Bioaccumulation and transfer of benzo(a)pyrene in a simplified marine food chain

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ABSTRACT: We examined the toxicokinetics of benzo(a)pyrene (BaP) and the trophic transfer in a marine planktonic food chain comprising phytoplankton, copepods, and fish. Kinetic parameters including the uptake rate from the dissolved phase, the assimilation efficiency (AE) from the ingested food, and the elimination rate were quantified. Influences of food quality and quantity and different routes of exposure (aqueous and food) on the biokinetics were also examined. The uptake rate constants were 390 to 1090 ml mg⁻¹ h⁻¹ in different species of phytoplankton, 1.2 ml mg⁻¹ h⁻¹ in the copepods, and 0.157 ml mg⁻¹ h⁻¹ in the fish, respectively. The assimilation efficiencies were 2 to 24% in the copepods and 32 to 51% in the fish ingesting different prey types. Increasing food concentration significantly reduced the AE, whereas the AE varied little among the different food diets tested at the same biomass. The elimination rate constants by the copepods were 0.8 to 1.7 d⁻¹, and comparable following uptake from the aqueous phase and the dietary phase. A kinetic modeling calculation suggests that more BaP accumulated in the copepods originates from the dietary intake than the aqueous intake. For fish, both trophic transfer and aqueous uptake are important in BaP bioaccumulation. Feeding rates contribute to the differences in the relative proportion of accumulated BaP in the fish.

KEY WORDS: PAHs · Bioaccumulation · Kinetic modeling · Food chain

INTRODUCTION

Many toxic xenobiotic compounds are eventually discharged into the sea, accumulated and transferred in the marine food chains. Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic compounds with considerable potential of biotransformation and can result in significant adverse health and environmental effects (Neff 2002). Elevated levels of PAHs have been detected in numerous coastal environments (Connell et al. 1998, Fisk et al. 2001, Klumpp et al. 2002, Maskaoui et al. 2002, Moore et al. 2002). Sources of PAHs in marine environment are usually related to human activities such as petroleum discharge or atmospheric deposition of incomplete combustion materials.

The bioaccumulation of PAHs by marine organisms has been extensively studied (van der Oost et al. 1991, Hellou et al. 1995, Meador et al. 1995, Kane & McElroy 1996, D’Adamo et al. 1997, Klosterhaus et al. 2002). However, the majority of laboratory and field studies focused on the uptake from water and the relationship between the bioconcentration factor (BCF, the ratio of contaminant concentration in an organism to the dissolved concentration) and the octanol–water partition coefficient (Kow). Barron (1990) indicated that the bioconcentration of PAHs from solution by marine organisms was directly proportional to their Kow. The quantified BCF increased with the Kow or molecular weight of PAHs (Veith & Kosian 1983), but the BCFs quantified in the field varied from those predicted simply from the Kow (Zaroogian et al. 1985, Neff & Burns 1996), suggesting that trophic transfer may also be important in the accumulation of these PAH compounds. Because of the biotransformation of PAHs by the mixed-function oxygenases (or oxidases) (MFOs), the PAHs can be converted to arene oxide intermediates followed by formation of derivatives of transdihydrodiols, phenols, and quinines, and are finally excreted.

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Phytoplankton organisms are the primary producers in the aquatic systems and therefore play a key role in the transport of organic contaminants to higher trophic levels. Marine copepods are the link between the primary producers and the higher trophic level such as fish, thus uptake by zooplankton presents another redistribution pathway for organic contaminants in the marine food chains. The bioaccumulation of PAHs by zooplankton has been considered in only a few early studies (Corner et al. 1976, Harris et al. 1977a,b). Fundamental processes of PAH transport in the lower trophic food chain need to be better understood to enable prediction of the environmental fate and food chain transport in the ecosystem of concern.

In this study, we examined the bioaccumulation and trophic transfer of benzo(a)pyrene (BaP, a 5 ring PAH compound) in a marine food chain consisting of phytoplankton, copepods and fish. We measured the uptake from the dissolved phase, the assimilation efficiency from the lower trophic level, and the elimination of BaP in the animals. After these experimental measurements, we constructed a simple food chain kinetic model to estimate the relative importance of aqueous and dietary exposure. The kinetic model has been used to evaluate the bioaccumulation of contaminants and to quantify the bioaccumulation factor (Landrum et al. 1992, Wang et al. 1996). With this model, the physiological process controlling contaminant accumulation can be identified and measured under varying environment conditions. It is also possible to predict the trophic transfer factor based on the kinetic measurements of contaminant uptake and elimination in the animals.

