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Bioconcentration of Organic Contaminants in *Daphnia* Resting Eggs

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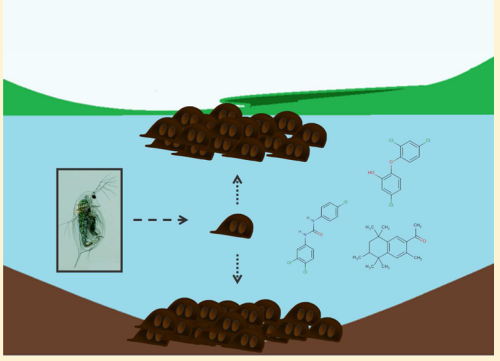
Supporting Information

ABSTRACT: Organic contaminants detected in sediments from Lake Greifensee and other compounds falling in the log D_{ow} range from 1 to 7 were selected to study the bioconcentration of organic contaminants in sediments in *Daphnia* resting eggs (ephippia). Our results show that octocrylene, tonalide, triclocarban, and other personal care products, along with pesticides and biocides can accumulate in ephippia with log BCF values up to 3. Data on the uptake and depuration kinetics show a better fit toward a two compartment organism model over a single compartment model due to the differences in ephippial egg content in the environment. The obtained BCFs correlate with hydrophobicity for neutral compounds. Independence between BCF and hydrophobicity was observed for partially ionized compounds with log D_{ow} values around 1. Internal concentrations in ephippia in the environment were predicted based on sediment concentrations using the equilibrium partitioning model and calculated BCFs. Estimated internal concentration values ranged between 1 and 68,000 $\mu g/kg_{lip}$ with triclocarban having the highest internal concentrations followed by tonalide and triclosan. The outcomes indicate that contaminants can be taken up by ephippia from the water column or the pore water in the sediment and might influence fitness and sexual reproduction in the aquatic key species of the genus *Daphnia*.



INTRODUCTION

During the last century, most European lakes with anthropogenic point sources went through a phase of eutrophication, which was accompanied by a shift in species composition and diversity of both pelagic and littoral communities, reduction of water quality, and even occasional fish kills.[1] The installation of sewage treatment plants in the 1980s[2] and their continuous upgrade enabled the recovery of many lakes to their original trophic state. Simultaneous to eutrophication, numerous new chemicals have been produced for use in households, agriculture, and industry.[3] Studies have revealed that chemical pollutants influence the aquatic food web,[4,5] but how this is happening and at which time scales chemical pollutants are influencing the aquatic food web, as well as the extent of the impact, is not well known. Measurements of emerging contaminants such as pharmaceutical and personal care products (PPCPs) started in the 1990s; thus the exposure before is not well studied.

In our previous work, we showed that sediments can be integrators in time of polar and medium polar organic compounds providing historical patterns of chemical deposition.[6] The ability of organic contaminants to sorb to sediments constitutes a primary source of exposure for benthic organisms. Benthic organisms can accumulate organic contaminants from the particulate and interstitial components of sediments as well as from the water column.[7,8] Sediment pore water plays an important role in sediment−water sorption mechanisms and bioavailability, given that it is captured during the sedimentation process and is essentially isolated from the water column.[7,9]

One of the most important planktonic grazers in pelagic food webs are species of the genus *Daphnia* (Crustacea, Anomopoda; water fleas). *Daphnia* species serve as food for fish and invertebrates, and they feed on algae and bacteria.[10] *Daphnia* normally reproduce clonally (parthenogenetic cycle), but they switch to sexual reproduction (sexual cycle) when environmental conditions are not ideal. Production of males and sexual eggs are then triggered by changes in food level, crowding, and photoperiod.[10,11] Sexual eggs are diapause stages, enclosed in a structure called an ephippium that can sink to the bottom of the lake and remain there until conditions become more favorable. Ephippia can also be transported by wind or water fowl to other water bodies and thus colonize new habitats. In the deeper parts of lakes, these diapausing eggs do not get a hatching stimulus and can remain in the sediment providing an

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unbiased archive of past populations. Ephippia can be extracted from sediment cores and either hatched for experimental purposes or directly analyzed with molecular genetic methods.[12] Analysis of the genetic architecture of ephippia revealed that lake eutrophication was associated with a shift in species composition and the population structure of evolutionary lineages.[10] However, whether the shift in species composition was directly caused by phosphate or if phosphate concentrations are only a proxy for other parameters like chemical contamination is not known. Chemical contaminants can affect natural *Daphnia* populations either by direct toxin uptake or by indirect ingestion of contaminated algae. Pollutants may bioaccumulate in their resting eggs already in the water column or after sedimentation, influencing the fitness and their sexual reproduction, and therefore the evolutionary potential of an aquatic key species. However, there is almost no information about bioconcentration in ephippia in literature. Wyn et al.[13] evaluated the temporal and spatial patterns of metal contamination in resting eggs from sediment cores. Analysis of organic contaminants in ephippia extracted from sediment cores has never been done due to the small number of ephippia available in the sediment extraction and the amount needed for the chemical analysis.

Therefore, the objectives of this study were to (i) clarify to what extent organic contaminants can bioconcentrate in *Daphnia* resting eggs (ephippia) in uptake and elimination experiments and (ii) reconstruct their contamination in the past based on sediment concentrations using an equilibrium partitioning model. The information obtained from the characterization of Lake Greifensee from our previous work[6] was used to select a range of relevant organic contaminants that was complemented with further compounds with different hydrophobicity.

EXPERIMENTAL SECTION

Details on the sources, preparation, and storage of reference standards and reagents are provided in the Supporting Information (SI).

