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Role of Particulate Organic Matter in Decreasing Accumulation of Polynuclear Aromatic Hydrocarbons by *Daphnia magna*

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Abstract. Accumulation of benzo[a]pyrene (BaP) and anthracene (A) by *Daphnia magna* in the presence of suspended yeast cells was analyzed using multicompartment models. The rate coefficient for uptake of polynuclear aromatic hydrocarbon (PAH) due to ingestion of yeast cells laden with sorbed chemical was only 3 to 15% of the rate coefficient for uptake of dissolved PAH. Uptake and accumulation of BaP was reduced 97% due to sorption of PAH to naturally occurring organic matter (humic acids). Accumulation of hydrophobic chemicals in aqueous systems appears to depend on the amount of chemical in solution and on the amount of chemical sorbed to particles entering the food chain. Chemicals sorbed to suspended organic matter, including dissolved or colloidal organic matter, have greatly reduced availability.

The possible future development of a large-scale synthetic fuels industry in the United States creates the potential for release of substantial quantities of organic wastes to aquatic ecosystems. The effluents and products from such processes are exceedingly complex mixtures of organic compounds (Gehrs 1976).

For many organic compounds in aqueous systems, there is a positive correlation between the hydrophobicity of the compound (*e.g.*, octanol-water partition coefficient) and both its potential for concentration in aquatic organisms (Ellgehausen *et al.* 1980; Southworth *et al.* 1978, 1980; Veith *et al.* 1979; Neely *et al.* 1974; Kenaga and Goring 1980) and its potential for sorption to suspended particulate matter or to sediment (Karickhoff *et al.* 1979; Means *et al.* 1979; Kenaga and Goring 1980). Sorption to suspended particulate matter could decrease the amount of xenobiotic organics directly available from solution, but permit entry via the food chain because filter feeders ingest particles laden with chemicals.

In this study, the net effect of sorption is examined on the accumulation of two polynuclear aromatic hydrocarbons (PAHs): the 5-ring benzo[a]pyrene (BaP), a known carcinogen, and the 3-ring anthracene (A) by the cladoceran, *Daphnia magna*. The purpose of this research is to develop a quantitative framework to assess the relative contribution of the two routes of accumulation: direct uptake of hydrophobic organic compounds from solution vs uptake by ingestion of PAH-laden particles.

Materials

Benzo[a]pyrene (BAP; 7,10-^{14}C; 21.7 mCi/mmol) and anthracene (A; 9-^{14}C; 32 mCi/mmol) were obtained from Amersham (Arlington Heights, IL). Non-radioactive BaP or A (Aldrich, 99+% gold label) was added to ^{14}C-BaP or ^{14}C-A, respectively, in dioxane, and 10 μL of either the BaP or A stock solution was dissolved per L of millipore-filtered well water (0.45 μm; Millipore Corp.) to nominal concentrations of approximately 1 μg/L (2×10^5 dpm/L) for BaP or 35 μg/L (5.6×10^5 dpm/L) for A. Solutions were covered, stirred overnight, and filtered twice through GF/F glass fiber filters (Whatman) prior to experiments. Concentrations of PAH were determined by liquid scintillation counting

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(see below) and by calculation based on the specific activity of the PAH stock solutions. All experiments were conducted at 21°C and under gold fluorescent lights (>500 nm) to minimize photodegradation. The water used in these studies had a pH of 7.6, hardness of 85 mg/L and dissolved organic carbon (DOC) concentration of 1.1 mg/L.

Daphnia magna used in experiments were approximately 10 days old, reproductively immature, and ranged from 0.74 ± 0.1 (SE) to 1.14 ± 0.2 mg wet wt per animal. The standard errors indicate the weight range within any particular experiment.

Yeast cells were used as particulate matter because of ease of culture and homogeneity of size, shape, and surface characteristics and because yeast is a known food source for *Daphnia*. Clones of yeast (*Saccharomyces cerevisiae*) were isolated by plating on maltose agar, then transferred and grown in Difco Broth (1.5 g beef broth, 2.5 g beef peptone/L) enriched with 5 g/L glucose. Immediately prior to use, cells were autoclaved, concentrated by centrifugation (1000 g × 10 min), rinsed twice in filtered well water by recentrifugation and suspended in a small volume of well water. Cell number and size was determined with a Model B Coulter Counter (Coulter Instruments). Appropriate volumes were then added to filtered ^{14}C-PAH solutions and allowed to equilibrate for use in sorption and uptake studies.

Methods

The fraction of ^{14}C-PAH bound to yeast was determined by filtration of the equilibrated suspension. The total ^{14}C activity of the suspension (A_T) was determined by liquid scintillation counting of 5 mL in 10 mL ACS cocktail (Amersham-Searle) using a Packard 460C scintillation counter. The ^{14}C activity on precombusted GF/F glass fiber filters (Whatman) after filtration of 10 or 25 mL of suspension (A_F) and the nonspecific sorption of ^{14}C after filtration of the same volume of particle-free ^{14}C-PAH solution (A_b) was determined by counting the filters in 10 mL of ACS. The fraction of ^{14}C sorbed to particles (f) was calculated:

$$f = (A_F - A_b)/(A_T). \quad (1)$$

The partition coefficients for sorption of BaP and A to yeast were determined from the least squares estimate of the slope of the adsorption isotherms.

The uptake of ^{14}C-BaP by *Daphnia* was measured in two types of experiments. In early experiments, designated batch exposures, groups of 100 to 150 animals were added to 3 L of ^{14}C-BaP solution (with or without yeast). At various times, groups of 5 to 10 *Daphnia* were removed, blotted dry, weighed on a Cahn Model 21 electrobalance, and added to 10 mL ACS cocktail. The cocktail was effective in extracting ^{14}C from the animals and gave results comparable to sonicating animals in dioxane and counting the dioxane. The total radioactivity of the ^{14}C-BaP solution and the fraction of PAH sorbed to particles were determined as described.

