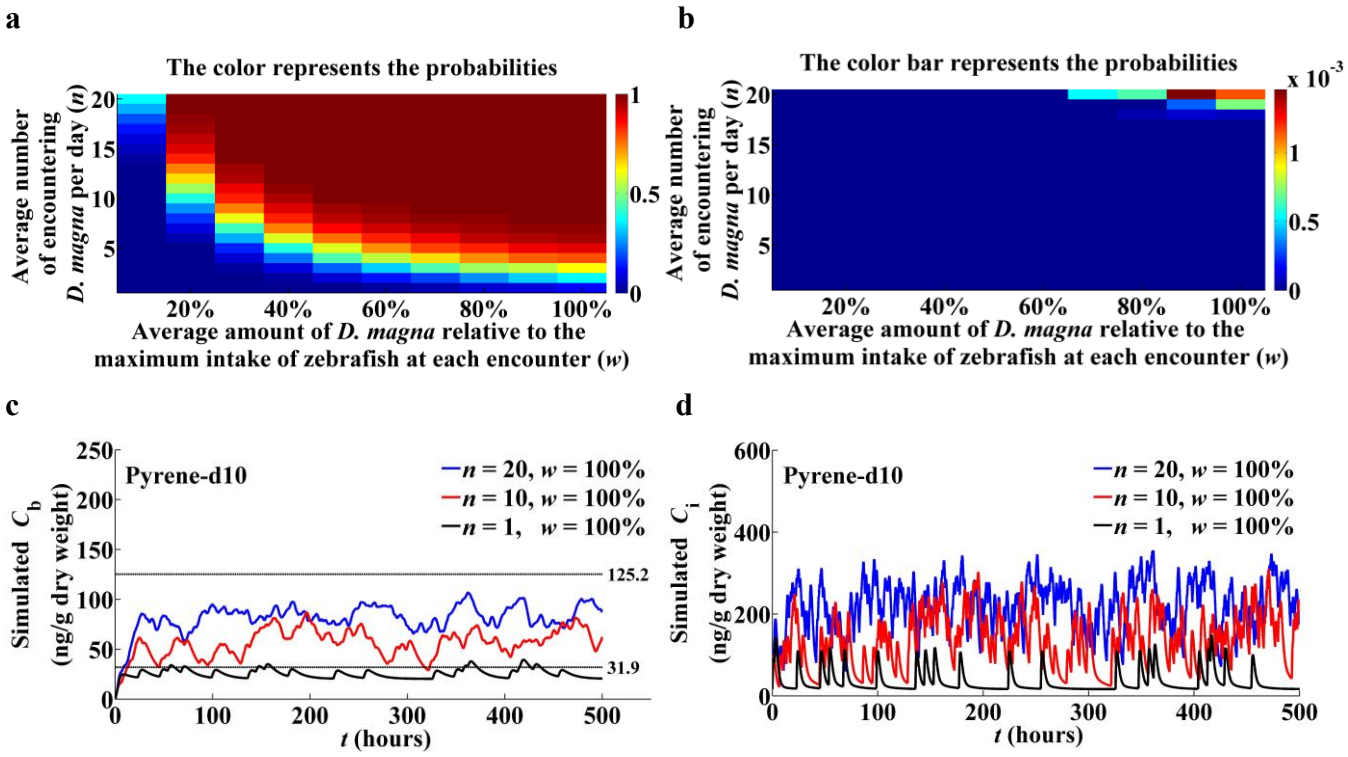

Figure S10. Probability distributions (a, b) and simulated concentrations (c, d) of pyrene-d_{10} in zebrafish under different dietary uptake patterns. (a) The probability distribution of the situation that additional dietary uptake from *D. magna* significantly increases the steady-state concentration of pyrene-d_{10} in the whole body (except GI tract) of zebrafish compared with waterborne-only uptake, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than the steady-state with waterborne-only uptake (i.e., 31.9, Table S11). (b) the probability distribution of biomagnification, which was obtained by calculating the frequencies that the simulated PAH-d_{10} concentrations in the whole body (except GI tract) of zebrafish were significantly higher than that in *D. magna* (i.e. 125.2, Table S5) after lipid normalization (Table S6).; (c) The simulation of pyrene-d_{10} concentrations in the whole body (except GI tract) (C_b). (d) The simulation of pyrene-d_{10} concentrations in the GI tract of zebrafish (C_i). Subsets c and d are the examples from repeated runs in Monte Carlo simulation.