Sample Collection and Preservation. Two sediment cores were taken from Lake Greifensee, located 11 km to the northeast of Zurich, Switzerland. Sediment cores were collected using a free fall gravity corer and stored vertically in the dark at 4 °C until analysis. Dating of the sediment cores was performed by counting yearly laminations as has been described elsewhere.[6] Total phosphorus concentrations for each sediment layer were measured using peroxodisulfate oxidation as described by Ebina et al.[14]

Ephippia from the *Daphnia longispina-galeata* species complex were collected with nets from Lake Greifensee during peaks of sexual reproduction in spring and fall 2011. Ephippia were stored in 10 L polyethylene (HD-PE) plastic containers (Hünersdorff GmbH, Germany) and kept at 12 °C before cleaning. Ephippia were cleaned using different sieves (mesh sizes: <1 mm) and rinsed with filtered and double autoclaved lake water to remove any residues of algae or other particles. After cleanup, ephippia were stored in the dark at 4 °C in 500 mL Schott bottles (Schott Duran, Germany) containing double autoclaved and filtered lake water.

Lake water was collected simultaneously with ephippia, filtered through a glass fiber filter (pore size: 0.7 μm, Sartorius Stedim Biotech, France), and subsequently double autoclaved at 120 °C for 30 min each with a Vapoklav 500 (HP Medizintechnik GmbH, Germany). The treated lake water contained none of the studied chemicals in background concentrations above the limits of detection.

Ephippia Exposure: Uptake and Depuration Kinetics. For determination of the uptake kinetics, between 30 and 100 mg of wet weight (ww) clean ephippia were transferred to 250 mL Schott bottles filled with 200 mL of exposure medium. The medium consisted of treated lake water containing a mixture of 16 analytes with a nominal final concentration between 150 and 250 μg/L. The analytes included pesticides, corrosion inhibitors, biocides, and personal care products. The chemical selection was based on the organic contaminants found in sediments of Lake Greifensee from our previous study.[6] In addition, seven compounds, which include pharmaceuticals and pesticides, were added to the study to have a broader range of analytes with different physical chemical properties (log D_{ow} 1−7, log octanol−water partition coefficient (log *K*ow) corrected for the dissociation at the pH 8.2 of the medium). The chemicals were spiked as a mixture in ethanol with a final ethanol content of 0.4% (v/v). The complete list of analytes used in the experiment are reported in Table 1. The assays with the exposed ephippia were placed on a shaker SM-30 Contra (Edmund Bühler GmbH, Hechingen, Germany) at 12 °C and stored in the dark. After a specific time of exposure (up to 120 h), based on an estimation time for accumulation, the assays were sieved (mesh size: <1 mm), rinsed, and transferred to 2 mL centrifuge tubes (Eppendorf AG, Germany). The samples were stored at −20 °C until extraction. In addition, 1 mL of aliquot of each exposure medium were transferred to 5 mL glass vials (Infochroma Ag, Switzerland) and stored at −20 °C until analysis. In total, samples were collected at 21 different time points in duplicates.

For depuration, previously exposed (for 120 h) ephippia were sieved and weighed, and between 30 and 100 mg_{ww} ephippia were transferred to individual 250 mL Schott bottles containing 200 mL of treated lake water (without contaminants). The medium was exchanged every 24 h to avoid reuptake of chemicals. Samples were collected at 21 different time points in duplicates. Clean up and preparation was carried out as described above.

Quality controls were taken during the experiment and include control medium consisting of treated lake water containing 0.4% ethanol (v/v), sample medium containing ephippia in treated lake water and 0.4% ethanol (v/v), and exposure medium. The controls were collected every 24 h and analyzed for possible cross contamination. No contamination was present in the controls or in the exposure medium. For all compounds, the concentration in the medium was stable during the entire experiment. The measured concentrations over the complete experiment are illustrated in Figure 1 as well as in Table S3 of the SI. In addition, pH (8.2 ± 0.2) and dissolved oxygen (10.1 ± 0.3) (mg/L) were monitored and kept constant during the experiment. An illustration of the complete experiment is provided in Figure S1 of the SI.

Lipid content (f_{lip}) was measured to be 1.5 ± 0.3% of the wet weight and was determined gravimetrically according to the method developed by Smedes et al.[15] Water content was determined gravimetrically and accounted for 89 ± 1% of the ephippia weight.

Clean-Up and Enrichment of Extracts. Frozen ephippia were transferred to individual 2 mL microcentrifuge tubes (Greiner Bio-One Ltd., Germany) containing approximately 200−300 mg of a 0.5 mm size beads mixture of zirconia and silica beads (BioSpec Products Inc., U.S.A.). Subsequently,

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ephippia were suspended in 440 μL of methanol, and 60 μL of an internal standard mix solution with a nominal final concentration in the vial of 240 μg/L was added. Ephippia were homogenized six times for 15 s at a speed of 6.0 m/s with a FastPrep FP120 instrument (Thermo Savant, California, U.S.A.).

After homogenization, the centrifuge tubes were rinsed with 500 μL of methanol, and the two extracts were combined to give a final extraction volume of 1 mL. The extracts were diluted with 19 mL of nanopure water and enriched by solid phase extraction (SPE) (Oasis HLB cartridges. Waters Corp., U.S.A.). Samples were eluted with a mixture of 50:50 methanol and isopropanol (v/v), evaporated to 100 μL using an EZ-2 personal evaporator (Genevac, U.S.A.), and diluted to 1 mL with a solvent mixture of 50:50 nanopure water and methanol (v/v).

