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AN EXPERIMENTAL DESIGN TO PROBE THE INTERACTIONS OF DISSOLVED ORGANIC MATTER AND XENOBIOTICS : BIOAVAILABILITY OF PYRENE AND 2,2',5,5'-TETRACHLOROBIPHENYL TO *DAPHNIA MAGNA*

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ABSTRACT

Experiments were conducted to probe the interactions between natural dissolved organic matter (DOM) and two xenobiotics, and to determine how DOM influences their bioavailability. The experimental set-up, using dialysis bags, was designed to expose test organisms to the same constant concentration of free dissolved chemical, while increasing the concentration of the bound-to-DOM fraction. *Daphnia magna* S. were exposed to pyrene or 2,2',5,5'-tetrachlorobiphenyl in the presence of 0, 1, 2, 5, 10 or 20 mg L^{-1} of a reference riverine humic acid (Suwannee River Humic Acid). The physico-chemical parameters were well constrained in the microcosm, demonstrating its potential usefulness. However bioaccumulation by *D. magna* showed important variability between replicate treatments, sufficient to mask any trends as a function of DOM concentration. The organic-carbon-normalised partition coefficients (K_{OC}) ranged from 52000 to 92000 L kg^{-1} for pyrene and from 8200 to 89000 L kg^{-1} for 2,2',5,5'-tetrachlorobiphenyl, with a marked "concentration effect" for the latter compound.

INTRODUCTION

Natural dissolved organic matter (DOM) can bind to individual organic compounds and this has important consequences for their environmental fate. Many studies have established that the binding generally reduces the bioavailability of chemicals (including [1,2,3,4]). For example, Aldrich humic acid reduced the bioaccumulation and acute toxicity to *Daphnia magna* of synthetic pyrethroid insecticides [5], and the uptake of benzo(a)pyrene and 2,2',5,5'-tetrachlorobiphenyl by *Salmo gairdneri* [6]. On the other hand, enhanced ecotoxicological effects of contaminants in the presence of humic material have also been

reported on occasion. Methyl parathion and carbaryl elicited an enhanced toxicity to a bacterial bioluminescence assay in the presence of Aldrich humic acid, while other compounds of the same classes showed a reduced toxicity [7]. Likewise, there are reports of enhanced toxicity of fenveralate and lindane to *D. magna* exposed in solutions containing lake humic matter [8], slight increase in the accumulation of 2,2',4,4',5,5'-hexachlorobiphenyl by *D. magna* in the presence of DOM from a lake [9], and increased accumulation of methylcholanthrene by *Daphnia* in the presence of Aldrich humic acid [10].

The reasons for this inconsistent behaviour are unclear. In some cases, there may be insufficient binding between the chemical and the DOM for quantitative effects to be observed: it is estimated that the effect of DOM is significant only for chemicals with a octanol-water partition coefficient, K_{ow}, greater than 10^4 [11]. Where a quantitatively important binding takes place, it is unlikely that the bound fraction could be available for bioaccumulation. Indeed, studies of the steric factors influencing bioaccumulation point to a molecular cut-off of 600 Da [12], or a cross-section cut-off of 9.5 Å [13]. Other hypotheses involve direct effects of the DOM on the biota (e.g., see [14]). For example, humic material has a positive effect on acid-stressed *D. magna* [15]. DOM may effect the physiology of the animal, and thus the uptake rate of a contaminant. A greater uptake rate means that the test species will die faster or at a lower concentration of chemical, thus yielding a higher measured acute toxicity. Such effects might well be unpredictable since the properties of DOM may vary regionally and seasonally [2, 16].

In the above studies, it is usually difficult to understand the mechanisms at play because both the freely dissolved and the bound fraction of the contaminant vary between tests. The purpose of this paper is to describe and evaluate an experimental set-up to assess the relative bioavailability of the freely dissolved and the DOM-bound fraction of a xenobiotic. The hypothesis tested is that the bioaccumulation of each xenobiotic is controlled by the free unbound aqueous concentration and is independent of the humic acid concentration. The test organism is exposed to a fixed concentration of freely dissolved pyrene or 2,2',5,5'-tetrachlorobiphenyl, and at the same time to increasing concentrations of the compounds bound to a reference riverine humic acid. Dialysis bags are used to segregate treatments with different concentrations of humic acid in the same microcosm. This allows the determination of both the binding coefficient to DOM and the bioaccumulation of the xenobiotic.

MATERIALS AND METHODS

Test species

The test species we used is the waterflea *Daphnia magna* Strauss, a micro-crustacean of the Cladoceran order that can reach 5 to 6 mm in length. *D. magna* is found in many freshwater bodies in the North and the West of North America and constitutes an important link in many aquatic foodwebs, notably as an important source of food for juvenile salmonids [17]. Test specimens were obtained from the culture maintained by the ecotoxicology unit of the Quebec Ministry of the Environment. Homogenous

populations aged 4-6 days after hatching were used to ensure that the animals were sexually immature and to minimise variations in physiology that might interfere with the uptake of contaminants. The average dry weight was 550 µg for 10 individuals. Before exposure, the animals were left for 1 hour in clean synthetic medium to allow them to clear their gut content.