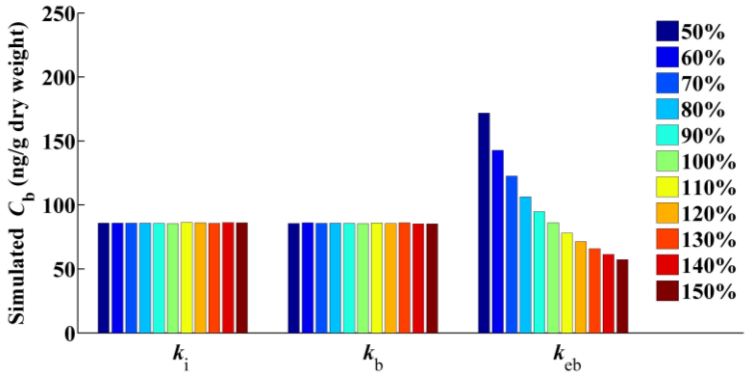

Figure S11. The sensitivity analysis of the kinetic parameters for pyrene-d_{10} in Monte Carlo simulation. The variation ranges for the transfer rate constant from the GI tract to the whole body (except GI tract) of zebrafish (k_i), the transfer rate constant from the whole body (except GI tract) to the GI tract (k_b), and the elimination rate constant (k_{eb}) including depuration rate constant and biotransformation rate constant in the whole body (except GI tract) were set from 50% to 150% of themselves. The Monte Carlo simulation was run with one of the tested parameters varied and the other two parameters fixed. For each variation, the simulation was run 100 times with 500 h each with n = 20 and w = 100%. The simulated concentration of pyrene-d_{10} in the whole body (except GI tract) (C_b) was the average value of that in the latter 300 hours of 100 times simulation.