Control and exposure medium were directly analyzed by transferring 940 μL of medium to 1 mL HPLC vials (BGB Analytics AG, Switzerland) followed by addition of 60 μL of an internal standard mix solution.

Extraction, enrichment, and analysis of sediment samples was performed according to Chiaia-Hernandez et al.[6] Briefly, sediment samples were sliced, freeze-dried, and extracted by means of pressurizing liquid extraction followed by liquid−liquid partitioning. Cleanup of sediment extract was performed by adding 5 mL of acetonitrile, followed by 1.6 g of $MgSO_4$, and 0.4 g of NH4Cl. The mixture was vortexed and centrifuged. After separation, the acetonitrile phase was transferred to a graduated centrifuge tube, evaporated, and brought to a volume of 500 μL by adding methanol. The final extract was filtered into 2 mL autosample vials using 0.2 μm PTFE filters (BGB analytics, Boeckten, Switzerland). Furthermore, sediment extracts were analyzed by LC-ESI/APPI-HR-MS as described elsewhere.[6]

Liquid Chromatography Tandem High-Resolution Mass Spectrometric Detection. Ephippia extracts were separated through a X-bridge C18 column (2.1 mm × 50 mm with particle size of 3.5 μm) with a flow rate of 200 μL/min and a linear gradient of 28 min starting with 95% of 0.1% formic acid (FA) (v/v) in HPLC water and 5% of 0.1% FA (v/v) in methanol. After 17 min, 10% isopropanol were added to improve elution of hydrophobic compounds. For generation of ions, electrospray ionization (ESI) in the negative and positive mode was used in two separate injections. Detection was performed using a LTQ (linear trap quadrupole) orbitrap mass spectrometer (Thermos Fisher Scientific Corp., U.S.A.). High-resolution mass spectrometry (HR-MS) with a resolution of 60000 and a mass accuracy of <5 ppm were used for peak detection followed by data-dependent acquisition of product ion spectra (HR-MS/MS) at a resolution of 7500 for peak identification. Data analysis was done with Xcalibur software (Thermo Scientific, U.S.A.).

Accuracy and precision of the method were determined in independent studies with an overall average method recoveries for ephippia and lake water of 92% and 82% and an average precision of 7% and 8%, respectively. Details and specifications on recoveries, quantification. and detection of analytes are reported in the SI and include internal standards, exact masses, ionization, and retention times (see Table S2 and S3, SI) as well as calibration curves and quality controls used.

Bioconcentration Factor (BCF). *One Compartment Organism Model.* The bioconcentration factor (BCF_i) is defined as the ratio of the concentration of a given compound *i* in an organism or organisms compartment (C_{iorg}) [mol/kg_{ww}] to the concentration in the surrounding medium (C_{imed}) [M] at steady state (eq 1).

Ephippia can uptake and eliminate organic contaminants from sediments or from the water column only by passive uptake and depuration mechanisms because ephippia represent a resting stage, and metabolic activities are not expected. Assuming first-order kinetics for uptake and elimination of each chemical, the C_{iorg} can be described by eq 2, and the BCF [L/kg_{ww}] can be expressed by the ratio of the uptake rate constant k_u [$L/(kg_{ww}\ h)$] and the elimination rate constant k_e [h^{-1}] (eq 1).

$$BCF_i = \frac{C_{iorg}}{C_{imed}} = \frac{k_u}{k_e} \tag{1}$$

$$\frac{dC_{iorg}}{dt} = k_u \times C_{imed}(t) - k_e \times C_{iorg}(t) \tag{2}$$

Two Compartment Organism Model. The one compartment organism model (eq 2) was used as a first attempt to determinate the uptake (k_u) and elimination (k_e) rates of chemicals in ephippia. When this model was used, we observed that the model fit could not capture the fast uptake, and elimination rates probably due to the difference in ephippia egg content.

Ephippia are made up of chitinous sheets that can be melanized and enclose the dormant embryos. *Daphnia* from the *longispina-galeata* complex species produce ephippia containing no eggs (empty), one egg, or two eggs (full). In our case, around 80% of ephippia were empty, while the remaining 20% were full. The percent values were obtained by taking an ephippia aliquot, opening the ephippia with dissecting needles and examining the number of eggs per ephippium by eye under a stereo microscope. Without destruction, empty and full ephippia cannot be distinguished as illustrated in Figure S2 of the SI. In order to describe the bioconcentration of this mixture of full and empty ephippia, a two compartment organism model was used, where the total $C_{iorg\ (total)}$ is described as slow uptake (k_{ufull}) in the full ephippia and fast uptake (k_{uempty}) in the empty ephippia (eqs 3 and 4). $C_{iorgfull}$ and $C_{iorgempty}$ [mol/kg_{ww}] are the concentrations of a given compound in the full and empty ephippia whereas C_{imed} [M] is the concentration in the surrounding medium. Assuming two well mixed compartments (full and empty) and similar lipid and mass, the $C_{iorg(total)}$ can be described by eq 5 according to the percentage of full and empty ephippia. The uptake (k_{ufull}, k_{uempty}) [$L/(kg_{ww}\ h)$] and elimination (k_{efull}, k_{eempty}) [h^{-1}] kinetics can be used to calculate BCFs (L/kg_{ww}) as expressed in eqs 6 and 7.

$$\frac{dC_{iorgfull}}{dt} = k_{ufull} \times C_{imed}(t) - k_{efull} \times C_{iorgfull}(t) \tag{3}$$

$$\frac{dC_{iorgempty}}{dt} = k_{uempty} \times C_{imed}(t) - k_{eempty} \times C_{iorgempty}(t) \tag{4}$$

$$C_{iorg(total)}(t) = 0.20 \times C_{iorgfull}(t) + 0.8 \times C_{iorgempty}(t) \tag{5}$$

$$BCF_{full} = \frac{k_{ufull}}{k_{efull}} \tag{6}$$

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$$BCF_{empty} = \frac{k_{uempty}}{k_{eempty}} \quad (7)$$

Recalculating BCFs for different fractions of full and empty ephippia depending on the different ephippia composition in the environment could be performed by changing the fractions (0.20/0.80) of full and empty ephippia in eq 5 and by recalculating BCFs using the same rate constants because rate constants are independent of the full and empty fraction. Additional lipid normalized BCFs are provided in Table 1 and S1 of the SI.