In the second type of experiment, designated rotating jar exposures, groups of five *Daphnia* were transferred to 4-oz glass jars (with teflon-lined caps) containing 100 mL of ^{14}C-PAH solution, with or without yeast. The jars were rotated end-over-end at 1 rpm (McCarthy 1983). At various times, all animals in the jar were removed, blotted, weighed and analyzed for ^{14}C-activity as described. The ^{14}C-solutions were analyzed as in batch exposures.

For experiments measuring the uptake of PAH by *Daphnia* in the presence of yeast, the yeast-PAH suspension was prepared by addition of concentrated, autoclaved yeast suspension to filtered ^{14}C-PAH solutions. The suspension was equilibrated for at least 4 hr before beginning an uptake experiment. Equilibration appeared to be complete by 4 hr; there was no increase in the fraction of ^{14}C-PAH bound to yeast between 4 and 26 hr.

Prior to adding *Daphnia* to particle-free ^{14}C-PAH solutions for uptake experiments, animals were maintained for 2 hr in filtered well water to permit clearance of their gut. Animals to be exposed to yeast-PAH suspension were prefed for 2 hr in a yeast suspension without PAH at the same concentration as the ^{14}C suspension to which they were to be exposed.

The uptake of ^{14}C-BaP was also measured in heat-killed *Daphnia* to assess the importance of filter feeding activity on the uptake of PAH. Animals were killed by exposure to 50°C for 30 min. Groups of five animals were transferred to jars contained particle-free ^{14}C-BaP solutions and uptake measured using the rotating jar exposure method. The heat treatment did not cause noticeable changes in the appearance of the animals (*e.g.*, no swelling, lysis, etc.) for at least the first 4 to 6 hr.

The uptake of ^{14}C-BaP was also measured in the presence of humic acids. Humic acids (HA; Aldrich) were purified by repeated dissolution in 0.1 N NaOH followed by precipitation due to acidification to pH 2 with HCl. The final precipitate was dissolved in distilled water and dialyzed against distilled water. Concentrated HA solution was added to 3 L of filtered ^{14}C-BaP solution to a final concentration of 20 mg HA/L and allowed to equilibrate for 1 hr before ~100 *Daphnia* were added. Groups of 5 to 10 animals were sampled at various times, and analyzed as described for batch exposures.

To measure the rate of depuration of ^{14}C-BaP, groups of 100 to 150 *Daphnia* were held for 3 hr in a filtered solution of ^{14}C-BaP, then transferred to 3.5 L of filtered well water. At various times, 5 to 10 animals were collected, blotted dry, weighed, and assayed for ^{14}C.

To measure the rate of metabolism of ^{14}C-PAH by *Daphnia*, groups of 5 animals were maintained in jars with 100 mL filtered ^{14}C-PAH solution for 0, 2, 4, or 24 hr. Solutions and *Daphnia* were then analyzed for the appearance of metabolites of ^{14}C-PAH. The total ^{14}C-activity of the uptake water was determined and 90 mL of solution was extracted twice in 10 mL hexane by shaking for 1 hr. The ^{14}C-activity of both the pooled hexane extracts and the aqueous phase was determined. The test animals were disintegrated by sonication in 1 mL dioxane. One-half the dioxane was analyzed for total ^{14}C activity. The remainder was diluted to 90 mL with distilled water and extracted with hexane.

^{14}C-activity in the aqueous phase above control (zero time) levels was assumed to be polar metabolites produced by *Daphnia* (Cohen *et al.* 1980); for BaP, 92.0 ± 1.3 (SE)% (n = 7) of the ^{14}C-BaP and 92.3 ± 13% (n = 8) of the ^{14}C-A in the control solutions were extracted into the hexane. The hexane extracts were concentrated by evaporation and analyzed for radiolabelled PAH metabolites by high performance liquid chromatography (HPLC) on a C-18 reverse phase column using a Varian Model 5000 liquid chromatograph and a Varian Vista 401 Chromatography Data System. The column was eluted with a linear gradient of 20 to 100% acetonitrile in water (Burdick and Jackson Labs, Muskegon, MI) over 30 min at a flow rate of 2 mL/min. Fractions (2 mL) of the column eluant were collected in scintillation vials and analyzed for ^{14}C-activity. In uptake experiments, total ^{14}C-activity in *Daphnia* and water was corrected by subtracting the percentage of radioactivity present in polar compounds; results are reported as concentration of parent compound in the animals or uptake solution.

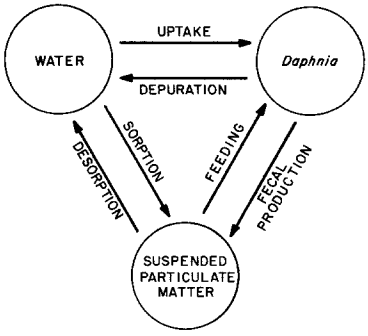

Fig. 1. Conceptual model describing the flux of hydrophobic chemicals among the dissolved, sorbed, and biotic components

Experimental Design and Analysis

These experiments were designed to determine rate coefficients for uptake of PAH from solution and from ingestion of yeast cells with sorbed PAH. Figure 1 illustrates the conceptual model used.