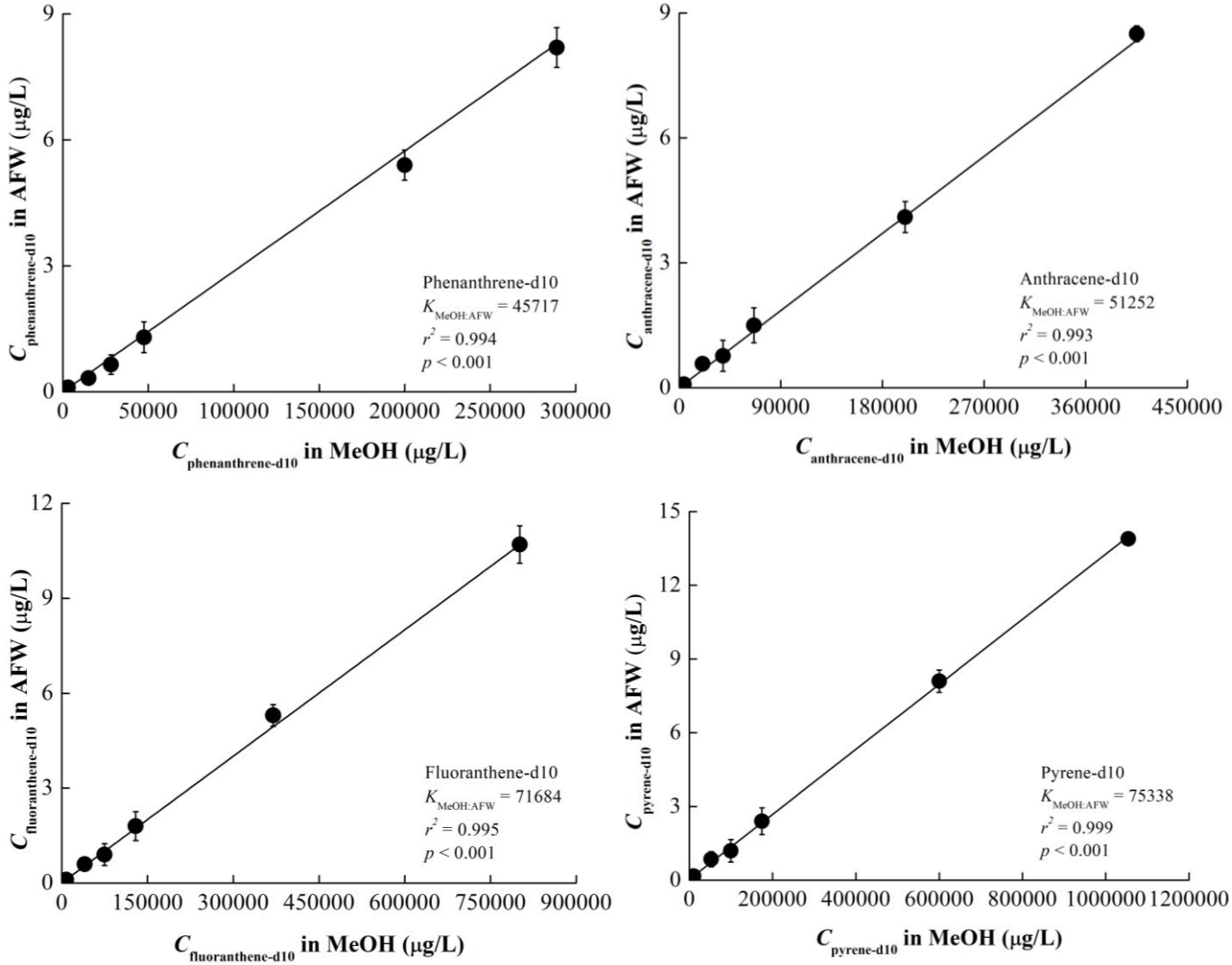

Figure S12. The relationship between the PAH-d_{10} concentrations in methanol loading solutions (MeOH) and artificial water (AFW), mean ± standard deviation, n = 3.