**MATERIALS AND METHODS**

**Chemicals and organisms.** $^3$H-benzo(a)pyrene (with a specific activity of 48 Ci mmol$^{-1}$, Log $K_{ow} = 6.13$, from Amersham) was dissolved in toluene solution (200 µl). Radiochemical purity determined by the HPLC on a Hypersil ODS column using a water:methanol gradient system was >99%. The chemicals were subsequently diluted 100× in acetone to facilitate their solubility in seawater (solvent-to-seawater ratio <0.04 %). Preliminary experiments demonstrated that a trace amount of acetone in seawater did not affect the test organisms, as shown by the antioxidation enzyme activities (X. H. Wang unpubl. data). Because only a small amount of toluene was added (i.e. 2 orders of magnitude less than the acetone or 0.0004 % of the solvent-seawater ratio), any influence of toluene on BaP uptake was ignored in our study.

Different species of phytoplankton (diatom *Thalassiosira pseudonana* and *T. weissflogii*, prasinophyte *Tetraselmis levis*, and dinoflagellate *Prorocentrum minimum*) were cultured in the laboratory and maintained in an f/2 medium (Guillard & Ryther 1962) at 18°C and at a light illumination of 70 µmol photon m$^{-2}$ s$^{-1}$ with a 14:10 h light:dark cycle. Copepods *Acartia erythraea* were collected by net tows (250 µm mesh size) from Port Shelter, Clear Water Bay, Hong Kong. The copepods were returned to the laboratory and transferred into 4 l of glass fibre filtered (GF/F) seawater, and fed with the mixed algae. Mangrove snappers *Lutjanus argentimaculatus*, 2 to 3 cm length with a wet weight of 0.5 to 0.7 g, were purchased from a local fish farm. The experimental seawater was collected from Clear Water Bay, Hong Kong, and was filtered through GF/F and 0.22 µm polycarbonate membranes before use. All experiments were carried out in glass vials to minimize sorption of organic contaminants onto the experimental glasswares.

**Kinetic uptake of BaP from the aqueous phase.** We examined the kinetic uptake of BaP by 4 species of marine phytoplankton: *Thalassiosira pseudonana* (3H), *T. weissflogii* (Thala, for both logarithmic and stationary phase), *Tetraselmis levis* (Tetra), and *Prorocentrum minimum* (Pro). When the cells reached the logarithmic or stationary (for *T. weissflogii* only) growth phase, they were filtered and resuspended in 100 ml of 0.2 µm filtered seawater (FSW) at a cell biomass of 1 mg l$^{-1}$. The algae were then spiked with 0.02 µCi $^3$H-BaP (1.1 ng l$^{-1}$) and then exposed under the dark condition. The spiked concentration was higher than those in clean waters, but was comparable to or lower than those measured in polluted environments such as the contaminated Chinese coastal waters (Neff 2002, Maskaoui et al. 2002). No nutrient was added to the solution. There were 3 replicates for each algal species. At 0.5, 1.0, 1.5 and 2.0 h of exposure, a 10 ml sample was filtered onto 2 stacked 25 mm GF/F filters and rinsed with 5 ml of FSW. The top filter retained the algae, while the bottom filter was used to calibrate the sorption of BaP during the filtration step. The algae were solubilized by Solvable (Perkin-Elmer) and the radioactivity was later determined by Wallace 1414 Liquid Scintillation Counter (LSC) using the external standard ratio method and corrected for background and quenching. Counting times were adjusted to yield a propagated counting error of <2 to 3 %. A 1 ml water sample was also taken for measurements of radioactivity (including both the water and the algae). All uptake experiments were conducted under red light and cocktails were added immediately to minimize losses associated with evaporation and photo-oxidation. The dry weight concentration factor (DCF) was calculated as the ratio of BaP concentration in algae to the BaP concentration in the water (dry wt per volume).