Water chemistry

The culture media for the *Daphnia* was a reconstituted water obtained by adding inorganic salts to deionised (milli-Q grade) water (organic free, DOC < 0.2 mg L^{-1}): $NaHCO_3$, 200 mg L^{-1}; $CaCl_2.2H_2O$, 297 mg L^{-1}; K_2SO_4, 27 mg L^{-1}. The pH was 7.6 (no adjustment), the ionic strength was 7.5 10^{-3} M and the temperature was kept at 20 ± 1 °C. The accumulation experiments were conducted in the same media supplemented with the studied compounds.

Model compounds

Table 1 lists the physico-chemical properties of the two model compounds studied. The chemicals chosen were pyrene, a polycyclic aromatic hydrocarbon (PAH) and PCB IUPAC No 52 (2,2',5,5'-tetrachlorobiphenyl). We will refer to these compounds as PYR and PCB. The acute toxicities of these compounds are not very high, but both families of compounds are an environmental threat because of their global distribution in the environment. Some PAHs are known or suspected carcinogens and PCBs are a threat to wildlife, particularly marine mammals. Radio-labelled compounds (^{14}C) were obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository, Chemsyn Science Laboratories (Lenexa, KS) for [4,5,9,10-^{14}C]pyrene (14.2 mCi $mmol^{-1}$ specific activity) and from Sigma Chemical Company (St. Louis, MO) for 2,2',5,5'-tetrachlorobiphenyl-UL (32.3 mCi $mmol^{-1}$ specific activity). Both compounds were of purity >98 % and were used as received without further purification. The chemicals were solubilised with a generator column as has been recommended for this type of study [18, 19]. Solutions of sparingly soluble organic substances are obtained by this method with a minimum of losses and without the drawbacks of using a co-solvent as carrier. The method used here involved coating the chemical onto glass beads (0.60-0.85 mm) in a column (15 cm long, 1 cm diameter) and letting water flow at a rate slow enough for saturation to be approached (22 mL min^{-1}). The concentrations obtained were close to the reported solubility of the chemicals (Table 1).

Dissolved Organic Matter

We chose to work with humic acids which have been shown to interact with apolar organic compounds. Various authors have cautioned against the use of poorly referenced commercial humic material as DOM [20]. The properties of these materials can be different from those of natural organic matter and their characteristics or properties may be variable from batch to batch. Furthermore, the characteristics and properties of natural DOM isolated from individual water bodies may also be variable. Hence we chose a

reference humic acid from the Suwannee River (1R101H), isolated and distributed by the International Humic Substances Society (Golden, CO). Elemental analysis indicates 54% C, 4% H, 41% O, 0.7% N and 0.82% ash. We will refer to this material as HA.

***Table 1*:** Physico-chemical properties of the chemicals studied.

	Molecular Weight	**Solubility ($\mu g\ L^{-1}$)**	**K_{OW}**	**Ref.**
PYRENE	202	150	5.2	[35]
PCB IUPAC No 52	292	30	6.1	[36]

Experimental set-up

The principle of the experimental system (Fig. 1) was to maintain a constant free dissolved concentration of the chemical, while submitting the test organisms to varying concentrations of HA and hence to varying concentrations of bound chemical. Equilibrium dialysis was employed to this effect, which also allowed the determination of the binding coefficient of the chemical to HA. Dialysis has been shown to be one of the best techniques to estimate the bioavailability of organics [21]. Dialysis bags (Spectra/Por 6, molecular weight cut-off 1000 Da) were filled with 40 mL of deionised water with varying concentrations of HA and put into a large (35 L) glass aquarium. Prior to use, the bags were rinsed thoroughly with deionised water to remove the sodium benzoate preservative. A saturated solution of the PYR or PCB was added to the aquarium. The large quantity of water in the aquarium outside the dialysis bags ensured that there was a large reservoir of chemical. Prior to the *Daphnia* exposure, the system was left to equilibrate so that the pollutants could reach equilibrium with each of the concentrations of HA. After four days the *Daphnia* were added to the bags. A large Teflon magnetic stirrer (10 cm) rotated slowly to ensure a gentle homogenisation of the system. A glass lid was used to limit evaporative losses. To avoid photodegradation of PYR, the system was kept in the dark and a gold fluorescent light (>500 nm) was used for illumination when necessary (when adding the test chemical, or the Daphnia).

In this system, the bioconcentration factor (BCF: the ratio of the concentration accumulated by *Daphnia*, C_d ($ng\ kg^{-1}$, wet weight), to the free dissolved aqueous concentration, C_f ($ng\ L^{-1}$), $BCF = C_d/C_f$) should be the same for all treatments because the free dissolved concentration is the same in all dialysis bags.

Accumulation experiments

The concentrations of chemicals were kept very low (30 to 110 $ng\ L^{-1}$) to ensure that they were close to environmentally realistic conditions. These concentrations were lower than those reported to be toxic to *Daphnia* [22, 23]. The concentrations of humic acid (0-20 $mg\ L^{-1}$) bracketed the concentrations of DOM

normally encountered in the aquatic environment: 1-5 mg C L^{-1} for freshwater, up to 20 mg C L^{-1} for very humic streams draining peatbogs [24].