In the absence of yeast, the net flux of BaP in *Daphnia* can be described in Eq. (2):

$$dD/dt = (k_u \times W) - (k_d \times D) \quad (2)$$

where D = μg PAH/(g wet wt), W = μg PAH/mL (*i.e.*, the amount of PAH in the *Daphnia* and water, respectively), k_u = uptake rate coefficient (mL/g wet wt/hr) and k_d = depuration rate coefficient (per hr). Equation (2) can be solved:

$$D(t) = [(k_u/k_d) \times W][1-e^{-k_d t}] \quad (3)$$

where t = time (hr). The concentration of A in water (W) did not decrease significantly over the course of the experiment, and Eq. (3) was used to obtain estimates for k_u and k_d. However, because BaP was so highly concentrated by *Daphnia*, the concentration of BaP in water decreased over time with kinetics approximated by:

$$W(t) = \alpha + \beta e^{-\lambda t}. \quad (4)$$

By substituting Eq. (4) into Eq. (2) and solving for D(t),

$$D(t) = \left(\frac{k_u\beta}{\lambda - k_d} - \frac{k_u\alpha}{k_d}\right)e^{-k_d t} - \left(\frac{k_u\beta}{\lambda - k_d}\right)e^{-\lambda t} + \frac{k_u\alpha}{k_d}. \quad (5)$$

Data on the decrease of BaP in water (total ^{14}C activity corrected for appearance of polar metabolites) were fitted to Eq. (4), then fitted values of α, β, and λ were substituted into Eq. (5) (after Southworth *et al.* 1978). The values of k_u and k_d were then estimated from data on the accumulation of BaP in *Daphnia* over time.

In experiments measuring uptake of PAH in the presence of yeast, yeast cells were pre-equilibrated with PAH solution before *Daphnia* were added. Because the fraction of PAH bound to yeast did not change over the experimental period, the sorption/desorption kinetics between water and yeast were ignored in these analyses. The concentration of yeast in solution did not decrease significantly during the experiments, although there was a decrease in the media cell size, presumably due to fragmentation of yeast cells as they passed through the gut (Kersting and Holterman 1973). The flux of PAH among the water, *Daphnia*, and yeast compartments is described by Eq. (6):

$$dD/dt = (k_u \times W) - (k_d \times D) + (k_f \times Y). \quad (6)$$

Where W and Y = μg PAH in dissolved and sorbed form per mL suspension (μg/mL), respectively, and k_f = rate coefficient for net assimilation of PAH resulting from ingestion of yeast cells (mL/g wet wt/hr). Y can be converted to units of μg PAH/(g yeast) (= Y′) by dividing Y by the yeast concentration used in each experiment; k_f can be converted to units of g yeast/g animal wet wt/hr (= k_f') by dividing the product of [$k_f \cdot$ Y] by Y′. Equation (6) can be solved:

$$D(t) = \frac{k_u W + k_f Y}{k_d}(1 - e^{-k_d t}). \quad (7)$$

The rate coefficients were estimated by the nonlinear iterative least squares procedure, NLIN, of SAS (Statistical Analysis Institute, Raleigh, NC). Data on the *Daphnia* [D(t), as μg/g wet wt] at various exposure times, as well as the concentration of PAH sorbed to yeast (Y; μg/mL suspension) and/or dissolved water (W as μg/mL in Eq. (3) or (7), or values of α, β and λ [Eq. (5)] estimated by a similar nonlinear regression procedure on the changing BaP concentration in water over time [Eq (4) with W(t) as μg/mL]) were given as inputs to the appropriate 2 or 3 compartment model. The rate coefficients estimated by fitting the data to the model are reported ± asymptotic standard error.

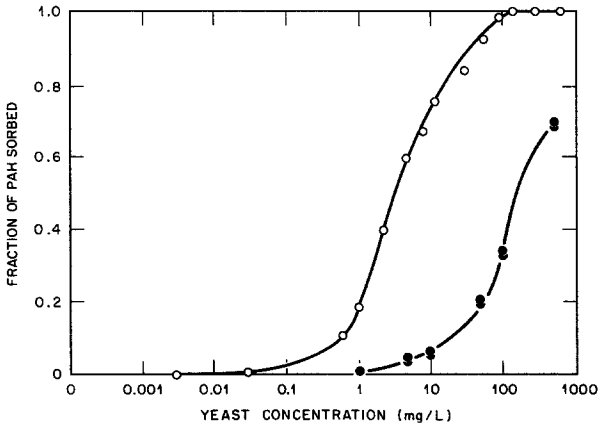

Fig. 2. The fraction of benzo[a]pyrene (open circles) and anthracene (closed circles) sorbed at equilibrium to yeast in relation to yeast concentration. Yeast was added to ^{14}C-BaP (1 μg/L) or ^{14}C-A (35 μg/L) and equilibrated with shaking for 24 hr

Results

Sorption of BaP to Yeast Cells

Partitioning of BaP is dramatically affected by the concentration of yeast cells, varying from 0 to 100% sorbed between 0.1 and 120 mg/L yeast. The region of greatest effect is typical of amounts of suspended solids found in many natural waters (10-100 mg/L; Figure 2). The fraction of A sorbed to yeast also increases with increasing concentrations of yeast,