The copepods were placed in 3 replicated 200 ml FSW containing 0.05 µCi $^3$H-BaP (1.3 ng l$^{-1}$), under the
dark condition. At 1, 2, 4, and 8 h of exposure, a 1 ml water sample was removed for measurement of the radioactivity. Afterwards, 20 copepods were removed from each replicate bottle, rinsed with GF/F seawater by placing them in non-radioactive seawater for 2 to 3 min. The copepods were then solubilized with Solvable and the radioactivity was quantified by LSC after adding the cocktails. The dry weights of the copepods were also quantified by drying the copepods overnight at 80°C.

Individual mangrove snappers were placed in 4 l FSW containing 1 μCi ³H-BaP (1.3 ng l⁻¹) under the dark condition. At 1, 2, 3, and 4 h, individual fish were taken from 3 replicated tanks and placed in non-radioactive seawater for 5 min to remove the loosely bound compounds. Any remaining water on the body surface was then blotted dry and the fish was immediately dissected into 3 body parts: gill, viscera, and remaining tissue. The wet weight of each body part was first determined and 0.5 to 1.0 ml of Solvable was subsequently added to solubilize the tissues. After 1 more day when the tissues (gills, viscera, or remaining tissues) were solubilized, a 10 ml cocktail was added and the radioactivity was counted. A 1 ml water sample was also collected at each time point. The dry weight to wet weight ratio of each body part was determined from 10 dissected fish after drying the tissues at 80°C for >1 d until a constant weight was reached.

Assimilation of BaP from the food. The assimilation efficiency (AE) of BaP in the copepods was quantified by pulse-feeding the animals with radiolabeled phytoplankton, whereas the AE in fish was quantified by feeding the animals with radiolabeled copepods. The phytoplankton were first filtered from their growth medium, suspended into 200 ml FSW, and spiked with ³H-BaP. No nutrient was added to the medium. After the radioactive feeding, the fish were exposed to the radiolabeled BaP either in the aqueous phase or in the dietary phase. In the aqueous exposure treatment, 3000 copepods were exposed to the radiolabeled Thalassiosira weissflogii for 12 h, and were then fed to the snappers for 60 min at 4 different densities (2, 5, 10, and 20 mg l⁻¹). After the radioactive feeding, the fish were removed and placed in nonradioactive seawater and allowed to depurate of their ingested food materials for 24 h. During the depuration period, the snap- pers were fed with copepods at the same density as that used during the radioactive feeding period, and the seawater was changed every 12 h. Feces were removed at 4, 7, 9, 19, and 24 h of the depuration.

The AE was operationally calculated as the percentage of BaP retained in the copepods after 12 h of depuration (i.e. the radioactivity in copepods at 12 h divided by the initial radioactivity ingested). The copepods complete their food digestion within this period of time (Wang & Fisher 1998). In this study, we used the assimilation efficiency instead of the absorption efficiency since we calculated the AE as % of BaP retained in the animals, which already excluded the excretion term. The absorption efficiency is the fraction of digestive products taken up across the cell membranes of the gut wall, whereas the assimilation efficiency is the fraction of absorbed products that is incorporated into the body tissues (by excluding the excretion term; Penry 1998).

The AE of BaP were also measured in fish feeding at different densities of copepod prey. The copepods were radiolabeled by feeding on the radiolabeled Thalassiosira weissflogii for 12 h, and were then fed to the snappers for 60 min at 4 different densities (2, 5, 10, and 20 mg l⁻¹). After the radioactive feeding, the fish were removed and placed in nonradioactive seawater and allowed to depurate of their ingested food materials for 24 h. During the depuration period, the snappers were fed with copepods at the same density as that used during the radioactive feeding period, and the seawater was changed every 12 h. Feces were removed at 4, 7, 9, 19, and 24 h of the depuration.

There were 8 replicates in each treatment. Similarly, the retention of BaP in the fish was determined by the mass balance method, as described for copepods above. Since digestion of food materials was completed within 24 h (e.g. very little radioactivity was detected after this period of depuration), the AE was calculated as the radioactivity retained in fish at 24 h divided by the initial radioactivity ingested.