After solving the differential eqs 2−4, the rate constants (k) from the experimental data for the one and the two compartment model were obtained by weighted least-squares minimization using the nls function in R;[16−18] starting values were calculated with Aquasim (Reichert, P., AQUASIM 2.1 Eawag, Switzerland). For the measured data, a 10% standard deviation was assumed.

The time to reach 95% steady state (SS) for the empty ($C_{iorgempty}$) and full compartment ($C_{iorgfull}$) was calculated using the analytical solution of eqs 3 and 4. For the combined empty and full compartment ($C_{iorg(total)}$), the time to reach 95% SS was calculated using eq 5 in combination with the function uniroot from the package rootSolve in R.[18−20]

Predicting Concentrations in Ephippia in the Environment. To evaluate the bioconcentration of hydrophobic organic compounds from sediment, the equilibrium partitioning model (EqP) is often used. EqP assumes that organic contaminants are distributed between the lipids of the organisms, pore water, and organic carbon of the sediment and that these compartments are in equilibrium.[21,22] On the basis of this concept, the accumulation of a chemical in aquatic organisms can be estimated by eq 8, where C_{lip} is the lipid normalized steady-state concentration in the biota ($\mu g/kg_{lip}$), C_{ipw} is the concentration in the pore water ($\mu g/L$), and BCF_{lip} is the lipid normalized aqueous bioconcentration factor (L/kg_{lip}), which is obtained from our experimental data.[22] C_{ipw} concentrations can be estimated by standard approaches using the organic carbon sorption coefficient values (K_{oc}), organic contaminant concentrations measured in sediments from Lake Greifensee (C_{ised}), and fraction of organic carbon (f_{oc}) as stated in eq 9.[5,22] Values used to calculate C_{lip} are provided in Table 1.

$$C_{lip} = BCF_{lip} \times C_{ipw} \quad (8)$$

$$C_{ipw} = \frac{C_{ised}}{K_{oc} \times f_{oc}} \quad (9)$$

RESULTS AND DISCUSSION

Sediment Analysis. Our earlier analyses of sediments from Lake Greifensee show that biocides, musk fragrances, and other personal care products were the most frequently detected compounds with concentrations ranging from pg/g_{dw} to ng/g_{dw}, with the highest concentrations for tonalide and triclosan observed in the 1970s.[6] In Table 1, the maximum and minimum concentrations of the organic contaminants detected in sediments from Lake Greifensee as well as the physicochemical properties of all analytes used in the study are compiled.

In this study, the biocide triclocarban (TCC) was quantified from sediments of Lake Greifensee as a complementary information to our previous work. TCC concentrations range from 2 to 150 ng/g_{dw}. The highest concentrations were found in the 1970s similar to the input pattern of triclosan (TCS) as illustrated in Figure S4 of the SI. TCC has been employed since 1957 as antimicrobial agent similar to TCS in a variety of consumer products. Although, TCC is not yet monitored in Switzerland, TCC is approved for use as an antimicrobial in cosmetic products at a maximum use concentration of 0.2% (Annex 2, Swiss Cosmetic Product Regulation).

Uptake and Depuration Kinetics. The two compartment organism model used as an alternative to the one compartment organism model (eq 2) assumes that the uptake (k_{uempty}) and elimination (k_{eempty}) of chemicals are faster in the empty ephippia because the dormant embryos are missing, and organic contaminants have to pass only through the outer layer shell to enter or exit the ephippium mainly consisting of chitin and only few lipids, while full ephippia containing one or two dormant embryos will have slower uptake (k_{ufull}) and elimination (k_{efull}) rates. This assumption was confirmed by comparing the one vs two compartment organism model as shown in Figure 1. In addition, for model selection, the Akaike information criterion (AIC) was applied to measure the relative goodness of the fit of the two models. Lower AIC values indicate the model that fits better with respect to the number of parameters included in the model.[23] The AIC test points toward the two compartment model as a preferable model for more than 80% of the analytes studied and, therefore, was used for the final BCF calculations.