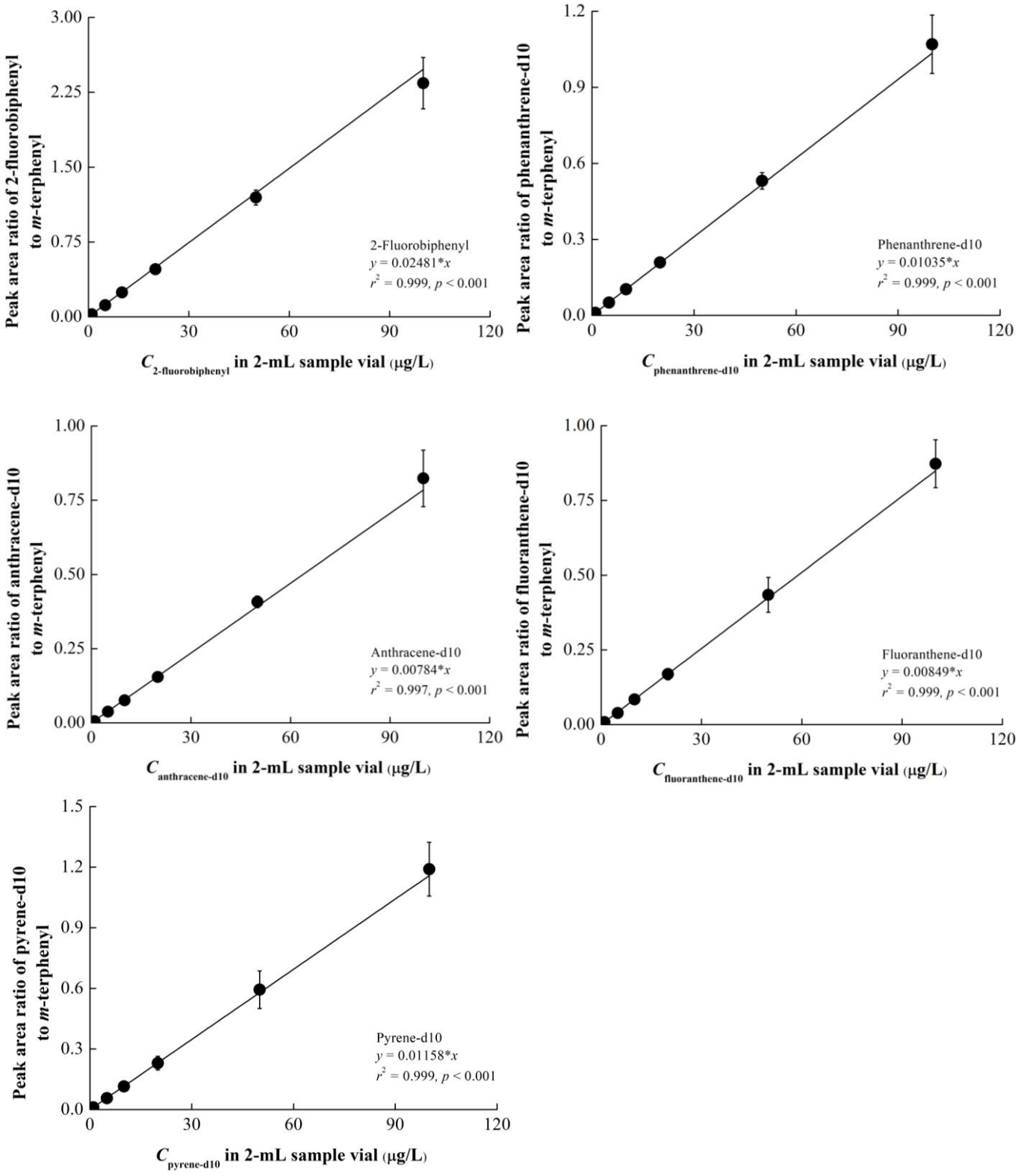

Figure S13. The internal standard calibration curves, mean ± standard deviation, $n = 3$.