Elimination of BaP from the copepods. Copepods were exposed to the radiolabeled BaP either in the aqueous phase or in the dietary phase. In the aqueous exposure treatment, 3000 copepods were exposed to 5 μCi of ³H-BaP (8.9 ng l⁻¹) for 12 h. In the food exposure treatment, 3000 copepods were fed radiolabeled
**Thalassiosira weissflogii** (2 µCi in 100 ml seawater for 2 h, 104 ng l⁻¹) at 4 food concentrations (0.1, 0.5, 2.5, 5.0 mg l⁻¹). After 12 h of radioactive feeding, the copepods were collected and rinsed with FSW, and then transferred to twelve 300 ml glass beakers (3 replicates × 4 food concentrations). Each beaker contained 250 copepods. At the beginning of depuration, 20 copepods were collected from each beaker to measure the initial radioactivity in the copepods. During the course of depuration, 20 copepods were collected at 2, 4, 8, 12, 24, 36, and 48 h. The seawater was renewed at 2 to 4 h intervals within the first 12 h, and then every 6 to 12 h afterwards. The food concentrations were maintained at 0.1, 0.5, 2.5, 5.0 mg l⁻¹ during the course of the depuration period. The elimination rate constant was calculated based on the regression between the natural log of the percentage of BaP retained in the animals and the time of depuration (12 to 48 h).

**Modeling BaP exposure.** With uptake from both the aqueous and food phases and assuming that both uptake pathways are additive, the bioaccumulation of BaP in the animals can be described by the following equation (Landrum et al. 1992):

\[ \frac{dC}{dt} = (k_u \times C_w) + (AE \times IR \times C_f) - (k_e \times C) \]

where C is the BaP concentration in the animals at time t, \(k_u\) is the uptake rate constant from the aqueous phase (ml mg⁻¹ d⁻¹), \(C_w\) is the BaP concentration in the aqueous phase (µg l⁻¹), \(AE\) is the BaP assimilation efficiency, \(IR\) is the ingestion rate (mg g⁻¹ d⁻¹), \(C_f\) is the BaP concentration in ingested food particles (µg mg⁻¹) and can be measured as the BaP bioconcentration factor (BCF, defined as the concentration in food particles to concentration in ambient water under equilibrium conditions) in ingested food × \(C_{sw}\), and \(k_e\) is the BaP elimination rate constant (d⁻¹). The \(k_u\) should include all the loss terms for the animals, including any biotransformation with the assumption that the water-soluble metabolites are excreted out of the body. With this equation, the fraction of BaP accumulated from the dissolved phase (R) and the food chain transfer factor TTF under steady-state conditions can be calculated as (Wang & Fisher 1999, Wang 2002):

\[ R = \frac{(k_u)}{(k_u + AE \times IR \times BCF)} \]

\[ TTF = \frac{(AE \times IR)}{k_e} \]

**RESULTS**

**Kinetic uptake of BaP from the aqueous phase**

Fig. 1 shows the kinetic uptake of BaP by different algal species at the same biomass of 1 mg l⁻¹. BaP exhibited an approximately linear pattern of uptake between 0.5 and 2 h of exposure, during which period no steady-state or equilibrium was reached. There was also an initial rapid sorption onto the algae. Given the linear pattern of uptake during the second phase, \(k_u\) was calculated from the slope of the linear regression between the DCF and the time of exposure (0.5 to 2 h), by excluding the initial sorption of BaP onto the cells (Table 1). Within the same diatom species (**Thalassiosira weissflogii**), the \(k_u\) was significantly higher for cells in the stationary phase than for those in the log phase (p < 0.05, t-test). The \(k_u\) differed by 2.8-fold and was the highest for the dinoflagellate **Prorocentrum minimum**.

The kinetic uptake of BaP by the marine copepods also exhibited a linear pattern over the 1 to 8 h exposure period (Fig. 2). Similarly, the \(k_u\) was calculated from the slope of the linear regression between the DCF and the time of exposure (1 to 8 h) by excluding the initial sorption of BaP. The \(k_u\) was >300-fold lower than in phytoplankton (Table 1). The \(k_u\) calculation assumed that there was negligible elimination of BaP from the copepods during the exposure period as compared to the uptake.