The experimental data obtained for the exposure and elimination of ephippia are shown in Figure 1. For discussion of the results, log D_{ow} (pH 8.2) values were used instead of log K_{ow} values to correct for pH dependency. The data indicates very fast uptake and elimination after a couple of hours from the start of the experiment for most of the compounds. Terbuthylazine was the fastest to reach 95% equilibrium within 74 h (see Table 1 and Figure S5, SI). The fast uptake of terbuthylazine could be attributed mainly to its log D_{ow} of 2.5. Irgarol and terbutryn, compounds with log D_{ow} values around 3 and having similar molecular structure, reached 95% SS after 92 and 200 h of exposure, respectively, which might indicate that interactions other than diffusion driven by hydrophobicity play a role for the elimination of terbutryn. Benzotriazole and valsartan reach 95% SS after 840 and 579 h, results that are not in agreement with their low log D_{ow} of 1 but can be attributed to their high polarity and partially ionized form. Uptake and elimination is not completely reached for clotrimazole, octocrylene, ritonavir, TCC, and tonalide. The 95% SS for these compounds is reached between 260 to up to 1290 h, which is consistent with their log $D_{ow} \geq 5$. For example, TCC has been reported to be quickly eliminated from fish tissue ($t_{1/2}$ = 1 h) mainly through metabolism with only 1% TCC remaining.[24] In ephippia, metabolism does not occur, and therefore TCC has a very slow elimination, which is consistent with our data. The difference in time to reach equilibrium (95% SS) between the full ($C_{iorgfull}$) and empty ($C_{iorgempty}$) compartments were between 50 and 500 times, and the 95% SS values for the combined compartment ($C_{iorg(total)}$) were comparable to the full compartment as reported in Table 1 and Table S4 and Figure S5 of the SI.

A variability in the uptake phase after 50 h is observed. This can be explained by the increasing growth of fungi with time around the ephippia as illustrated in Figure S3 of the SI. Ephippia are directly in contact with the environment, so

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Table 1. Maximum and Minimal Concentration of Organic Contaminants Found in Sediments of Lake Greifensee and Toxicokinetics Parameters To Calculate Their Bioconcentration in Ephippia[a]

name	compound class	log D_{ow}[b] at pH 8.2	K_{oc}[c] (L/kg)	$C_{isedmax}$ (pg/g_{dw})	$C_{isedmin}$ (pg/g_{dw})	$C_{ipw\ max}$ (ng/L)	$C_{ipw\ min}$ (ng/L)	k_{ufull} ($L/(h\ g_{ww})$)	k_{efull} (h^{-1})	BCF_{full} (L/kg_{ww})	log BCF_{full}	BCF_{full} (L/kg_{lip})	95% SS_{total} (h)	$C_{iorgmax}$ ($\mu g/kg_{lip}$)	$C_{iorgmin}$ ($\mu g/kg_{lip}$)
benzotriazole	corosion inhibitor	1.2	6.1×10^{01}	1400	1200	660	570	1.04×10^{-05}	3.56×10^{-03}	2.9	0.5	200	720	130	110
clotrimazole	pharmaceutical	5.8	–	–	–	–	–	4.09×10^{-03}	8.25×10^{-03}	500	2.7	33100	328	–	–
diazinon	pesticide	4.2		–	–	–	–	3.08×10^{-05}	1.23×10^{-02}	2.5	0.4	170	138	–	–
fenofibrate	pharmaceutical	5.3	–	–	–	–	–	1.37×10^{-03}	1.25×10^{-02}	110	2.0	7300	198	–	–
fenpropimorph	pesticide	4.7	–	–	–	–	–	3.45×10^{-04}	1.34×10^{-02}	26	1.4	1720	144	–	–
irgarol	biocide	3.0	4.3×10^{02}	3100	600	210	41	$2\ 33 \times 10^{-05}$	1.40×10^{-02}	1.7	0.2	110	92	23	5
octocrylene	pcp	6.8	1.1×10^{05}	9000	9000	2	2	3.31×10^{-03}	2.30×10^{-03}	1440	3.2	95900	1289	220	220
prochloraz	pesticide	3.6	3.3×10^{03}	4000	3000	35	26	9.37×10^{-04}	1.67×10^{-02}	56	1.7	3730	142	130	98
propiconazole	biocide	4.3	7.5×10^{02}	620	620	24	24	$8\ 25 \times 10^{-05}$	$9\ 93 \times 10^{-03}$	8.3	0 9	560	204	13	13
ritonavir	pharmaceutical	5.2	–	–	–	–	–	2.17×10^{-04}	2.86×10^{-03}	76	1.9	5060	1007	–	–
terbuthylazine	pesticide	2.5	–	–	–	–	–	$5\ 93 \times 10^{-06}$	9.00×10^{-03}	0.7	−0.2	44	74	–	–
terbutryn	biocide	2.9	6.1×10^{02}	600	60	28	3	6.09×10^{-05}	1.09×10^{-02}	5.6	0.7	370	200	11	1
tonalide	pcp	5.0	1.9×10^{04}	332000	31000	510	48	2.57×10^{-03}	9.83×10^{-03}	260	2.4	17400	266	8900	830
triclocarban	biocide	4.9	5.3×10^{03}	152000	2400	820	13	8.23×10^{-03}	6.62×10^{-03}	1240	3.1	82900	432	68200	1100
triclosan	biocide	4.4	8.4×10^{03}	92000	10000	320	34	9.56×10^{-04}	1.28×10^{-02}	74	1.9	4970	183	1560	170
valsartan	pharmaceutical	1.1	–	–	–	–	–	4.93×10^{-05}	5.17×10^{-03}	9.5	1.0	640	455	–	–

[a] C_{ised} is the maximum and minimum concentration measured in sediments from Lake Greifensee, K_{oc} is the organic carbon sorption coefficient values, C_{ipw} is the concentration in the water, k_{ufull} and k_{efull} are the uptake and elimination rate constants, respectively, BCF_{full} is the bioconcentration factor, 95% SS_{total} is the time to reach 95% steady state for the full and empty compartment combined, C_{iorg} is the minimal and maximum predicted concentrations in ephippia. C_{ipw} was calculated using the fraction of organic carbon (f_{oc}) obtained by averaging different f_{oc} values (0.034 kg_{oc}/kg) measured from Lake Greifensee sediment cores between 1940−1999 reported by Zennegg et al.,[32] and the lipid fraction (f_{lip}) is determined experimentally with a value of 0.015 kg_{lip}/kg_{ww}. [b] Values calculated using MarvinSketch (http://www.chemaxon.com/). [c] K_{oc} values were calculated using Estimation Program Interface (EPI Suite 4.1) using the log K_{ow} approach[33] (http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm).