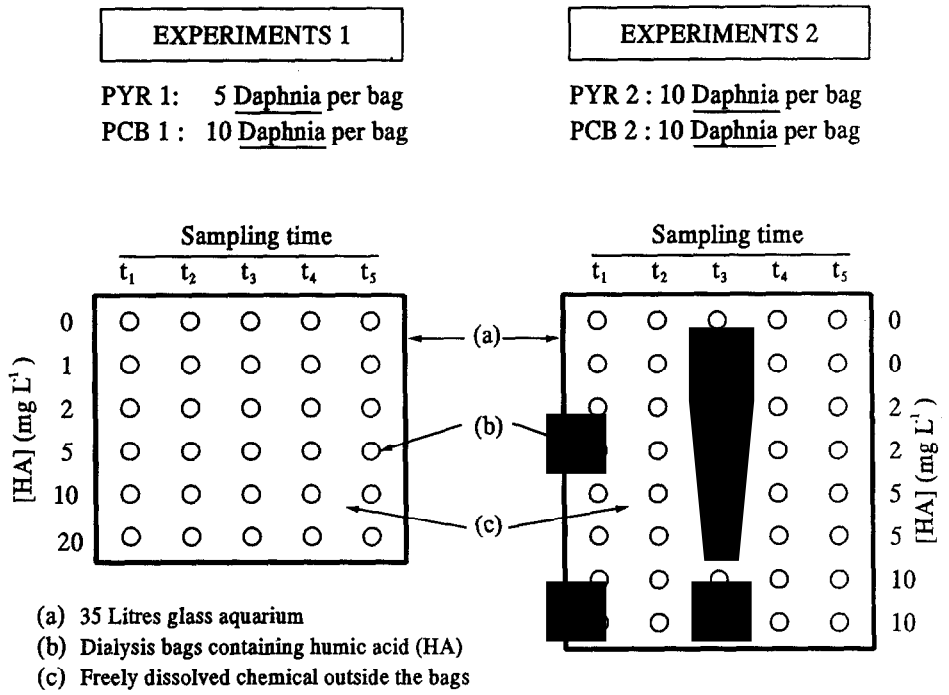

Figure 1: Schematic representation of the experimental set-up. *D. magna* are exposed to increasing concentrations of bound chemical corresponding to increasing concentrations of humic acid, while the level of free dissolved chemical remains the same among the treatments.

Following previous authors [6], the chemicals and the DOM were allowed to equilibrate for four days. Then 5 or 10 animals (depending of the experiment) were added to each dialysis bags and sampled at 5 time steps over the course of a 24-hour bioaccumulation experiment. This duration minimises the depletion of lipid reserves in *Daphnia* that is known to result from prolonged starvation. At five time intervals a dialysis bag was recovered, the animals removed on a net, and water samples taken from the bulk solution of the microcosm as well as inside the bag. The exact conditions of the four experiments referred to as PYR1, PCB1, PYR2 and PCB2 are summarised in Table 2.

Table 2: Experimental conditions for the four experiments.

	PYR1	PCB1	PYR2	PCB2
Start ($ng\ L^{-1}$) concentration	760	850	1500	1700
Mean exposure ($ng\ L^{-1}$) concentration (nM)	31 0.15	67 0.23	77 0.38	110 0.36
HA ($mg\ L^{-1}$)	0, 1, 2, 5, 10, 20	0, 1 ,2, 5, 10, 20	0, 2, 5, 10 in duplicate	0, 2, 5, 10 in duplicate
Number of Daphnia	5	10	10	10
Time steps t_1	3.75 h	4.25 h	3.50 h	4.50 h
t_2	6.58 h	7.00 h	7.25 h	8.00 h
t_3	10.50 h	10.50 h	11.75 h	14.00 h
t_4	18.33 h	14.50 h	19.00 h	18.75 h
t_5	24.50 h	24.00 h	24.00 h	24.00 h

Comparison of dead and living Daphnia

Differences in accumulation by living and dead *Daphnia* were evaluated. The organisms were killed by addition of NaN_3 (0.5-1%). Batch exposure experiments were conducted in triplicate with 20 organisms dead or alive, for both PYR and PCB. Dialysis bags or DOM were absent from this experiment. The exposure concentrations of the chemicals were as follow : PYR dead 780, 760, 780 $ng\ L^{-1}$; PYR alive 550, 510, 500 $ng\ L^{-1}$; PCB dead 3500, 3600, 3700 $ng\ L^{-1}$; PCB alive 2200, 2200, 2100 $ng\ L^{-1}$. The exposure was limited to 4.5 h to minimise the degradation of the dead animals. The latter were checked for damage under a microscope after exposure and there was no noticeable change in their appearance.

Chemical analysis

For the aqueous samples, 10 mL of scintillation cocktail (Aquasol fluor) were added to 10 mL samples and the resulting emulsion analysed using a liquid scintillation counter (LKB Wallac 1215 Rackbetta). The counting error was <2%. The daphnia were not left to dry to minimise the possible loss of contaminants. They were netted, washed rapidly with milli-Q water (10 mL), blotted dry, crushed with a Pasteur pipette and added to 5 mL of milli-Q grade water and 10 mL of scintillation cocktail.. This mixture was counted using the same apparatus. Due to the low level of radioactivity of the samples, they were counted for an hour each in order to obtain a satisfactory precision (<2%). Blanks (10 mL water + 10 mL scintillation cocktail) were run each time to measure the background level that was subtracted from the results. Quenching was corrected by measuring the response for an external standard (^{14}C-hexadecane) for each of the treatments (varying concentrations of humic acid).