Aqueous uptake of BaP by the mangrove snappers was quantified both for the whole fish and different parts of the fish. A linear increase in DCF with increasing exposure time (1 to 4 h) was found (Fig. 3). The calculated DCF was highest for the viscera, and was comparable between the whole fish and the remaining tissues. Over the course of exposure, there were notable differences in the relative distribution of BaP in different fish bodies. Almost equal distribution was found for the viscera and remaining tissues, but after 4 h of exposure,
the fraction distributed in the viscera increased. The $k_\text{u}$ in the whole fish and different body parts was also calculated from the slope of the linear regression between the DCF and the time of exposure (1 to 4 h) by excluding the initial sorption of BaP (Table 1). The highest $k_\text{u}$ was found for the viscera, whereas the remaining tissues had the lowest $k_\text{u}$. For the whole fish, the $k_\text{u}$ was 7.6 times lower than the $k_\text{u}$ of copepods.

### Assimilation of BaP by copepods and fish

Generally, rapid egestion of unassimilated BaP by copepods was found within the first few hours of depuration (Fig. 4). At a higher food concentration, the depuration by the copepods was much faster. After the initial rapid loss, BaP was then depurated from the copepods at a slower rate. The calculated $AE$s decreased significantly with increasing food concentrations ($p < 0.001$, Table 2). The greatest change in $AE$ occurred at the food concentration between 0.1 to 0.5 mg l$^{-1}$. The $AE$s decreased by 13.6$\times$ with increasing food concentrations from 0.1 to 5.0 mg l$^{-1}$. Depuration of BaP in copepods feeding on different algal types followed similar patterns (Fig. 4). The tested algal biomass in this experiment was relatively high (1 mg l$^{-1}$), thus the calculated $AE$s were rather low (3 to 9%), and the differences among different algal diets were small.

For the same diatom diet ($Thalassiosira weissflogii$), the $AE$s were 5.6% for the stationary cells as compared to 2.9% for the log growing cells.

### BaP elimination from copepods

The copepods were exposed to radiolabeled water or diatom food for 12 h and their retention was followed.
for 2 d at different diatom food concentrations (Fig. 6). By the end of the depuration, the % of BaP that remained in the copepods was similar for the aqueous and dietary exposure. For the aqueous exposure experiment, the BaP was retained at a higher efficiency at lower food concentrations. The elimination rate constant (k_e) was similar for the aqueous and dietary exposure (Table 3). Differences in food concentrations did not significantly affect the BaP elimination from the copepods (p > 0.05, 1-way ANOVA).

**Modeling BaP exposure in copepod and fish**

Eq. (2) was used to calculate the relative exposure (aqueous vs. food) of BaP in the copepods and fish. Values of k_e and AE were derived from this study, and the ingestion rates of copepods and fish were obtained from the literature (Table 4). The BCFs in phytoplankton were selected based on our short-term measurement of BaP uptake. However, we assume that the BCF varies by one order of magnitude (10^6 to 10^7 l kg⁻¹)

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**Table 2. Assimilation efficiency (AE) of BaP by marine copepods (Acartia erythraea) and mangrove snappers (Lutjanus argentimaculatus) under different food conditions. Significant influence of food concentration on AE is indicated by *(p < 0.05) and ***(p < 0.001, 1-way ANOVA). Data are mean ± SD, n = 3 for copepods and 8 for fish**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Copepods</strong></td>
<td></td>
</tr>
<tr>
<td>Food concentration (mg l⁻¹ Thalassiosira weissflogii) (***)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>24.4 ± 5.2</td>
</tr>
<tr>
<td>0.5</td>
<td>7.9 ± 3.3</td>
</tr>
<tr>
<td>2.5</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>5.0</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Food type (1 mg l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Thalassiosira weissflogii (log phase)</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Thalassiosira weissflogii (stationary)</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>Tetraselmis levis</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>Prorocentrum minimum</td>
<td>8.4 ± 1.8</td>
</tr>
<tr>
<td>Thalassiosira pseudonana</td>
<td>4.4 ± 1.6</td>
</tr>
<tr>
<td><strong>Mangrove snappers</strong></td>
<td></td>
</tr>
<tr>
<td>Copepod prey concentration (mg l⁻¹) (*')</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>51.2 ± 4.7</td>
</tr>
<tr>
<td>5</td>
<td>47.4 ± 4.1</td>
</tr>
<tr>
<td>10</td>
<td>33.6 ± 2.4</td>
</tr>
<tr>
<td>20</td>
<td>31.9 ± 1.6</td>
</tr>
</tbody>
</table>