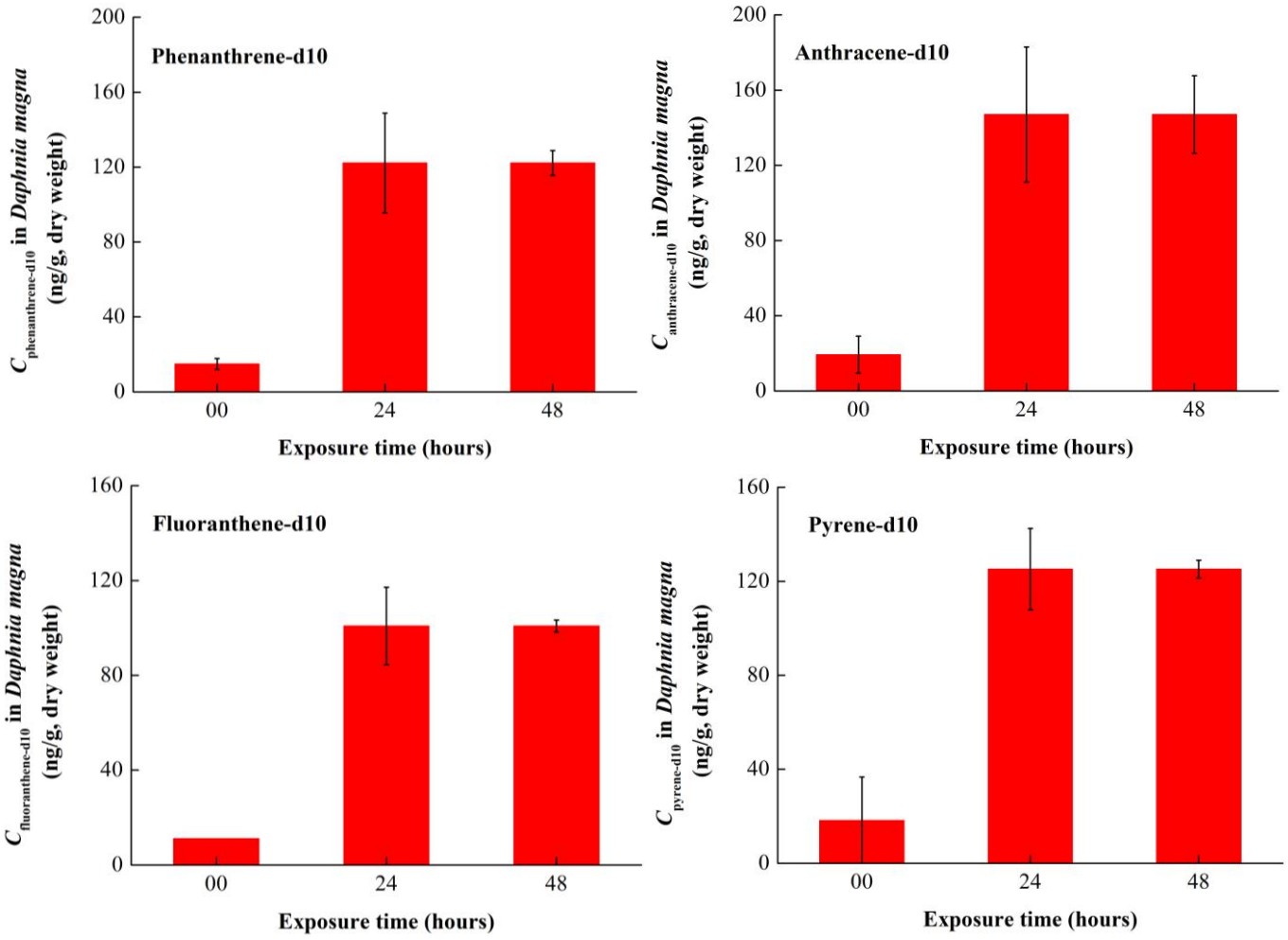

Figure S14. The concentrations of the PAHs-d_{10} in *D. magna* during a 48h-exposure. No significantly statistical differences were observed in the concentrations of the PAHs-d_{10} in *D. magna* at time point 24 h and 48 h (one-way ANOVA with a Tukey *posthoc* test, $p = 0.542$ for phenanthrene-d_{10}, $p = 0.858$ for anthracene-d_{10}, $p = 0.619$ for fluoranthene-d_{10}, and $p = 0.408$ for pyrene-d_{10}).

Table S1. The design of exposure experiments.

Exposure treatments	Design		
	Exposure time	Waterborne exposure	Dietary exposure*
Control	0–13th day	none	none
Single dietary uptake from spiked fish food	0–24th hour	none	spiked fish food at 1.5% wet weight of zebrafish at 0^{th} hour
Only dietary uptake from *D. magna*	0–13th day	none	10 pre-exposed *D. magna* (whose dry weight was about 0.4% of the wet weight of zebrafish) per zebrafish on a daily basis for 13 times.
Waterborne uptake and dietary uptake from *D. magna*	0–15th day	100 ng L^{-1} PAHs-d_{10}	none
	15–21th day	100 ng L^{-1} PAHs-d_{10}	10 pre-exposed *D. magna* (whose dry weight was about 0.4% of the wet weight of zebrafish) per zebrafish on a daily basis for 7 times
	22–25th day	100 ng L^{-1} PAHs-d_{10}	none
	25–34th day	none	none
Waterborne uptake and dietary uptake from spiked fish food	0–15th day	100 ng L^{-1} PAHs-d_{10}	none
	15–21th day	100 ng L^{-1} PAHs-d_{10}	spiked fish food at 1.5% wet weight of zebrafish on a daily basis for 7 times
	22–25th day	100 ng L^{-1} PAHs-d_{10}	none
	25–34th day	none	none

* When there was no dietary uptake, the zebrafish fed on non-spiked fish food every other day (1.5% of the wet weight of zebrafish).