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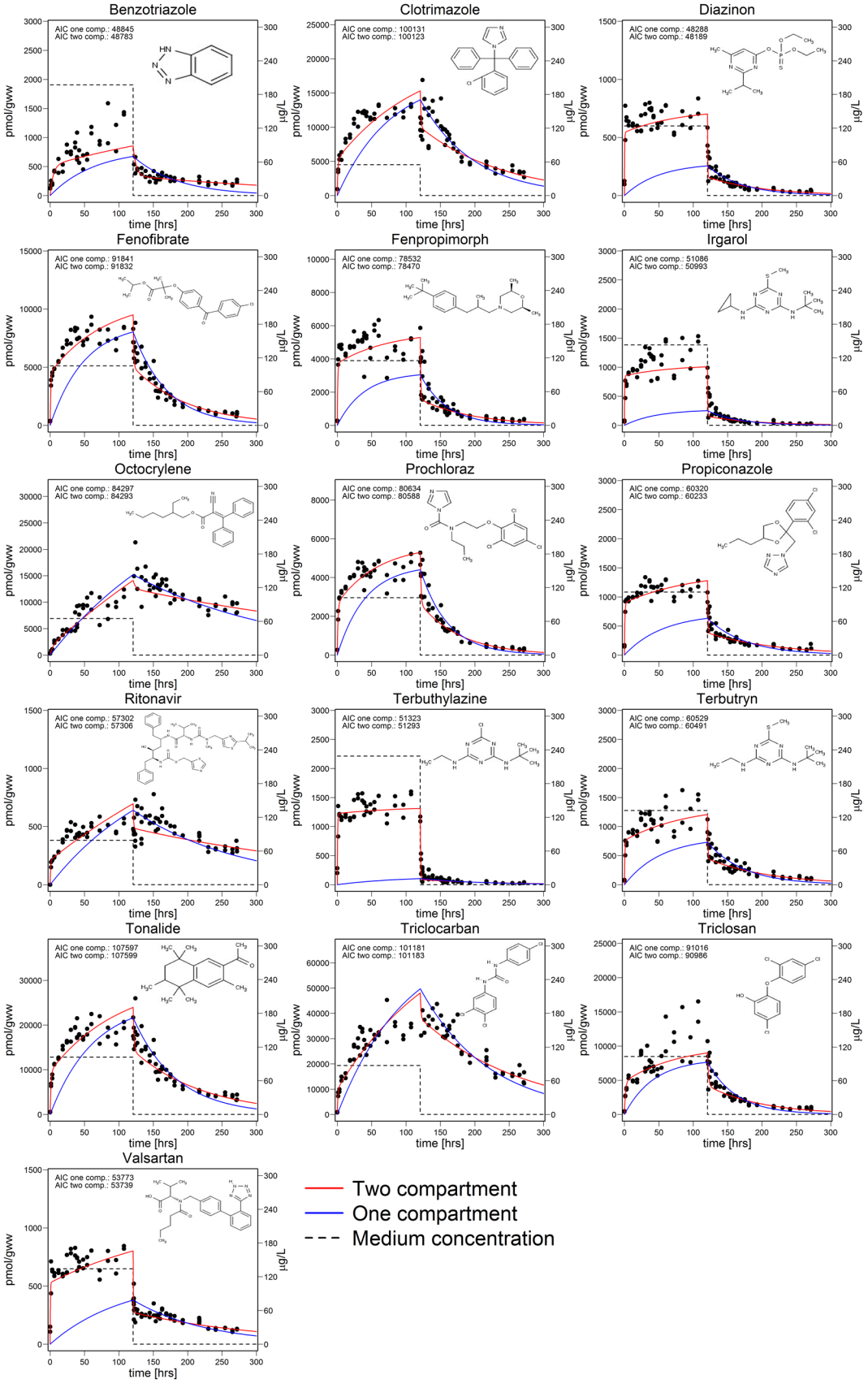

Figure 1. Ephippia uptake and elimination kinetics for 16 analytes. The one and two compartment organism models are illustrated with solid lines in blue and red, respectively. Measured exposure medium concentrations are shown with dashed lines in black color. The Akaike information criterion (AIC) is shown in the upper left corner with lower values indicating the "best" model.

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bacteria and fungal spores are regularly seen associated with them.[25] In some cases, the growth of fungi can be controlled to a certain degree by treating ephippia with bleach. However, treating ephippia from the *Daphnia longispina-galeta* species complex with bleach damages the ephippial sheets and eggs in an unpredictable way, and therefore, we could only limit fungal growth by using filtered and autoclaved medium. As a result, we could not distinguish the fraction of the compounds accumulated in fungi from that in ephippia, likely resulting in overestimated concentrations in ephippia in the later phase of the uptake experiment.