**Table 3. Acartia erythraea. Elimination rate constants (k_e) of BaP after aqueous and dietary exposure (mean ± SD, n = 3)**

<table>
<thead>
<tr>
<th>Diatom concentration (mg l⁻¹)</th>
<th>Food Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.82 ± 0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>0.96 ± 0.12</td>
</tr>
<tr>
<td>2.5</td>
<td>1.66 ± 0.12</td>
</tr>
<tr>
<td>5.0</td>
<td>1.30 ± 0.14</td>
</tr>
</tbody>
</table>
given the uncertainty of this parameter (especially given the initial sorption). The BCF of BaP in the copepods as the prey of fish was calculated as \((k_u + AE \times IR \times BCF_{phytoplankton})/k_e\). Using a phytoplankton BCF of \(4 \times 10^6\) l kg\(^{-1}\) and an \(AE\) of 5%, the BCF in the copepods was calculated as \(9 \times 10^4\) l kg\(^{-1}\). We therefore use \(5 \times 10^4\) to \(5 \times 10^5\) l kg\(^{-1}\) as the range of copepod BCFs in the fish accumulation model calculation. Thus, under the likely conditions encountered by the copepods, the uptake for BaP was variable and greatly dependent on the \(AE\) (Fig. 7). Assuming an average \(AE\) (5%, Table 4), >50% of BaP are derived from the food intake under most BCF conditions, suggesting that trophic transfer is more important than the aqueous uptake. For the mangrove snappers, the relative importance is related to the ingestion rate of the fish and the BCF in the prey (Fig. 8). Assuming a median ingestion rate (0.05 g g\(^{-1}\) d\(^{-1}\), Zhao et al. 2001) and \(AE\) (40%), trophic transfer contributes 21 to 72% to the total BaP accumulation in fish over the BCF range simulated, implying that both aqueous uptake and dietary uptake can be equally important for the overall BaP accumulation in the fish.

It is also possible to calculate the trophic transfer factor (TTF) for the copepods using Eq. (3) with known \(AE\), \(IR\), and \(k_u\). The TTF in the copepods fed with phytoplankton is consistently <0.1 under all conditions, thus the potential for trophic transfer of BaP from phytoplankton to copepods is very low.

### DISCUSSION

Fan & Reinfelder (2003) indicated that the sorption of phenanthrene occurred within the first 20 min of contact with the diatoms, followed by passive diffu-

<p>| Table 4. Parameters used in modeling the exposure of BaP in the marine copepod \textit{Acartia erythraea} and the mangrove snapper \textit{Lutjanus argentimaculatus}. (IR): ingestion rate, (k_u): dissolved uptake rate constant, (AE): assimilation efficiency, (k_e): elimination rate constant, (BCF): bioconcentration factor in prey. |</p>
<table>
<thead>
<tr>
<th>Range</th>
<th>Mean</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IR) (g g(^{-1}) d(^{-1}))</td>
<td>0.2–0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>(k_u) (l g(^{-1}) h(^{-1}))</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>(AE) (%)</td>
<td>2–25</td>
<td>5</td>
</tr>
<tr>
<td>(k_e) (d(^{-1}))</td>
<td>0.82–1.66</td>
<td>1.20</td>
</tr>
<tr>
<td>(BCF) (l kg(^{-1}))</td>
<td>(10^0–10^7)</td>
<td></td>
</tr>
<tr>
<td>Mangrove snappers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IR) (g g(^{-1}) d(^{-1}))</td>
<td>0.01–0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>(k_u) (l g(^{-1}) h(^{-1}))</td>
<td></td>
<td>0.157</td>
</tr>
<tr>
<td>(AE) (%)</td>
<td>30–50</td>
<td>40</td>
</tr>
<tr>
<td>(BCF) (l kg(^{-1}))</td>
<td>(5 \times 10^4–5 \times 10^5)</td>
<td></td>
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Fig. 6. \textit{Acartia erythraea}. Retention of BaP following dietary or aqueous exposure for 12 h, at different diatom densities. Values are mean ± SD, n = 3