Quenching and background corrections were used to transform the measured counts per minute (cpm) into disintegrations per minute (dpm). Knowing the specific activity of the chemicals, their molecular weight, and the dry weight of the animals, we could then calculate the concentrations of exposure and the quantities that had been bioaccumulated (ng L^{-1} and $\mu g\ kg^{-1}$).

RESULTS AND DISCUSSION

Behaviour of the experimental microcosm

The generator column was well adapted to deliver an aqueous solution of the chemicals. However, shortly after introduction into the aquarium, most of the aqueous solute had left the water column, presumably through evaporation and adsorption to the walls of the aquarium and to the dialysis bags. These losses were monitored. For example for PYR1, the original concentration of 760 ng L^{-1} dropped to 95 ng L^{-1} after 12 hours and to 46 ng L^{-1} after 65 hours. After the four days of equilibration, the decrease in concentration reached an asymptote and could be considered constant during the subsequent 24 hours accumulation phase (e.g. for PYR1, the exposure concentration varied from 35 ng L^{-1} to 29 ng L^{-1}, with a mean of 31 ng L^{-1}). The mean exposure concentration (Table 2) is the average of the concentrations measured over the time of the accumulation experiment (from t_0 to t_5).

The evolution of the concentrations inside and outside the bags was monitored and was found to evolve in parallel, indicating a rapid equilibration of the system. The binding of PAHs by DOM is rapid [25, 26]. The results of the duplicate water samples for PYR2 and PCB2 are in close agreement (Table 3). The system is therefore suitable for the study of the interactions of chemicals and DOM.

Binding of PYR and PCB by dissolved organic matter

Table 3 lists the analytical results for the aqueous phase for the four sets of experiments. The outside concentration (bulk of the microcosm) is the freely dissolved organic contaminant C_f (ng L^{-1}). The difference between the inside of the bag (C_t) and the outside is assumed to be the concentration of the chemical bound to HA, C_b (ng L^{-1}). The partition coefficient normalised to organic carbon, K_{OC}, is : $K_{oc} = C_b/(C_f \times DOC)$, where DOC is the concentration of dissolved organic carbon in kg C L^{-1}, with DOC = 0.54 [HA]. K_{oc} is expressed in units of L kg^{-1}.

Table 4 displays the K_{OC} values calculated for both series of experiments (PYR1/PCB1 and PYR2/PCB2) for each HA concentration and at each sampling time (t_1 to t_5). Because the amount of bound contaminant is small for the humic acid concentration of 1 mg L^{-1}, the determination of the bound fraction by difference is likely to be distorted by an important error; this is apparent in the observation that the standard deviation of the K_{OC} is larger for the lower concentrations of HA. Thus we only report K_{OC} for the experiments at 2, 5, 10 and 20 mg L^{-1} HA. The K_{OC} ranges from 52000 to 92000 L kg^{-1} for PYR and from 8200 to 89000 L

kg^{-1} for PCB. The K_{OC} values reported in the literature (Table 5) vary greatly with the type of organic matter and the measurement technique employed, nevertheless our results fall within the same range.

Table 3: Total aqueous (free + bound) concentrations of PYR or PCB (ng L^{-1}) in the dialysis bags.

	Concentration of HA (mg L^{-1})									
Sampling	0	0	1	2	2	5	5	10	10	20
	PYR1 (ng L^{-1})									
t_1	32	-	35	34	-	37	-	48	-	57
t_2	32	-	34	34	-	40	-	50	-	54
t_3	32	-	35	36	-	41	-	47	-	53
t_4	32	-	34	35	-	41	-	48	-	53
t_5	29	-	32	35	-	34	-	42	-	48
	PCB1 (ng L^{-1})									
t_1	77	-	85	79	-	80	-	87	-	89
t_2	76	-	79	76	-	72	-	78	-	85
t_3	69	-	67	72	-	72	-	72	-	72
t_4	57	-	68	70	-	69	-	73	-	71
t_5	56	-	57	55	-	57	-	67	-	63
	PYR2 (ng L^{-1})									
t_1	75	66	-	78	85	75	87	98	97	-
t_2	74	74	-	74	78	89	88	98	110	-
t_3	77	78	-	84	83	89	89	100	100	-
t_4	93	76	-	87	81	89	93	95	110	-
t_5	84	79	-	72	80	89	75	98	98	-
	PCB2 (ng L^{-1})									
t_1	125	120	-	130	120	120	110	140	120	-
t_2	110	120	-	125	130	130	120	110	125	-
t_3	100	87	-	96	83	96	97	97	93	-
t_4	94	100	-	93	98	110	96	105	110	-
t_5	100	97	-	90	96	97	96	99	100	-

Table 4: Organic-carbon-normalised partition coefficients of pyrene and PCB IUPAC No 52 with Suwannee River humic acid.