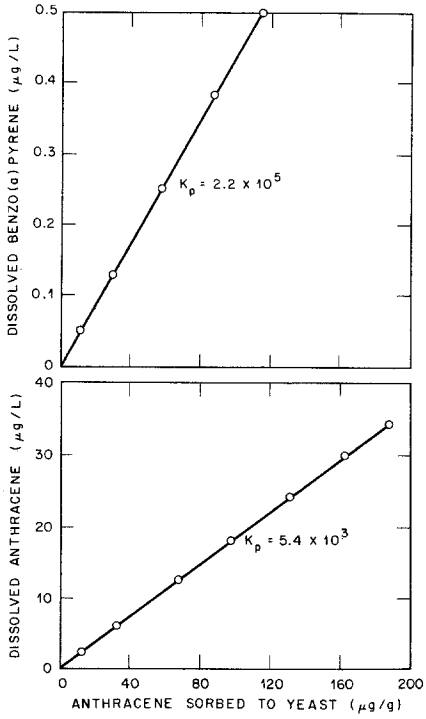

Fig. 3. Isotherms for (A) benzo[a]pyrene and (B) anthracene sorption to yeast constructed by equilibrating yeast (6 mg/L) in solutions of ^{14}C-BaP (0.12 to 1.2 μg/L), or equilibrating ^{14}C-A (1.7 to 40.8 μg/L) with yeast (35 mg/L)

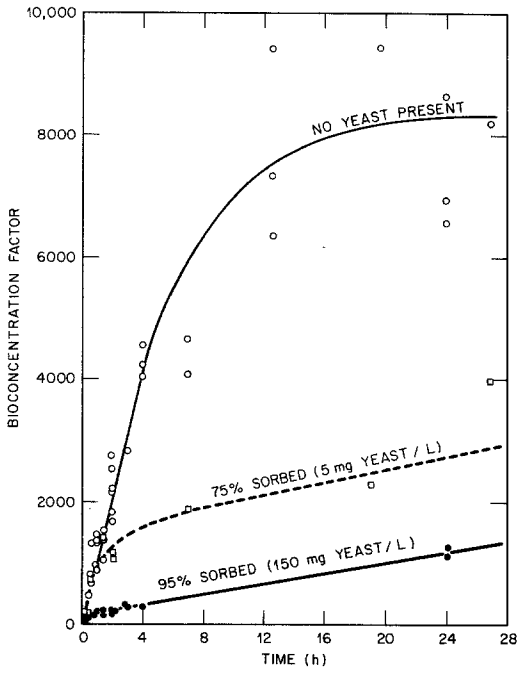

Fig. 4. Accumulation of benzo[a]pyrene by *Daphnia* in particle-free water (open circles) and when BaP was equilibrated with 5 mg yeast/L (open squares) or 150 mg yeast/L (closed circles)

although relatively less A is sorbed. Freundlich isotherms for both BaP and A were a straight line with a unit slope, which indicates that the number of adsorption sites exceeded the number of PAH molecules adsorbed. The partition coefficient, K_p, for sorption of BaP on yeast cells is 2.2 × 10^5, while the K_p for the less hydrophobic PAH, A, is 5.4 × 10^3 (Figures 3A,B).

Metabolism of PAH by Daphnia

(a) Benzo[a]pyrene—The percentage of ^{14}C activity remaining in the aqueous phase after partitioning against hexane increased over time both in *Daphnia* and their uptake solution. Polar metabolites in *Daphnia* increased from 12.2 ± 4.5 (SE)% (n = 6) to 20.1 ± 3.0% (n = 6) of the total ^{14}C-activity between 4 and 24 hr. Polar metabolites were not detected at 4 hr (n = 7), but by 24 hr, 21 ± 2% (n = 5) of the ^{14}C in the uptake solution was polar. HPLC analysis of the hexane extracts confirmed that no additional ^{14}C metabolites extracted into the hexane. The rate coefficient for biotransformation of BaP by *Daphnia,* calculated from the body burden of BaP in *Daphnia* and the amount of BaP metabolites accumulated in *Daphnia* and released into the water between 4 and 24 hr, is approximately 0.08 per hr, or, on a molar basis, 1.1 nmole/g wet wt/hr at 4 μmole/L.

(b) Anthracene—A much smaller percentage of total ^{14}C-activity in the uptake medium remained in the aqueous phase after partitioning against hexane because of the much greater concentration of A (35 μg/L) in solution. Only 0.22 ± 0.02 (SE)% (n = 7), 0.60 ± 0.16% (n = 7), and 2.93 ± 0.06% (n = 8) of the ^{14}C-activity in the uptake water remained in the aqueous phase at 2, 4, and 24 hr. In the *Daphnia,* 12.0 ± 5.0% (n = 7), 10.0 ± 3.5% (n = 7), and 14.5 ± 3.7% (n = 8) of the total ^{14}C-activity in the animals was extracted into the aqueous phase at 2, 4, and 24 hr. HPLC analysis of the hexane extracts showed that no additional ^{14}C metabolites of A were extracted into the hexane. The rate coefficient for biotransformation of A by *Daphnia* is 0.11 per hr, or, on a molar basis, 4.2 nmole/g wet wt/hr at 185 nmole/L.

Accumulation of PAH by Daphnia

The accumulation of BaP by *Daphnia* in the absence of particulate matter and in the presence of 5 mg yeast/L (75% of the BaP sorbed to yeast) and 150 mg yeast/L (95–98% sorbed) is shown in Figure 4. The bioconcentration factor [BCF = ratio of μg