Fig. 7. \textit{Acartia erythraea}. Predicted percentage of BaP uptake from the dietary phase in marine copepods as a function of BaP bioconcentration factor in phytoplankton. \(AE\): assimilation efficiency from food, \(IR\): ingestion rate of copepods (g g\(^{-1}\) d\(^{-1}\))
sorption into the cells with increasing exposure. Lei et al. (2002) examined the uptake of pyrene by several algae and found that >65% of pyrene was adsorbed onto the cell walls of *Selenastrum capricornutum*, suggesting that the major binding sites for these PAH compounds were on the cell surfaces. In our study, we also found that the initial sorption dominated the overall accumulation of BaP by the organisms. Because of the passive physico-chemical biosorption, the specific surface area may critically influence the sorption of BaP by the phytoplankton. At the beginning of exposure, the quantified concentration factors were generally the highest for the diatom *Thalassiosira pseudonana*, which may be attributed to their small size (3 to 5 µm) and large surface area to volume ratio. By excluding the initial sorption, we calculated the uptake rate constant based on the linear regression between the second phase of accumulation and the time of exposure. The internal accumulation of hydrophobic organic contaminants, especially those with *K*<sub>ow</sub> > 6, by phytoplankton can be very slow (Swackhamer & Skoglund 1993, Stange & Swackhamer 1994, Jabusch & Swackhamer 2004). Thus, the short-term exposures of phytoplankton to dissolved PAHs may not have fully labelled internal compartments and may result in the overestimation of accumulation rates (which reflect mainly surface accumulation). The phytoplankton BaP uptake rates reported in Table 1 are indeed an order of magnitude higher than those predicted (64 ml mg<sup>-1</sup> h<sup>-1</sup>) for the intracellular accumulation of the most hydrophobic PAHs, including BaP, for a similarly sized cell (Del Vento & Dachs 2002).

The *DCF* of BaP in copepods was lower than that in phytoplankton, again probably due to their lower surface area to volume ratios. The lipid composition in marine copepods varied from 4 to 43% of the dry weight (Buhring & Christiansen 2001, Fisk et al. 2001), and was comparable to or higher than those in marine phytoplankton (5 to 6%, Wahbeh 1997). Given the much higher *DCF* for phytoplankton than for copepods, the biosorption was presumably much more important in determining the BaP uptake than the lipid content. Among the different tissues of fish, the accumulation was the highest for the viscera, most likely due to the highest lipid content and the rapid transport to this tissue. Spacie et al. (1983) found that the uptake of BaP by the bluegill sunfish was linear, although the biotransformation increased between 1 and 2 h of exposure. At 4 h, only 11% of the <sup>14</sup>C activity represented the parent compound and 25% of the BaP was distributed into the liver and gall bladder (Spacie et al. 1983).

The *AEs* by copepods varied over the range of 2 to 24% within the food concentration range tested (0.1 to 5.0 mg l<sup>-1</sup>). Our study demonstrated that the *AEs* of BaP were dependent on the food quantity, and were generally very low at a high food concentration. Because the *AEs* for different algal foods were quantified at a high food concentration (1.0 mg l<sup>-1</sup>), they were too low to result in a meaningful comparison among the different algae. In earlier experimental studies, it was found that the marine copepods typically had a low retention of PAHs. For example, the copepods lost >35% of naphthalene accumulated from the food over the 24 h depuration period (Corner et al. 1976, Harris et al. 1977a,b). Furthermore, the slow intracellular accumulation of BaP by phytoplankton may affect the interpretation of the *AE* experiments in which phytoplankton were labeled for 2 h before fed to copepods. It is possible that the low BaP *AEs* measured in copepods may have resulted from the insufficient labeling of intracellular compartments in the phytoplankton.

The measured *AEs* of BaP (30 to 50%) for the mangrove snappers were higher than those determined for the marine copepods. For fish, enzymes responsible for biotransformation included Phase I cytochrome P450 monooxygenase or MFO system. Livingstone (1998) found that fish metabolized BaP at a faster rate than the other animals, consistent with the higher levels of...
total cytochrome P450 and inducible cytochrome P450 1A (CYP1A) activity. Given the higher P450 activity in the fish and the higher AE compared to the copepods, biotransformation was not the only factor affecting the retention of these PAH compounds in the animals. Digestive physiology may also play an important role in the assimilation of these dietary PAH chemicals. In contrast, previous studies found that the AEs of the PAH compounds in fish measured using similar methodology were relatively low (<15% for naphthalene and BaP; Corner et al. 1976, Fair & Fortner 1987, Lemaire et al. 1992).