HA	t_1	t_2	t_3	t_4	t_5	Average	Std.-Dev.	n
(mg L^{-1})	**PYRENE**							
2 (exp 1)	35000	60000	110000	98000	160000	92000	48000	5
2 (exp 2)	160000	25000	72000	n.d.	n.d.	52000	24000	3
5 (exp 1)	49000	96000	95000	110000	58000	81000	26000	5
5 (exp 2)	58000	75000	57000	26000	n.d.	54000	20000	4
10 (exp 1)	90000	100000	84000	91000	80000	89000	7900	5
10 (exp 2)	74000	70000	57000	35000	39000	55000	18000	5
20 (exp 1)	71000	65000	60000	61000	58000	63000	5100	5
	PCB IUPAC No 52							
2 (exp 1)	21000	n.d.	41000	210000	n.d.	89000	100000	3
2 (exp 2)	n.d.	80000	n.d.	n.d.	n.d.	80000	-	1
5 (exp 1)	11000	n.d.	18000	75000	8800	28000	31000	4
5 (exp 2)	n.d.	25000	12000	19000	n.d.	19000	6800	3
10 (exp 1)	23000	4600	7500	50000	36000	24000	19000	5
10 (exp 2)	7500	4800	4000	21000	3800	8200	7200	5
20 (exp 1)	15000	11000	4900	21000	12000	13000	6000	5

n.d.: not determined (the counts in the dialysis bags with HA were lower than in the bags without HA)

Interestingly, PCB seems to have a lower K_{OC} for Suwannee River humic acid than does PYR. The PYR2 and PCB2 experiments are directly comparable as they have very similar exposure concentrations (0.38 nM and 0.36 nM respectively). The only result showing $K_{OC}^{PCB} > K_{OC}^{PYR}$ is for [HA] = 2 mg L^{-1}, but as we have seen the determination of K_{OC} is not very reliable for the lower concentrations of HA. The $K_{OC}^{PCB} < K_{OC}^{PYR}$ trend holds true if we compare PYR1 and PCB1, even though the molar concentration of pyrene is lower than that of PCB (0.15 nM and 0.23 nM respectively). One might have expected the extent of binding to be related to the respective Kow values of the chemicals (log K_{OW} = 5.2 for PYR and 6.1 for PCB). The discrepancy could be due to steric factors (PYR is smaller than PCB) and/or to selective chemical affinity between the compounds and the Suwannee River humic acid. Indeed it has been shown that benzo(a)pyrene and PCB bind selectively to different fractions of fractionated organic matter [27]. Similarly, the binding of benzo(a)pyrene to DOM of boreal origin is stronger than that of 3,3',4,4'-tetrachlorobiphenyl [2].

Table 5: K_{OC} values (L kg^{-1}) in the literature for pyrene and PCB IUPAC No 52.

K_{OC}	Type of humic material	Experimental technique	Ref.
PYRENE			
17000	Suwannee river humic acid	Fluorescence quenching	[26]
170000	Podzolic soil humic acid	Fluorescence quenching	[25]
81000-180000	Aldrich HA	Reverse phase separation	[3]
170000	Soil HA	Fluorescence quenching	[37]
PCB IUPAC No 52			
25000	Total DOC, stream river	Dialysis	[27]
58000	Aldrich HA	Reverse phase separation	[6]
35000	Aldrich HA	Reverse phase separation	[29]
26000	Aldrich HA	Dialysis	[29]
24000-64000	Soil and water humic acids	Volatilisation	[38]

In the case of PCB, there is a marked "concentration effect" where the partition coefficient decreases with increasing amounts of HA. Other authors have reported this phenomenon [28, 29, 30]. The most likely explanation is that there is increased humic-humic interaction as the HA concentration increases, which reduces the number of sites available for contaminant binding. In contrast, there seems to be little or no concentration effect with pyrene. This result can be interpreted in the light of the earlier arguments that pyrene and PCB do not bind to the same fractions of HA: these fractions do not necessarily interact with themselves in the same way.

Note too that, on average (mean of all the values measured at different time steps for a given [HA]), the K_{OC} values measured for different concentrations of HA are systematically higher for the first series (PYR1 and PCB1) than the second (PYR2 and PCB2), the first series having been carried out at slightly lower contaminant concentrations. This is a little surprising since the concentration of HA in our experiments is from 20000 to 300000 times greater than the pyrene or PCB concentrations on a mass basis. Since the standard deviation of our results is large, we can only make a suggestion which warrants further investigation: there might be a saturation of the hydrophobic sites. This could explain why Schlautman and Morgan [26] measured a lower K_{OC} for pyrene (Table 5) with the same HA (concentrations between 0 and 25 mg L^{-1}), as they used a much higher pyrene concentration (0.41 μM compared with 0.15 nM and 0.38 nM in the present experiments). This also has ecological relevance as it means that the proportion of bound

contaminants in the environment could be higher than suggested by laboratory experiments, since these are almost always done with contaminant concentrations that exceed ambient environmental levels.

Table 6: Bioconcentration factor in *D. magna*. BCF is the ratio of the concentration in *Daphnia*, C_d (ng kg^{-1} wet weight), to the free dissolved aqueous concentration, C_f (ng L^{-1}).