Table S2. The measured freely dissolved PAHs-d_{10} concentrations in water during exposure to zebrafish (ng/L, mean ± standard deviation, n = 3).

PAHs-d_{10}	treatment	0d	13d	15d	22d	25d
Phenanthrene-d_{10}	only dietary uptake from *D. magna*	nd*	2.3 ± 0.4	-	-	-
	waterborne-only uptake	127 ± 15	122 ± 7	-	-	-
	waterborne uptake and dietary uptake from *D. magna*	-	-	134 ± 11	116 ± 15	123 ± 21
	waterborne uptake and dietary uptake from spiked fish food	-	-	123 ± 21	142 ± 12	123 ± 21
Anthracene-d_{10}	only dietary uptake from *D. magna*	nd	2.3 ± 0.7	-	-	-
	waterborne-only uptake	119 ± 13	103 ± 9	-	-	-
	waterborne uptake and dietary uptake from *D. magna*	-	-	133 ± 11	109 ± 13	117 ± 13
	waterborne uptake and dietary uptake from spiked fish food	-	-	117 ± 13	129 ± 12	117 ± 13
Fluoranthene-d_{10}	only dietary uptake from *D. magna*	nd	nd	-	-	-
	waterborne-only uptake	99 ± 5	93 ± 6	-	-	-
	waterborne uptake and dietary uptake from *D. magna*	-	-	112 ± 18	93 ± 10	95 ± 6
	waterborne uptake and dietary uptake from spiked fish food	-	-	95 ± 6	96 ± 7	95 ± 6
Pyrene-d_{10}	only dietary uptake from *D. magna*	nd	9.5 ± 8.3	-	-	-
	waterborne-only uptake	99 ± 6	99 ± 6	-	-	-
	waterborne uptake and dietary uptake from *D. magna*	-	-	101 ± 1	99 ± 7	91 ± 5
	waterborne uptake and dietary uptake from spiked fish food	-	-	91 ± 7	91 ± 7	91 ± 5

*nd: below the detection limits.

Table S3. The kinetic model inputs.

		Phenanthrene-d_{10}	Anthracene-d_{10}	Fluoranthene-d_{10}	Pyrene-d_{10}
m_{Dm} (ng)	The amount of the PAHs-d_{10} ingested into the GI tract via *D.* magna at each dietary uptake	0.191	0.229	0.157	0.195
m_{sp} (ng)	The amount of the PAHs-d_{10} ingested into the GI tract via spiked fish food at each dietary uptake	30.4	39.7	45.4	50.5

W_i (g)	The dry weight of the GI tract of zebrafish	0.00326
W_b (g)	The dry weight of the whole body (except GI tract) of zebrafish	0.0784
W_{Dm} (g)	The dry weight of the *D.* magna ingested by zebrafish at each dietary uptake (10 *D.* magna per zebrafish)	0.00158
W_{sp} (g)	The dry weight of the spiked fish food ingested by zebrafish at each dietary uptake (1.5% wet weight of zebrafish)	0.00428
k_w (h^{-1})	The first-order elimination rate constant of the food weight in the GI tract of zebrafish (Figure S6)	1.28

The freely dissolved concentrations of the PAHs-d_{10} in each treatment (ng/mL)				
	A 13-day control	single dietary exposure to spiked fish food	A 34-day exposure, of which the bioaccumulation is from *D. magna*	A 34-day exposure, of which the bioaccumulation is from spiked fish food
Phenanthrene-d_{10}	0	0	0.1239	0.1286
Anthracene-d_{10}	0	0	0.1198	0.1176
Fluoranthene-d_{10}	0	0	0.0998	0.1032
Pyrene-d_{10}	0	0	0.0979	0.0991

Table S4. The measured concentrations of the PAHs-d_{10} in commercial fish food (ng/g dry weight, mean ± standard deviation).

