

ETox 5084

# Humic Acids Reduce Bioaccumulation of Some Polycyclic Aromatic Hydrocarbons<sup>1</sup>

G. J. LEVERSEE<sup>2</sup>, P. F. LANDRUM<sup>3</sup>, J. P. GIESY<sup>4</sup>, AND T. FANNIN<sup>5</sup>

Savannah River Ecology Laboratory Drawer E, Aiken, SC 29801, USA

LEVERSEE, G. J., P. F. LANDRUM, J. P. GIESY, AND T. FANNIN. 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fish. Aquat. Sci.* 40(Suppl. 2): 63-69.

In laboratory studies, *Daphnia magna* were exposed for 6 h to five polycyclic aromatic hydrocarbons (PAH) (0.1-2.0 µg/L) in water with and without Aldrich<sup>®</sup> humics (2 mg DOC/L). Compared to results in nonhumic water, accumulation of PAH by *Daphnia* in water with humics was significantly reduced for benzo[a]pyrene (-25%) while it was increased for methylcholanthrene (+210%). Humics did not significantly alter *Daphnia* accumulation of anthracene, dibenzanthracene or dimethylbenzanthracene. In additional studies, humics reduced *Daphnia* accumulation of benzo[a]pyrene (B[a]P) over a range of B[a]P concentrations (1.1-5.4 µg/L) exceeding the reported limit for water solubility (1.1-1.2 µg/L). Humics consistently increased *Daphnia* accumulation of methylcholanthrene (MC) over a range of humic concentrations from 0.2 to 10.0 µg DOC/L. Particulates and DOC (10-12 mg TOC/L) occurring naturally in two South Carolina streams reduced *Daphnia* accumulation of B[a]P by 38-66%, with about 40% of the overall reduction attributable to DOC. We conclude that dissolved refractory organics may significantly affect bioavailability and environmental transport of some PAH in fresh waters.

LEVERSEE, G. J., P. F. LANDRUM, J. P. GIESY, AND T. FANNIN. 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fish. Aquat. Sci.* 40(Suppl. 2): 63-69.

Dans des études de laboratoire, des *Daphnia magna* ont été exposées pendant 6 h à cinq hydrocarbures aromatiques polycycliques (HAP) (0,1-2,0 µg/L) dans de l'eau contenant ou non des dérivés humiques Aldrich<sup>®</sup> (2 mg COD/L). Comparativement à ce qui se produit dans une eau non humique, l'accumulation de HAP par *Daphnia* dans de l'eau contenant des dérivés humiques est nettement réduite en ce qui a trait au benzo[a]pyrène (-25 %), tandis qu'elle est plus élevée dans le cas du méthylcholanthrène (+210 %). Les dérivés humiques ne modifient pas pour la peine l'accumulation de l'anthracène, du dibenzanthracène ou du diméthylbenzanthracène. Dans des études supplémentaires, les dérivés humiques ont réduit l'accumulation du benzo[a]pyrène (B[a]P) dans une gamme de concentration (1,1-5,4 µg/L) dépassant la limite connue de solubilité dans l'eau (1,1-1,2 µg/L). Les dérivés humiques causent une plus forte accumulation par *Daphnia* du méthylcholanthrène (MC) sur une gamme de concentration humique de 0,2-10,0 µg COD/L). Les matières particulières et le COD (10-12 mg COD/L) présents naturellement dans deux cours d'eau de la Caroline du Sud réduisent d'environ 38-66 % l'accumulation de B[a]P par *Daphnia*, 40 % de la réduction globale étant attribuables au COD. Nous concluons que des substances organiques réfractaires dissoutes peuvent influencer de façon significative sur l'accessibilité aux organismes et le transport dans l'environnement de certains HAP présents en eaux douces.

Received March 18, 1982  
Accepted October 12, 1982

Reçu le 18 mars 1982  
Accepté le 12 octobre 1982

<sup>1</sup>This paper forms part of the Proceedings of the Conference on Pollution in the North Atlantic Ocean, convened at Halifax, Nova Scotia, October 19-23, 1981.

<sup>2</sup>Present address: Keene State College, Keene, NH 03431, USA.

<sup>3</sup>Present address: Great Lakes Environmental Research Lab, NOAA, Ann Arbor, MI 48104, USA.

<sup>4</sup>Present address: Pesticide Research Center, Michigan State University, East Lansing, MI 48824, USA.

<sup>5</sup>Present address: Wyoming Game and Fish Lab, Lander, WY 82540, USA.