Concentration of HA (mg L^{-1})										
	0	0	1	2	2	5	5	10	10	20
	PYR1									
t_1	490	-	570	1200	-	840	-	n.d.	-	620
t_2	750	-	570	1200	-	1300	-	930	-	1000
t_3	530	-	800	930	-	890	-	1100	-	620
t_4	2800	-	n.d.	2000	-	2600	-	1900	-	2300
t_5	1400	-	1900	1900	-	n.d.	-	1700	-	2800
	PCB1									
t_1	430	-	250	380	-	350	-	460	-	470
t_2	350	-	670	480	-	370	-	690	-	770
t_3	770	-	820	1200	-	820	-	660	-	970
t_4	1100	-	640	960	-	790	-	830	-	930
t_5	1700	-	1100	1500	-	1300	-	2400	-	1700
	PYR2									
t_1	920	1300	-	890	1200	1100	780	1100	1000	-
t_2	1500	1400	-	770	1700	840	960	1100	1300	-
t_3	1800	960	-	970	1800	1100	1100	1100	2100	-
t_4	1900	1100	-	530	1600	1700	1600	1800	510	-
t_5	1400	n.d.	-	820	990	640	380	890	710	-
	PCB2									
t_1	540	690	-	610	330	690	690	940	1100	-
t_2	1300	690	-	980	960	960	1400	1500	1400	-
t_3	2400	2600	-	1600	1900	1500	1200	2400	2100	-
t_4	2500	990	-	3100	2300	1800	2700	2600	2500	-
t_5	2400	2100	-	2300	2200	1900	2700	2300	3700	-

n.d.: not determined (some animals were trapped in the closures of the dialysis bags and could not be recovered).

Accumulation of PYR and PCB by *Daphnia*

The bioconcentration factors (BCF) of PYR and PCB by *Daphnia* were calculated by using a dry weight/wet weight ratio for *D. magna* of 0.1 following Kukkonen *et al.* [31]. For each t_i and each HA treatment, the free aqueous concentration used to calculate the BCF was taken to be the total concentration in the dialysis bag for the treatment [HA] = 0. The average wet weight BCF (all treatments) after 24 h is 1900 ± 550 (n = 5) and 840 ± 330 (n = 7) for PYR1 and PYR2 respectively, and 1600 ± 450 (n = 6) and 2400 ± 550 (n = 8) for PCB1 and PCB2. These latter values are close to the BCF of 2000 reported by Dillon *et al.* [23] for the same PCB.

The variation of BCF values over time for various concentrations of HA is shown in Table 6. If the free dissolved contaminants only were bioavailable, the BCFs would be similar in all dialysis sacs, with no trends as a function of the HA concentration. If on the other hand the BCFs were higher with higher concentrations of HA, this could indicate that some bound chemical is bioavailable (unlikely), or that the presence of HA has an influence on the uptake rate (e.g. by affecting membrane permeability). In this latter case, one should see a higher uptake at the beginning of the exposure period, but the equilibrium BCF values should be the same. Under the present experimental conditions, there is no obvious pattern in the differences in BCF values among treatments. Thus, we cannot confirm or infirm the hypothesis that only the freely dissolved contaminant is bioavailable. There is sufficient scatter in the bioaccumulation data for the *Daphnia* to mask any subtle differences that might exist in accumulation among the treatments: in the second series of experiments, where duplicates were sampled, the variations between the BCF values for the same HA concentration are as high as the differences between treatments (Table 6). Other authors [32] have reported a high biological variability in the accumulation of polychlorinated biphenyls by *Daphnia.*

Table 7: Average and standard deviation (n = 3) of the BCF (wet weight basis) for the accumulation of pyrene and PCB by dead and living *D. magna* after 4.5 h in separate batch experiments.

	PYR	**PCB**
Alive	2200 ± 270	2600 ± 130
Dead	170 ± 9	130 ± 10

To gain some understanding of this biological variability, we studied the differential accumulation of PYR and PCB by living and dead *Daphnia.* The results (Table 7) demonstrate that the uptake of both chemicals by dead *Daphnia* is much lower than by living Daphnia. Heat-killed *Daphnia* had a slower uptake rate for benzo(a)pyrene [34]. In this latter experiment, the difference for BCF values between living and dead animals was similar to our observations: from 4600 to 500. This presumably reflects the importance of water filtration in the bioaccumulation process. Variations in filtration rate among individual *Daphnia* (e.g. in animals stressed from lack of food) could explain some of the variability in our BCF values. The marked

difference between living and dead organisms also suggests that, in the absence of ingestion of food, the uptake of the chemicals is predominantly via the filtration of water and that the transfer of PYR or PCB through the external carapace/membrane, and their adsorption on the surface of the animal, are negligible. Changes in the average BCF values (all treatments) with time (Fig. 2) differ between PYR and PCB. The accumulation curve for PCB increases over time and suggests that steady-state is not yet reached after 24 h, whereas the BCF for PYR shows a clear tendency to increase initially and then decrease with time. This decrease could be a sign of metabolic breakdown of PYR by *Daphnia*, with subsequent excretion of polar metabolites. Polar metabolites of benzo(a)pyrene represented 20 % of the ^{14}C activity in *D. magna* after 24 h exposure and 15 % of the activity of anthracene [34].