PAHs-d_{10}	Non-spiked fish food (n = 3)	Spiked fish food (n = 9)
Phenanthrene-d_{10}	1.96 ± 0.675	$10.5 \times 10^3 \pm 1.01 \times 10^3$
Anthracene-d_{10}	2.27 ± 0.585	$11.0 \times 10^3 \pm 1.23 \times 10^3$
Fluoranthene-d_{10}	3.16 ± 0.725	$46.2 \times 10^3 \pm 3.72 \times 10^3$
Pyrene-d_{10}	3.74 ± 0.595	$46.7 \times 10^3 \pm 5.54 \times 10^3$

Table S5. The measured PAH-d_{10} concentrations in *D. magna* (ng/g dry weight, mean ± standard deviation) and corresponding logarithmic lipid normalized BCF at steady-state ($logBCF_{ss\text{-}lip}$).

PAHs-d_{10}	*D. magna* (n = 7)*	$logBCF_{ss\text{-}lip}$
Phenanthrene-d_{10}	122.2 ± 26.7	3.63
Anthracene-d_{10}	147.0 ± 35.9	3.75
Fluoranthene-d_{10}	100.8 ± 16.3	3.69
Pyrene-d_{10}	125.2 ± 17.3	3.78

**D. magna* were exposed to the PAHs-d_{10} of which the concentrations in water are shown in Table S10, and the concentrations of the PAHs-d_{10} in *D. magna* here were corresponding to the ones after exposure for 24 h.

Table S6. The lipid contents in zebrafish, *D. magna*, and spiked fish food (%, mean ± standard deviation).

	zebrafish (n = 7)	*D. magna* (n = 5)	spiked fish food (n = 5)
Lipid contents in dry weight	18.2 ± 5.47	20.5 ± 7.00	$5.64 \pm 9.28 \times 10^{-1}$
Lipid contents in wet weight	3.95 ± 1.19	$1.42 \pm 4.01 \times 10^{-1}$	-

Table S7. The assimilation efficiency of the PAHs-d_{10} in zebrafish.

PAHs-d_{10}	Assimilation efficiency (%)*
Phenanthrene-d_{10}	85.7
Anthracene-d_{10}	87.6
Fluoranthene-d_{10}	72.3
Pyrene-d_{10}	67.5

*The assimilation efficiency was obtained by calculating the ratio of the peak amount in the whole body (except GI tract) to the intake amount of the PAHs-d_{10} after single dietary exposure to spiked fish food.

Table S8. The logarithmic octanol-water partitioning coefficients (logk_{ow}), lipid-normalized BCF at steady-state (log$BCF_{ss\text{-}lip}$), and lipid-normalized BAF at steady-state (log$BAF_{ss\text{-}lip}$), as well as lipid normalized BMF at steady-state ($BMF_{ss\text{-}lip}$) of the PAHs-d_{10} in zebrafish under different treatments (mean ± standard deviation).

PAHs-d_{10}	logk_{ow}	treatment	log$BCF_{ss\text{-}lip}$ (n = 8)	log$BAF_{ss\text{-}lip}$ (n = 6)	$BMF_{ss\text{-}lip}$ (n = 6)
Phenanthrene-d_{10}	4.54	waterborne-only uptake	3.12 ± 0.10	-	-
		only dietary uptake from *D. magna*	-	-	0.017 ± 0.016
		waterborne uptake and dietary uptake from *D. magna*	-	3.25 ± 0.11	0.38 ± 0.11
		waterborne uptake and dietary uptake from spiked fish food	-	3.93 ± 0.14	0.010 ± 0.002
Anthracene-d_{10}	4.57	waterborne-only uptake	3.32 ± 0.12	-	-
		only dietary uptake from *D. magna*	-	-	0.018 ± 0.011
		waterborne uptake and dietary uptake from *D. magna*	-	3.32 ± 0.14	0.37 ± 0.11
		waterborne uptake and dietary uptake from spiked fish food	-	4.21 ± 0.10	0.010 ± 0.002
Fluoranthene-d_{10}	5.18	waterborne-only uptake	3.13 ± 0.13	-	-
		only dietary uptake from *D. magna*	-	-	0.019 ± 0.014
		waterborne uptake and dietary uptake from *D. magna*	-	3.16 ± 0.15	0.31 ± 0.11
		waterborne uptake and dietary uptake from spiked fish food	-	4.18 ± 0.12	0.002 ± 0.001
Pyrene-d_{10}	5.22	waterborne-only uptake	3.16 ± 0.10	-	-
		only dietary uptake from *D. magna*	-	-	0.002 ± 0.002
		waterborne uptake and dietary uptake from *D. magna*	-	3.12 ± 0.11	0.22 ± 0.06
		waterborne uptake and dietary uptake from spiked fish food	-	3.92 ± 0.17	0.001 ± 0.001