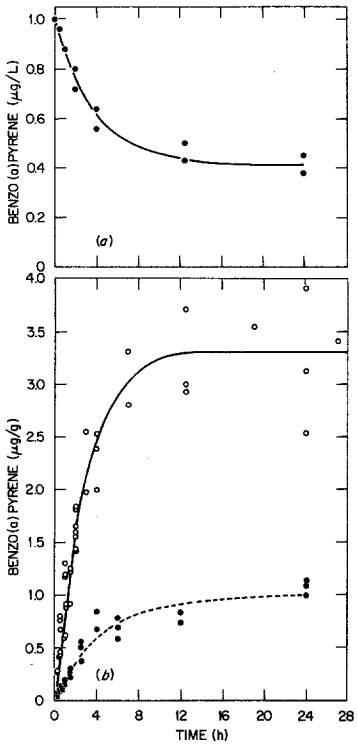

Fig. 5. (A) Decrease in the concentration of benzo[a]pyrene in particle-free water after addition of *Daphnia* at time zero. Closed circles indicate observed data and the solid line is the curve predicted by fitting the data to Eq. (4). (B) Uptake of benzo[a]-pyrene by *Daphnia* (0.90 ± 0.09 (SE) mg wet wt per animal) in particle-free water (open circles) and an equilibrated suspension of 25 mg yeast/L (89% of BaP sorbed) (closed circles). The solid and dashed lines are the curves predicted by fitting the data to Eq. (5) or Eq. (7), respectively

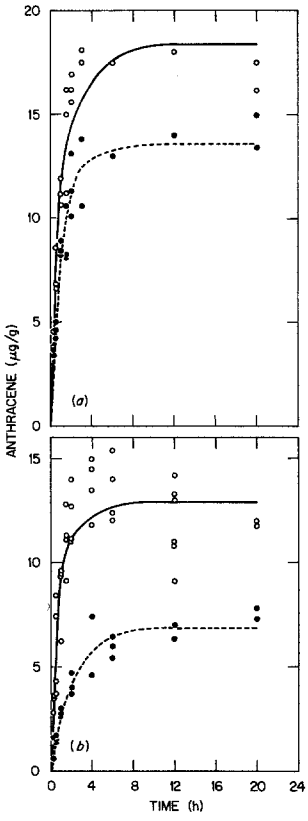

Fig. 6. (A) Uptake of anthracene by *Daphnia* in particle-free water (open circles) and an equilibrated suspension of 67 mg yeast/L (30% of A sorbed) (closed circles). Anthracene concentration is 38 μg/L. The solid and dashed lines are the curves predicted by fitting the data to equations (3) or (7), respectively. (B) Same as (A), but the yeast concentration is 190 mg/L (53% of A sorbed) and the anthracene concentration is 30 μg/L

BaP per g wet wt of *Daphnia vs* μg BaP per mL of solution or suspension (sum of the dissolved and sorbed form)] decreased from an apparent steady-state level of approximately 8000 in the absence of sorptive particles to approximately 1000 and 2500 in the presence of yeast sorbing 95% and 75% of the BaP, respectively. These results clearly indicate that sorption of BaP to suspended particulate organic matter (yeast) decreases availability of the PAH to *Daphnia*.

The data in Figure 4 was obtained in three non-simultaneous experiments with the batch exposure system (cf. Methods). Since these experiments used different groups of *Daphnia* (slightly different sizes and possibly different nutritional conditions) and since some yeast settled and became unavailable as food source over the course of the experiment, these data were not used to estimate the rate coefficients for uptake of PAH from the dissolved and sorbed compartments. These rate coefficients were estimated from data collected in simultaneous experiments (with or without yeast) using the same population of animals; furthermore, the rotating jar exposure system (cf. Methods) was used to prevent settling of yeast (Figures 5 and 6).

The rapid accumulation of BaP by *Daphnia* in particle-free water decreased the aqueous concentration of BaP (Figure 5A). The nonlinear regression procedure fit these data to Eq. (4) and yielded estimated values (±asymptotic standard error) for α, β and λ of 0.67 ± 0.02, 0.30 ± 0.01, and 0.28 ± 0.09, respectively. These values were substituted into Eq. (5), and the values of k_u and k_d estimated for the uptake of dissolved BaP (Table 1). Values of k_f, the rate coefficient describing the uptake of BaP sorbed to the food particles, was estimated in two ways: (a) the estimated value of k_u determined from Eq. (5) was substituted into Eq. (7) and the values of k_d and k_f estimated by the nonlinear regression procedure; and (b) the values of all three rate coefficients were estimated from Eq. (7) (Table 1). Estimated uptake of sorbed BaP (k_f) is only 5 to 10%

Table 1. Rate coefficients for uptake and depuration (± asymptotic standard error) of benzo[a]pyrene (1 μg/L); k_u and k_f are reported in units of mL/g animal wet wt/hr, k_d in units of per hr

		25 mg/L yeast (89% sorbed)	
k	No yeast	k_u fixed	All k's estimated
k_u	1040 ± 42	1040	1418 ± 623
k_d	0.13 ± 0.008	0.22 ± 0.02	0.22 ± 0.02
k_f		118 ± 20	74 ± 42

of the rate for uptake of dissolved BaP (k_u). The observed BCF for BaP decreased from 8500 in the absence of particles to 1000 in the presence of yeast sorbing 89% of the BaP.

The uptake of anthracene by *Daphnia* was examined in the same way at two concentrations of yeast. The two sets of experiments differed in the size of *Daphnia* used and in the final concentration of the anthracene solutions (Table 2, Figures 6A,B). The rate coefficients for uptake of A in particle-free water (k_u) is approximately only half that for BaP, and the depuration rate (k_d) is 7- to 10-fold faster for A than for BaP. The observed BCF for A is approximately 450–500 in the absence of particles, but is decreased to approximately 370 and 230 in the presence of yeast sorbing 30% and 53% of the A, respectively.

The values of k_u and k_d estimated from experiments in which yeast were present were lower than those estimated in the absence of yeast. The difference increased with increasing concentrations of suspended particulates and probably reflects changes in filtration rates of different concentrations of yeast (cf. Discussion). As was evident for BaP, the rate coefficient for uptake of A sorbed to yeast (k_f) was only 3 to 15% of the rate coefficient for uptake of dissolved A (k_u).