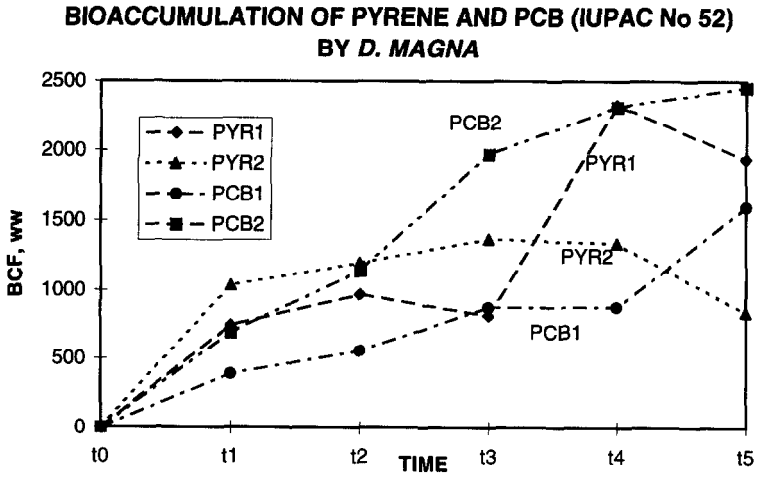

Figure 2: Bioaccumulation of pyrene and PCB IUPAC No 52 by *D. magna*. Mean BCF after each time step (for all [HA]) for each of the experiments.

CONCLUSION

The experimental set-up described was successfully employed to study the interactions of chemicals and DOM. In particular the physico-chemical factors were well constrained. It could be possible, for instance, to use this procedure to study simultaneously different types of DOM, while ensuring that other parameters such as the freely dissolved aqueous concentration of the chemical remain constant. Using *Daphnia* as test organism proved however to be a challenge because of the great variability in the measured bioconcentration factors. Other organisms (e.g., phytoplankton) might be more appropriate for demonstrating subtle differences among treatments. The accumulation of the chemicals by *Daphnia*

appears to be linked to water filtration, in the absence of ingestion, since the BCF values for dead animals are very small. The K_{OW} value was not the only predictor of the extent of binding between DOM and xenobiotics, and specific chemical affinity has to be considered to account for the greater K_{OC} of pyrene than that of 2,2',5,5'-tetrachlorobiphenyl.

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REFERENCES

1. G. E. Carlberg, K. Martinsen, A. Kringstadt, E. Gjessing, M. Grande, T. Källqvist and J. Skåre, Influence of aquatic humus on the bioavailability of chlorinated micropollutants in Atlantic salmon, *Arch. Environ. Contam. Toxicol.* **15**, 543-548 (1986).
2. J. Kukkonen and A. Oikari, Bioavailability of organic pollutants in boreal waters with varying levels of dissolved organic material, *Water Res.* **25**, 455-463 (1991).
3. P. F. Landrum, M. D. Reinhold, S. R. Nihart and B. J. Eadie, Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter, *Environ. Toxicol. Chem.* **4**, 459-467 (1985).
4. M. R. Servos and D. C. G. Muir, Effect of dissolved organic matter from Canadian shield lakes on the bioavailability of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin to the amphipod *Crangonyx laurentianus*, *Environ. Toxicol. Chem.* **8**, 141-150 (1989).
5. K. Day, Effects of dissolved organic carbon and acute toxicity of fenvalerate, deltamethrin and cyhalomethrin to *Daphnia magna* (Straus), *Environ. Toxicol. Chem.* **10**, 91-101 (1991).
6. M. C. Black and J. F. McCarthy, Dissolved organic macromolecules reduce the uptake of hydrophobic contaminants by the gills of Rainbow Trout, *Environ. Tox. Chem.* **7**, 593-600 (1988).
7. W. H. Benson and S. F. Long, Evaluation of humic-pesticide interactions on the acute toxicity of selected organophosphate and carbamate insecticides, *Ecotoxicol. Environ. Saf.* **21**, 301-307 (1991).
8. A. Oikari, J. Kukkonen and V. Virtanen, Acute toxicity of chemicals to *Daphnia magna* in humic waters, *Sci. Total Environ.* **118**, 367-377 (1992).
9. H. E. Evans, The influence of natural DOC on the uptake of 2,2',4,4',5,5'- hexachlorobiphenyl (PCB-153) by *Daphnia magna*, *Abstracts of Papers of the American Chemical Society*, Vol. 199, pp. 508-512 (1990).
10. G. J. Leversee, P. F. Landrum, J. P. Giesy and T. Fannin, Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons, *Can. J. Fish. Aquat. Sci.* **40**, 63-69 (1983).

11. J. F. McCarthy, Bioavailability and toxicity of metals and hydrophobic organic contaminants, In *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants* (Edited by I. H. Suffet and P. MacCarthy), American Chemical Society Symp. Series, Vol. 219, pp. 263-277 (1989).

12. D. N. Brooke, A. J. Dobbs and N. Williams, Octanol: water partition coefficients (P): measurement estimation and interpretation, particularly for chemicals with $P > 10^5$, *Ecotoxicol. Environ. Saf.* **11**, 251-260 (1986).

13. A. Opperhuizen, E. W. Velde, F. A. P. C. Gobas, D. A. K. Liem, J. M. D. Steen and O. Hutzinger, Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals, *Chemosphere* **14**, 1871-1896 (1985).

14. P. G. C. Campbell, M. R. Twiss and K. J. Wilkinson, Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota, *Can. J. Fish. Aquat. Sci.* **54**, 2543-2554 (1997).