Ephippia Bioconcentration Factor (BCF). The toxicokinetic parameters are reported in Table 1. The BCFs of 16 different chemicals were calculated using the rate constant for uptake (k_{ufull}) and elimination (k_{efull}). Values of BCF_{empty} were lower than BCF_{full} mostly due to the very fast elimination rates (k_{eempty}) as reported in Table S5 of the SI. Only rates and BCFs for full ephippia ($k_{ufull} = k_u$, $k_{efull} = k_e$, and $BCF_{full} = BCF$) are considered and discussed further because empty ephippia are not relevant for reproduction and are not used for additional discussion in this manuscript. Greater BCFs were obtained for compounds with higher log D_{ow} values showing a correlation ($r^2 = 0.59$, $p = 0.0005$) between log BCF and hydrophobicity as illustrated in Figure 2. A better correlation was observed

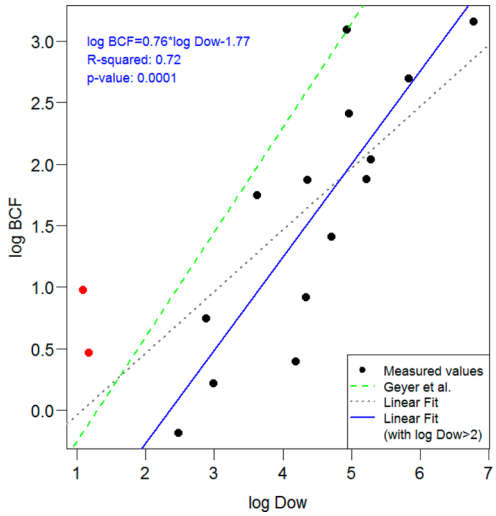

Figure 2. Correlation between log D_{ow} and log BCF in full ephippia on wet weight basis (ww) (L/kg_{ww}). Measured values are shown in black dots. Green line is the predicted relationships obtained from Geyer et al. (log BCF = 0.850 × log D_{ow} − 1.100) on ww. [28] Gray line represents the linear fit for all compounds studied, and blue line represents the linear fit for compounds with log D_{ow} > 2. Red points correspond to benzotriazole, and valsartan with log D_{ow} values below 2.

($r^2 = 0.72$, $p = 0.0001$) when the two polar and partially ionized compounds benzotriazole and valsartan were excluded from the linear fit. Highest BCFs were obtained for the personal care product octocrylene (1440 L/kg) and the biocide TCC (1240 L/kg), while the lowest BCF was found for the pesticide terbuthylazine. The correlation between BCF and hydrophobicity was further studied by comparing the relationship between k_u and k_e with hydrophobicity separately as shown in Figure 3. The model fit was obtained from the rate constant for adsorption and minimum elimination equations proposed by Hendrinks et al.[26] for accumulation of organic substances

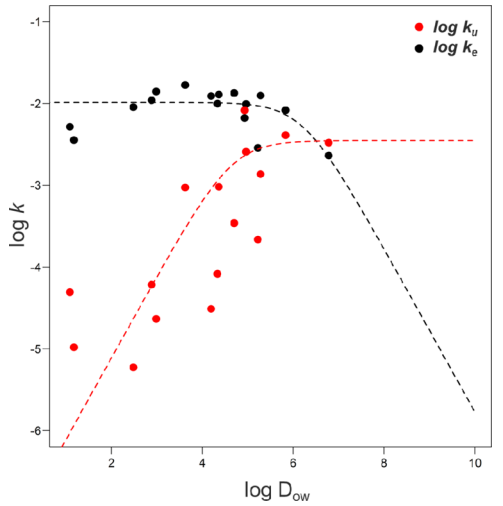

Figure 3. Relationship between ephippia uptake (k_u) and elimination (k_e) rate constants and hydrophobicity in full ephippia (full compartment) expressed as log D_{ow}. The model fit was obtained from the rate constant for adsorption and minimum elimination equations reported by Hendriks et al. [26].

related to K_{ow} of the chemical and the weight, lipid content, and trophic level of the species. Parameters and equations used for the model fit are reported in the SI. The relationship between k_u and k_e with hydrophobicity establishes that for compounds with log D_{ow} between 2 and 5, k_u increases with hydrophobicity. When k_u reaches maximum values (log D_{ow} around 5), k_u becomes independent from hydrophobicity as well as for compounds with log D_{ow} values lower than 2. For elimination rate constants, the k_e reaches a plateau for compounds with log D_{ow} values between 2 and 6 and for higher values becomes inversely proportional to hydrophobicity corresponding to a membrane and a diffusion layer controlled elimination process, respectively.[27] Therefore, an increase in BCF for more hydrophobic compounds is the result of a decreasing k_e, while k_u remains constant, and for less hydrophobic compounds, an increase in BCF results in an increase in k_u, while k_e remains constant. A decline of k_e with decreasing hydrophobicity is observed as in the cases of benzotriazole and valsartan, which do not follow the model fit. For hydrophilic compounds for which k_e increases with hydrophobicity, the BCF becomes independent of hydrophobicity as has been speculated previously by Gobas et al.[27] but not confirmed due to the absence of data. They explained this phenomenon by the fact that concentration partitioning of hydrophilic compounds in the lipid phase in the organism becomes less important as compared with the concentration in nonlipid phases, and therefore, hydrophobicity and BCF cannot be extrapolated to extremely low hydrophobic compounds. Even though the data presented in this study show similar trends, Gobas' hypothesis cannot be clearly confirmed due to the limited data.

Overall, our findings are in agreement with Hendrinks et al.,[26] who found similar trends between absorption and elimination rate constants and hydrophobicity for different organic compounds.