Depuration of BaP from Daphnia

The depletion of ^{14}C-activity from *Daphnia* after transfer to uncontaminated well water is shown in Figure 7. The shape of the depuration curve suggests that there is more than one kinetic component involved. The data were fitted by the nonlinear regression procedure to a double exponential model:

$$f = ae^{-k_1 t} + be^{-k_2 t}, \quad (8)$$

where f = fraction of BaP remaining in the *Daphnia* at time t, and k_1 and k_2 are rate coefficients for depuration from arbitrarily defined, fast and slow compartments. The estimated values (± asymptomatic standard error) of parameters in Eq. (8) were: a = 0.21 ± 0.05, b = 0.80 ± 0.01, k_1 = 3.7 ± 1.3 per hr, and k_2 = 0.06 ± 0.002 per hr. These results suggest that approximately 20% of the BaP is depurated rapidly, with the remainder in a form that depurates more slowly.

Bioaccumulation of BaP in the Presence of Humic Acid

Sorption to yeast decreases accumulation of the PAH by *Daphnia;* however, PAHs can also be adsorbed to naturally occurring dissolved or colloidal organic matter. Therefore, the effect of HA on accumulation of BaP by *Daphnia* was examined (Figure 8). In the presence of 20 mg/L HA, *Daphnia* accumulate BaP only 200-fold, or only ~2.5% of the levels accumulated in the absence of HA. The rate coefficient for uptake is approximately 29 mL/g wet wt/hr or ~2.7% of the estimated value of k_u in the absence of HA. Because of the difficulty in distinguishing the fraction of BaP sorbed to dissolved HA, no attempt was made to estimate rate coefficients for the 2 or 3 compartment models.

Bioaccumulation of BaP by Heat-Killed Daphnia

The accumulation of BaP by killed *Daphnia* (0.80 ± 0.04 mg wet wt per animal) is slower than by live *Daphnia* (Figure 9). The rate coefficient for uptake, estimated from the initial slope of the bioaccumulation curve, is approximately 110 mL/g wet wt/hr *vs* 1000 mL/g wet wt/hr for live *Daphnia* from the same cohort. The lowered uptake rate is not due to depletion of BaP from an immobile layer of water in the microenvironment surrounding the animals because the jars were rotated during the experiment. The decreased rate of bioaccumulation might result from the absence of water filtration motions by the thoracic appendages or lack of active metabolism or both.

Discussion

To satisfactorily predict the transport, fate and effect of a chemical in natural water systems, it is important to consider its speciation, *i.e.,* the chemical form in which the compound occurs. Most work on speciation has dealt with heavy metals and has demonstrated that association with naturally occurring particulate or dissolved organic matter can affect the transport and toxic effects of the metals (Sholkovitz 1978; Preston and Riley 1982; Sundra

Table 2. Rate coefficient for uptake and depuration (± asymptotic standard error) of anthracence; k_u and k_f are reported in units of mL/g animal wet wt/hr, k_d in units of per hr

Daphnia wt (mg)	[A] μg/L	[Yeast] mg/L	Fraction sorbed	k	No yeast	Yeast
0.80 ± 0.25	38	67	0.30	k_u	627.3 ± 64.0	480.6 ± 71.5
				k_d	1.29 ± 0.18	0.86 ± 0.15
				k_f	—	15.3 ± 4.9
1.14 ± 0.19	30	190	0.53	k_u	482.8 ± 47.8	184.5 ± 23.0
				k_d	0.97 ± 0.17	0.48 ± 0.06
				k_f	—	28.3 ± 33.6

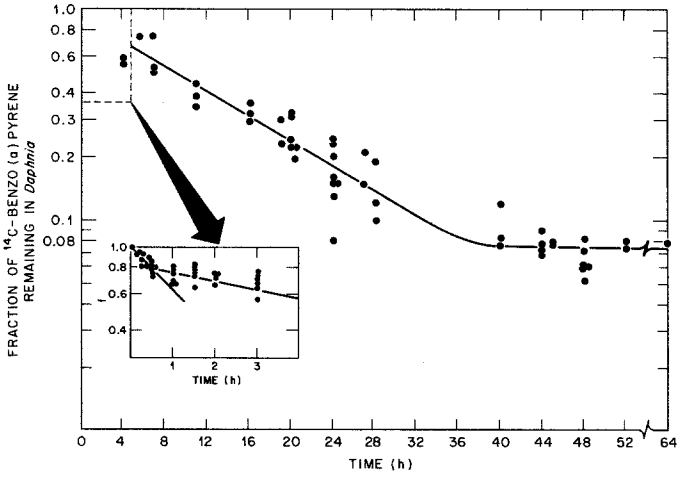

Fig. 7. The fraction of ^{14}C-activity remaining in *Daphnia* exposed to ^{14}C-BaP (1 μg/L) for three hr before being transferred to uncontaminated water at time zero

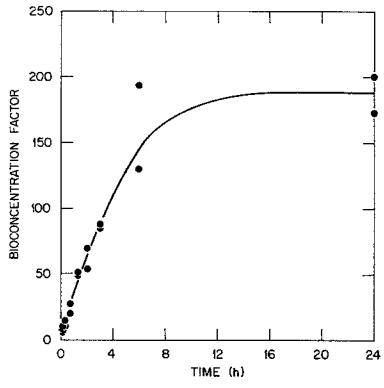

Fig. 8. Accumulation of benzo[a]pyrene (1 μg/L) by *Daphnia* in particle-free water containing 20 mg/L of humic acid