15. R. C. Petersen and U. Persson, Comparison of the biological effects of humic materials under acidified conditions, *Sci. Total Environ.* **62**, 387-398 (1987).

16. S. Johnsen, K. Martinsen, G. E. Carlberg, E. T. Gjessing, G. Becher and M. Laegreid, Seasonal variation in composition and properties of aquatic humic substances, *Sci. Tot. Env.* **62**, 13-25 (1987).

17. Environnement Canada, Méthode d'essai biologique: essai de létalité aiguë sur *Daphnia* spp., Série de la protection de l'environnement, Report SPE 1/RM/11 (1990).

18. J. W. Billington, G.-L. Huang, F. Szeto, W. Y. Shiu and D. Mackay, Preparation of aqueous solutions of sparingly soluble organic substances: 1. Single component systems, *Environ. Toxicol. Chem.* **7**, 117-124 (1988).

19. B. G. R. Webster, M. R. Servos, G. G. Choudhry, L. P. Sarna and D. C. G. Muir, Methods for dissolving hydrophobic compounds in water - Interactions with dissolved organic matter. In *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants* (Edited by I. H. Suffet and P. MacCarthy), American Chemical Society Symp. Series, Vol 219, pp. 251-262 (1989).

20. C. T. Chiou, D. E. Kile, T. I. Brinton, R. L. Malcolm, J. A. Leenheer and P. MacCarthy, A comparison of water solubility enhancements of organic solutes by aquatic humic materials and commercial humic acids, *Environ. Sci. Technol.* **21**, 1231-1234 (1987).

21. J. Kukkonen and J. Pellinen, Binding of organic xenobiotics to dissolved organic macromolecules - Comparison of analytical methods, *Sci. Total Environ.* **152**, 19-29 (1994).

22. A. M. Bobra, W. Y. Shiu and D. Mackay, A predictive correlation for the acute toxicity of hydrocarbons and chlorinated hydrocarbons to the water flea (*Daphnia magna*), *Chemosphere* **12**, 1121-1129 (1983).

23. T. M. Dillon, W. H. Benson, R. A. Stackhouse and A. M. Crider, Effects of selected PCB congeners on survival, growth, and reproduction in *Daphnia magna*, *Environ. Tox. Chem.* **9**, 1317-1326 (1990).

24. E. M. Thurman, *Organic Geochemistry of Natural Waters,* Martinus Nijhoff, 497 pp. (1985).

25. T. D. Gauthier, E. C. Shane, W. F. Guerin, W. R. Seitz and C. L. Grant, Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials, *Environ. Sci. Technol.* **20**, 1162-1166 (1986).

26. M. A. Schlautman and J. J. Morgan, Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials, *Environ. Sci. Technol.* **27**, 961-969 (1993).

27. C. W. Carter and I. H. Suffet, Binding of DDT to dissolved humic materials, *Environ. Sci. Technol.* **16**, 735-740 (1982).

28. J. Kukkonen, J. F. McCarthy and A. Oikari, Effects of XAD-8 fractions of dissolved organic carbon on the sorption and bioavailability of organic micropollutants, *Arch. Environ. Contam. Toxicol.* **19**, 551-557 (1990).

29. P. F. Landrum, S. R. Nihart, B. J. Eadie and W. S. Gardner, Reverse-phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters, *Environ. Sci. Technol.* **18**, 187-192 (1984).

30. R. Lara and W. Ernst, Interaction between polychlorinated biphenyls and marine humic substances - Determination of association coefficients, *Chemosphere* **19**, 1655-1664 (1989).

31. J. Kukkonen, A. Oikari, S. Johnsen and E. Gjessing, Effects of humus concentrations on benzo[a]pyrene accumulation from water to *Daphnia magna* - Comparison of natural waters and standard preparations, *Sci. Total. Environ.* **79**, 197-207 (1989).

32. H. E. Evans, The influence of water column dissolved organic carbon on the uptake of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) by *Daphnia magna*, In *Organic Substances and Sediments in Water Vol. 3* (Edited by R. A. Baker), Lewis, pp. 95-108 (1991).

33. J. F. McCarthy, Role of particulate organic matter in decreasing accumulation of polynuclear aromatic hydrocarbons by *Daphnia magna*, *Arch. Environ. Contam. Toxicol.* **12**, 559-568 (1983).

34. M. M. Miller, S. P. Wasik, G. L. Huang, W. Y. Shiu and D. Mackay, Relationships between octanol water partition coefficient and aqueous solubility, *Environ. Sci. Technol.* **19**, 522-529 (1985).

35. W. Y. Shiu and D. Mackay, A critical review of aqueous solubilities, vapor pressures, Henry law constants, and octanol-water partition coefficients of the polychlorinated biphenyls, *J. Phys. Chem. Ref. Data* **15**, 911-929 (1986).

36. B. E. Herbert, P. M. Bertsch and J. M. Novak, Pyrene sorption by water soluble organic carbon, *Environ. Sci. Technol.* **27**, 398-403 (1993).

37. M. A. Jota and J. P. Hassett, Effects of environmental variables on binding of a PCB congener by dissolved humic substances, *Environ. Toxicol. Chem.* **10**, 483-491 (1991).