In our radiolabeling of phytoplankton used for the AE measurements, we used a rather short exposure period (2 h) and it was assumed that the biotransformation was negligible over the relatively short period. Similarly, the copepods were radiolabeled with BaP for 12 h before they were fed to the fish. It was also assumed that biotransformation was minor within this period, but this would require further experimental confirmation. We calculated the AE by fish after 24 h of depuration and assumed that the hydroxyl metabolism can be ignored during the second phase of depuration. A recent study demonstrated that a significantly higher liver EROD activity was only detected after the fish (Acanthopagrus schlegeli) were exposed to 1.0 µg l–1 of BaP for 2 d (Wang, in press), suggesting that biotransformation was not significant within the first 2 d (within our AE measurement period). Since the AE was calculated after 24 h, the biotransformed products (if any) may have been excreted, and the effects on AE calculation may be reduced. However, further experimental studies are required to assess the implication of biotransformation on the biokinetics of BaP in aquatic animals.

The high elimination rate constants (0.8 to 1.7 d–1) of BaP in copepods were presumably related to their rapid metabolism and the small body sizes of the animals. In examining the physiological efflux of metals, it has also been shown that the marine copepods (Acartia spinicauda) were able to eliminate the metals rapidly (at rates similar to those found for BaP in this study) as a result of their small body sizes (Xu et al. 2001). Landrum et al. (2003) reported that the kα in freshwater amphipods, Diporeia spp., was 0.12 to 0.55 d–1 for naphthalene, 0.12 to 0.22 d–1 for phenanthrene, and 0.05 to 0.07 d–1 for pyrene, respectively. These rate constants were much lower than the kα of BaP in copepods measured in this study. Lotufo (1998) quantified the depuration of fluoranthene with 4 benzene rings (log Kow = 5.23) in 2 copepod species (Schizoperos knabeni and Coullana sp). The biological retention half-life of this compound was 4.8 to 7.4 h (with a corresponding elimination rate constant of 2.2 to 3.4 d–1), again illustrating that the freshwater zooplankton had an efficient elimination system to remove or metabolize the PAH compounds.

With the use of a simple kinetic model, we found that the BaP uptake by copepods was dominated by food uptake instead of dissolved exposure. Dietary accumulation plays an important role under most conditions simulated in this study. The significance of trophic transfer was principally due to the very high BCF of phytoplankton diets, despite the fact that the AE was low and the kα was high. For the mangrove snappers, dietary intake also plays an important role under conditions likely encountered by the fish, but it depends greatly on the feeding rate of the fish as well as the concentration of BaP in the copepod prey. It has been considered that uptake via food rather than dissolved phase is the dominant exposure route for compounds with log Kow > 5 (Thomann 1989, Connolly & Pedersen 1988). Dietary exposure is, however, not taken into consideration in the laboratory studies because of the technical difficulty in creating a realistic dietary exposure regime, even though some studies have shown the dominance of dietary exposure for poorly water-soluble compounds such as PCBs (Randall et al. 1998). Laboratory studies with rainbow trout, however, did not reveal significant accumulation through dietary exposure, apparently as a result of their poor assimilation efficiency from the diets and the rapid elimination of PAHs (Niimi & Dookhram 1989). Overall, our modeling results imply that trophic transfer should be considered in examining the bioaccumulation of BaP in marine animals and that the relative importance of trophic transfer is dependent on various biological and environmental conditions.

Our calculations of the trophic transfer factor (TTF) with typically <0.1 are consistent with many field observations that PAH is not biomagnified in the marine zooplankton (Fisk et al. 2001). Fisk et al. (2001) demonstrated that the concentrations of hydrophobic persistent organic pollutants (log Kow = 3.8 to 6.0) in zooplankton are likely to reflect water concentrations and that these compounds do not biomagnify in zooplankton. One main mechanism for the low TTF was the biotransformation of BaP, which may metabolize a fraction of the absorbed BaP to polar metabolites or phenolic compounds, resulting in a rapid elimination of these compounds by the marine copepods. Measurements of the biokinetics of PAH compounds can thus aid in understanding the trophic interactions of PAHs in marine food chains.

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