Table S9. The setup parameters in analyzing PAHs-d_{10} by GC-MS/MS.

Group-event	Target	Start time	End time	Retention time	Mode	Target ions	Ch1	Reference ions	Ch2
		min	min	min		m/z	CE	m/z	CE
1-1	2-Fluorobiphenyl	11.6	15.09	12.435	MRM	172.0 > 170.1	24	172.0 > 151.1	24
2-1	Phenanthrene-d_{10}	15.09	19.28	17.740	MRM	188.0 > 160.1	21	188.0 > 158.1	30
2-2	Anthracene-d_{10}	15.09	19.28	17.875	MRM	188.0 > 160.1	24	188.0 > 158.1	30
3-1	Fluoranthene-d_{10}	19.28	21.93	20.645	MRM	212.0 > 208.0	39	212.0 > 210.1	24
3-2	Pyrene-d_{10}	19.28	21.93	21.175	MRM	212.0 > 208.1	33	212.0 > 210.1	30
3-3	*m*-Terphenyl	19.28	21.93	21.430	MRM	230.0 > 228.1	27	230.0 > 215.1	21

Table S10. The measured freely dissolved PAH-d_{10} concentrations in water during the exposure to *D. magna* (ng/L, mean ± standard deviation, n = 3).

PAHs-d_{10}	0 h	48 h	average*
Phenanthrene-d_{10}	142 ± 8	137 ± 11	140 ± 9
Anthracene-d_{10}	131 ± 7	126 ± 11	128 ± 9
Fluoranthene-d_{10}	94 ± 2	107 ± 17	100 ± 13
Pyrene-d_{10}	99 ± 17	104 ± 17	101 ± 15

*No significantly statistical differences were observed in the concentrations of the PAHs-d_{10} at time point 0 h and 48 h (independent samples t test, p = 0.564 for phenanthrene-d_{10}, p = 0.512 for anthracene-d_{10}, p = 0.281 for fluoranthene-d_{10}, and p = 0.716 for pyrene-d_{10}), and the average is the mean concentrations of the PAHs-d_{10} at time point 0 h and 48 h (n = 6).

Table S11. The measured steady-state concentrations of the PAHs-d_{10} (ng g^{-1}) in the whole body (except GI tract) of zebrafish with waterborne-only uptake as well as with both waterborne uptake and dietary uptake from *D. magna*, and the calculated steady-state concentrations (ng g^{-1}) in the whole body (except GI tract) of zebrafish.

PAHs-d_{10}	waterborne-only uptake (n = 8)		waterborne uptake and dietary uptake from *D. magna* (n = 6)		$t_{0.95}(l)$	Significant value*
	mean	std.	mean	std.		
Phenanthrene-d_{10}	30.42	6.57	37.23	8.51	2.262	39.9
Anthracene-d_{10}	47.65	11.84	52.09	9.36	2.179	60.0
Fluoranthene-d_{10}	25.78	7.75	23.72	4.07	2.201	32.8
Pyrene-d_{10}	26.35	6.03	20.52	3.20	2.201	31.9

*The calculated steady-state concentrations with waterborne uptake and dietary uptake from *D. magna* which are significantly higher than that with waterborne-only uptake.

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