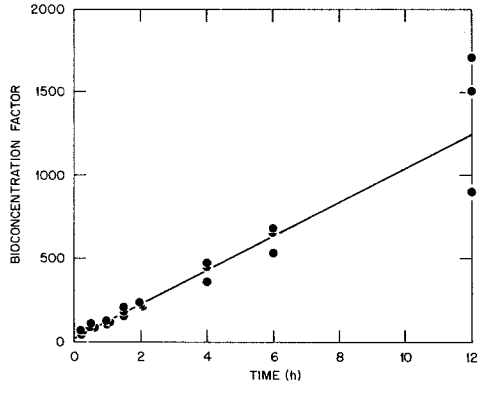

Fig. 9. Accumulation of benzo[a]pyrene by heat-killed *Daphnia*

and Gullard 1976). Since hydrophobic organic contaminants, such as PAHs, have a high affinity for sorption to particulate or dissolved organic matter, the effect of this sorptive interaction on bioavailability was examined.

The results demonstrate that sorption of PAH either to particles (yeast) or dissolved humic acids decreases their potential for uptake and accumulation in *Daphnia*. Since the tendency both for bioaccumulation and for sorption are positively correlated, the presence of naturally occurring sorptive materials might tend to ameliorate the biological impact of those contaminants which would have the greatest potential for bioaccumulation.

The sorptive affinity of BaP and A to yeast was related to the hydrophobicity of the contaminant. The octanol-water partition coefficients of the two PAHs [K_{ow} of 1.1×10^6 *vs* 2.8×10^4 for BaP and A, respectively (Leo 1975)] differ by approximately the same 40-fold factor separating their K_p's with yeast (Figure 3). The K_p for sorption of PAHs to sediment particles has also been correlated with their hydrophobicity (Karickhoff *et al.* 1979; Means *et al.* 1979). The previously observed correlation between K_{ow} and the potential for bioaccumulation (Kenaga and Goring 1980) was confirmed for the uptake of BaP and A in *Daphnia*. The BCF for BaP in particle-free water was approximately 8000. This BCF is somewhat higher than the BCF of ~2500 reported by Leversee *et al.* (1981); the discrepancy may be due to the 5-fold higher concentration of DOC in the water used by the Savannah River group (Giesy *et al.* 1978). The BCF for A in particle free

water was approximately 500, which is in agreement with values reported for *Daphnia pulex* (Herbes and Risi 1978).

In nature, however, appreciable quantities of suspended particulate matter (10–100 mg/L) are not uncommon. As demonstrated in Figure 2, a substantial fraction of BaP is sorbed by yeast concentrations in this range, although less A is sorbed at these concentrations of yeast. While yeast cells may not be a commonly occurring particulate in nature, significant amounts of PAH can sorb to naturally occurring particulate matter. In 10 reservoirs, streams and wastewater effluents, (where total suspended solids ranged from 7–38 mg/L), 24 to 67% of added ^{14}C-BaP and 3 to 20% of added ^{14}C-A were sorbed to naturally occurring suspended particulate matter (personal communication, S. E. Herbes, Oak Ridge National Laboratory). Eighty to 99% of BaP was removed by glass-fiber filtration of waters from two British rivers (Lewis 1975). The use of the three-compartment model [Eq. (7)] is, therefore, more appropriate for considering the actual accumulation of a hydrophobic organic toxicant by zooplankton in natural waters. The steady-state concentration factor can be predicted by rearrangement of Eq. 6:

$$dD/dt = (k_u \times W) - (k_d \times D) + (k_f \times Y) = 0. \quad (6')$$

If T is the total concentration of toxicant in both the dissolved (W) and sorbed (Y) form, and f is the fraction of chemical in the sorbed form,

$$\text{realized BCF} = \frac{D}{T} = \frac{D}{W + Y} = \frac{k_u + f(k_f - k_u)}{k_d}. \quad (9)$$

The BCF observed in the experiments in which different concentrations of yeast were present with BaP or A agree with the "realized BCF" calculated from Eq. (9) and Tables 1 and 2. The observed BCF for BaP (Figure 5) is ~1000 compared to a BCF of 965 predicted from Eq. 9 using data from Table 1. For A, observed BCF's of 370 and 230 (in the presence of 67 and 195 mg yeast/L; Figure 6) compare well to calculated values of 395 and 211 (Eq. 9, Table 2). If 24 to 67% of BaP introduced into natural waters (*e.g.*, by an oil spill) were sorbed to particles, Eq. (9) predicts that the realized BCF would be about two- to four-fold less than that expected if the effect of sorption on availability were ignored. The effect of sorption of hydrophobic pollutants should, therefore, be considered in assessing the accumulation of the chemical to aquatic organisms.

The distribution of a chemical between water and suspended particulate matter can be measured directly, as in this study; however, simpler and more general parameters can predict the relative tendency of chemicals to partition between water and suspended solids. Since sorption of hydrophobic pollutants, expressed as the partition coefficient (K_p), has been related to the organic carbon content of the particles and the K_{ow} of the pollutant by regression analysis (Karickhoff *et al.* 1979; Means *et al.* 1979), the fraction of a hydrophobic pollutant sorbed to suspended particles (f) can be estimated from the K_{ow} of the chemical:

$$f = (K_p \times P)/[1 + (K_p \times P)] \quad (10)$$

where T is the total concentration of the chemical P is the concentration of suspended particulates in the water (kg/L) and K_p is estimated from K_{ow}.