The obtained log BCF values were compared with values obtained via a quantitative structure−activity relationship (QSAR) based on log K_{ow} for bioconcentration of organic chemicals in *Daphnia magna* described by Geyer et al.[28] Kretschmann et al. [29] reported a lipid content between 1.5%

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and 1.8% for *Daphnia magna* (5−6 d), with lipid content similar to ephippia. The estimated log BCF values are systematically higher than the measured ones (Figure 2) indicating that *Daphnia* is not a good model to predict BCF in their ephippia. To the best of our knowledge, this is the first study that has obtained experimental BCFs of organic contaminants in ephippia. The linear regression of our data on log BCF versus log D_{ow} for ephippia can be used to predict accumulation of other organic pollutants in ephippia. Alternatively, uptake and elimination rate constants can be calculated for untested compounds based on our fit of the Hendriks at al.[26] model (Figure 3 and SI).

Estimated Concentrations in Ephippia in the Environment. Estimated C_{ipw} and C_{lip} in ephippia were obtained using the calculated BCF (BCF_{full}) values normalized to lipid content (BCF_{lip}) in combination with the lowest and highest organic contaminant concentrations found in sediments from Lake Greifensee. Results are reported in Table 1. The estimated internal concentrations in ephippia (C_{lip}) range from 1 to 68,000 $\mu g/kg_{lip}$. Highest values were obtained for TCC, followed by tonalide and TCS, while lower values were obtained for terbutryn, propiconazole, and irgarol in accordance with the log D_{ow}. Figure 4 shows the time series of estimated

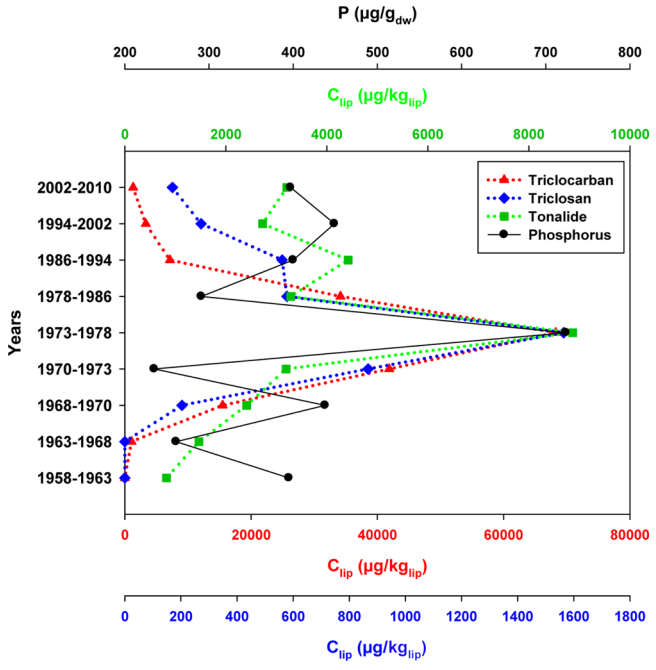

Figure 4. Estimated ephippia lipid normalized internal concentrations (C_{lip}) for triclocarban, triclosan, and tonalide in Lake Greifensee. Maximum C_{lip} concentrations are observed in the 1970s with a correlation between the highest C_{lip} of organic contaminants and highest phosphorus concentrations. Different colors correspond to scale units.

C_{lip} for TCS, TCC, and tonalide with a similar pattern to the total phosphorus (P) input into the lake and the highest concentrations around the 1970s. Anthropogenically increased levels of phosphorus from urban and industrial sewage, erosional runoff, and leaching from agricultural areas has been associated with a shift in *Daphnia* species composition in Lake Greifensee.[10] However, if this shift of species composition was caused by total phosphorus input or by organic contaminants still remains a challenging question.

Although TCC appears to be equally abundant but more persistent in the environment than TCS, the potential impact of TCC on organisms is almost unknown.[30] Estimated C_{ipw} for TCC range from 13 to up to 820 ng/L. The lowest observed effect concentration (LOEC) for TCC in *D. magna* has been reported at 4700 ng/L and a half maximal effect concentration (EC_{50}) of 10,000 ng/L.[31] Even though the EC_{50} values for TCC are more than 12 times higher and for the rest of compounds more than 1000 times higher when compared to predicted C_{ipw}, more studies are needed to understand the mixture toxicity and co-occurrence of organic contaminants in the environment. On the basis of our fit of the Hendriks et al.[26] model to ephippia data, one can calculate uptake and elimination rate constants for further organic contaminants and use those to predict the time course of concentrations in ephippia. This is possible, even for short or transient exposures that may occur in the water column. For diazinon, irgarol, and terbuthylazine, 50% SS is reached in about 20 min; therefore, these types of exposures could be significant. The use of the EqP to predict C_{lip} has its limitations because more parameters need to be taken into account such as the stability of the compounds in the water column and in the sediments, which was neglected in this study. However, the chemicals addressed in this study are known to be persistent, and additionally, degradation of organic contaminants in sediments usually take place at much slower rates than bioconcentration processes. The use of predicted D_{ow} and K_{oc} values add an extra uncertainty to the C_{lip} prediction. Nevertheless, the results obtained in this study give the first insight into the past and present concentrations in *Daphnia* resting eggs.

We have shown to what extent organic contaminants can bioconcentrate in *Daphnia* resting eggs and how this can be predicted using physical-chemical properties. This is a starting point to understand the potential impact of organic contaminants on aquatic organisms relying on resting eggs during their life cycle and moreover to comprehend and predict the impact of these compounds on ecosystems by their effects on benthic−pelagic coupling and evolvability of species.

■ ASSOCIATED CONTENT

Supporting Information

Additional information on analytical methods, temporal pattern of triclocarban, 95% steady state time predictions, experimental illustrations, equations, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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