If, for illustrative purposes, the rate coefficient for uptake of PAHs via ingestion of food particles (k_f) is assumed to be approximately 10% of the value of k_u (k_f ranged from 3-15% of k_u in Tables 1 and 2), Eq. (9) can be approximated:

$$\text{realized BCF} \simeq \frac{k_u(1 - 0.9f)}{k_d} = (1 - 0.9f)\ (\text{potential BCF}). \quad (11)$$

That is, for every fraction of chemical sorbed to particles, approximately 90% is unavailable for accumulation. Since the potential BCF of a compound has also been related by regression analysis to the K_{ow} (Kenaga and Goring 1980), the effect of sorption on accumulation of hydrophobic pollutants can be estimated from simple chemical and environmental parameters.

The relative contribution of food ingestion to the total accumulation can be estimated from the ratio of the flux of PAH into *Daphnia* via yeast versus the total flux:

$$\text{Relative contribution of food} = \frac{k_f \times Y}{(k_f \times Y) + (k_u \times W)} = \frac{0.1\,f}{1 - 0.9f}. \quad (12)$$

The latter portion of Eq. (12) continues the illustrative assumption that k_f is 10% of k_u. For filter feeders like *Daphnia*, therefore, uptake of very hydrophobic chemicals via food can be an important source of contamination. Assuming that natural waters have 10 to 100 mg/L of particulate organic matter, it can be calculated that chemicals with K_p's of 10^4 to 10^6 will result in the food chain contributing 25% ($P = 100$ mg/L, $K_p = 10^4$) to 50% ($P = 10$ mg/L, $K_p = 10^6$) of the total accumulation. For BaP (Table 1), for example, half of the uptake was due to ingestion of yeast; for A (Table 2), only 1.3% and

14.8% of the total uptake was due to ingestion of yeast when 30% or 53%, respectively, of the PAH was sorbed.

Implicit in the preceeding analysis is the assumption that *Daphnia* or other zooplankton can filter and ingest all size fractions of particles laden with sorbed pollutant. This assumption appears to be valid for *Daphnia* over a wide size range (15–165 μ^3, Kersting and Holterman 1973). However, hydrophobic pollutants can also sorb to naturally occurring colloidal or dissolved organic matter such as humic or fulvic acids (Boehm and Quinn 1973; Seitz 1982). If the pollutants are introduced as a complex mixture (petroleum or synthetic fuel spills), the hydrophobic chemicals can act as their own "solubilizers" by formation of colloidal micelles into which the lipid soluble chemicals concentrate.

From size considerations, it is unlikely that zooplankton would be able to filter and ingest colloidal or dissolved complexes as they do particles such as yeast cells. Sorption of BaP to humic acids (Figure 8) reduced concentration to a level far below that predicted from Eq. (9); even assuming f = 1.0, the realized BCF predicted from that model would be approximately 800 *vs* an observed BCF of less than 200. Reduced BCFs in the presence of HA were also reported for *Daphnia* by Leversee *et al.* (1980). Thus, available pollutant appears to be limited to that fraction of the total chemical in true solution and, to a far lesser extent, that fraction bound to particles that can be filtered and ingested.

The concentration of particulate matter in the water can also affect the rate of uptake of hydrophobic chemicals through its effect on rates of filtration by zooplankton. For *Daphnia,* rates of filtration of water are depressed in the absence of particles, then increase with increasing concentration of yeast cells up to a critical concentration. Above this level ($\sim 10^5$ cells/mL), the feeding rate is maintained at $\sim 2 \times 10^8$ cells ingested/g *Daphnia*/hr by a reduction in the rate of filtration (Rigler 1961; McMahon and Rigler 1963; Sorokin 1966; Gliwicz and Siedlar 1978). High yeast concentrations appeared to affect the uptake of A (Table 2). The estimated values of k_u and k_d decreased at increasing concentrations of yeast. If the rate of filtration of water through the food groove decreases to maintain a feeding rate of $\sim 2 \times 10^8$ cells/g *Daphnia*/hr, then the change in yeast cell concentration from 67 to 190 mg/L (Table 2) should result in a three-fold decrease in the filtration rate, which compares to a decrease of ~2.6-fold in the estimated values of k_u. The data in Figure 9 also suggest that the rate of accumulation of dissolved PAH is related to the rate at which water is washed over the food groove area by movements of thoracic appendages. Heat-killed *Daphnia* accumulate very little PAH even though water washed over the external carapace because of rotation of the jars. Crosby and Tucker (1971) and Wildish and Zitko (1971) also found that heat-killed *Daphnia* or *Gammarus* accumulated far less DDT and PCB, respectively, than did healthy individuals. The concentrations of yeast used in the experiments on A (Table 2) were higher than would be expected for particulate loads in most natural waters. The decrease in uptake due to reduced filtration rates would probably be relatively minor under most conditions.

The efficiency with which PAH ingested on yeast cells is assimilated by *Daphnia* was estimated from the rate of accumulation from the food (k_f'Y), the amount of PAH per cell [Y/(cells/mL)] and the feeding rate of *Daphnia* [taken from Rigler (1961); I measured feeding rate by Rigler's method at 5 and 160 mg yeast/L and obtained results within 20% of Rigler's (data not shown)]. Approximately 20% of the BaP ingested on yeast was assimilated. For A, approximately 7% was assimilated at 67 μg/L and 34% was assimilated at yeast concentrations of 190 mg/L. These estimates compare favorably with estimates of an overall assimilation efficiency of 20 to 30% for *Daphnia* and *Diaptomus* fed on algae (Sorokin 1966) and suggest that sorbed organic contaminants are assimilated with approximately the same efficiency as any carbon source.

In summary, the accumulation of a hydrophobic organic pollutant in natural waters can be considerably less than that measured in a laboratory situation using particle-free water. In natural waters, accumulation will depend on the amount of pollutant present in available forms, that is, in true solution or sorbed to particles capable of entering the food chain.

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