EVIDENCE suggests that the most important pathway of bioaccumulation of polycyclic aromatic hydrocarbons (PAH) is direct uptake from water by simple partitioning into lipids (Neely et al. 1974). It has been demonstrated that humics in freshwater systems may form stable complexes affecting the transport of trace organic compounds including dialkyl phthalates (Ogner and Schnitzer 1970), pesticides (Wershaw et al. 1969), amino acids (Lytle and Perdue 1981), and cholesterol (Hassett and Anderson 1979). In marine systems, dissolved organic matter (DOM) reportedly increased the solubility of *n*-alkanes (Boehm and Quinn 1974) and decreased the uptake of aromatic and other petroleum hydrocarbons by the bivalve *Mercuraria mercenaria* (Boehm and Quinn 1976). It is generally believed that DOM in rivers is precipitated on exposure to seawater and deposited in estuarine and nearshore marine sediments (Gardner and Menzel 1974). The potential for deposition of PAH associated with DOM precipitated in estuarine sediments may account for some presently unknown fraction of organic trace contaminants found in estuarine sediments.

It is therefore of interest to determine the effects of freshwater humic and fulvic acids on PAH environmental transport. Of particular interest are the effects of humics on direct uptake of PAH by freshwater biota. Studies on humics as they affect PAH accumulation in *D. magna* were conducted to determine (1) if a "standard" humic acid (Aldrich<sup>®</sup>) at one concentration (2 mg DOC/L) affected the bioaccumulation of several PAH, (2) if observed effects in some PAH varied predictably with humic concentration, (3) if observed effects changed over a range of PAH concentrations, some of which exceeded limits of water solubility, and (4) if humics in natural waters behaved similarly.

### Materials and Methods

Six radiolabeled PAH were studied: naphthalene, anthracene, benzo[*a*]pyrene, dibenzanthracene, dimethylbenzo[*a*]anthracene, and methylcholanthrene (Table 1). PAH were selected to provide a broad range of molecular weights, water solubilities, octanol-water partition coefficients, structural configurations, and substitutions. Stocks of PAH were diluted in solvents as shipped and added to test water in volumes of 1–30  $\mu$ L/L. The <sup>3</sup>H-dibenzanthracene specific activity was adjusted to 38 GBq/mole by addition of unlabeled dibenzanthracene (Aldrich<sup>®</sup>, 97% pure) to water at the time of labeling. Water was labeled in bulk (1–4 L) and thoroughly shaken for several minutes before use. All preparative, analytical, and experimental procedures were carried out under gold fluorescent light ( $\lambda \geq 500$  nm) to minimize photodegradation of PAH.

For benzo[*a*]pyrene, experimental water was analyzed after 6 h to insure that *Daphnia* were being exposed primarily to parent compound and not to B[*a*]P degradation products. Triplicate 200-mL aliquots were acidified to pH 4 with glacial acetic acid. Each aliquot was extracted three times with 50-mL volumes of benzene and then three times with 50-mL volumes of ethyl acetate. All benzene and ethyl acetate extracts were combined (total volume  $\approx$  300 mL) and dried by passing through anhydrous Na<sub>2</sub>SO<sub>4</sub>. The water-free extracts of each aliquot were sampled in triplicate (3  $\times$  0.5 mL) to

determine total radioactivity. Extracts were then reduced in volume to  $\approx$  100  $\mu$ L by rotary flash evaporation and evaporation under a nitrogen stream. Extracts were spotted on Merck<sup>®</sup> silica gel thin-layer chromatography plates. Plates were developed in 9:1 (v/v) hexane:benzene, and spots were visualized using ultraviolet light. B[*a*]P was identified using co-chromatography of authentic standards. Plates were scraped and counted using a Beckman LS8100 liquid scintillation counter. Degradation products were identified as non-B[*a*]P<sup>14</sup>C.

Test water was prepared by adding inorganic salts to Milli-Q<sup>®</sup> deionized water as follows (all concentrations mg/L): NaHCO<sub>3</sub> = 48, CaSO<sub>4</sub>·2H<sub>2</sub>O = 30, MgSO<sub>4</sub> = 30, KCl = 2. With these inorganic additions, water quality was as follows: pH 7, hardness 20 mg/L as CaCO<sub>3</sub>, alkalinity 120 mg/L as CaCO<sub>3</sub>, conductivity 130  $\mu$ S/cm.

A humic stock solution was prepared from Aldrich<sup>®</sup> humic acid (H-1675-2, Lot #082091) by repeating the following steps three times. Humic acid was dissolved in 0.1 mol NaOH/L. The crude solution was centrifuged at 13 000  $\times$  *g* for 30 min. The pH was adjusted to 2 with 6.0 mol HCl/L and the solution allowed to stand for 18 h. Humic acid precipitate was collected by centrifugation (4000  $\times$  *g* for 20 min). The final humic solution was adjusted to pH 7 and dialyzed repeatedly against deionized water until conductivity was < 10  $\mu$ S/cm. Stock humics were added to labeled test water to a final, nominal concentration of 2.0 mg/L dissolved organic carbon (DOC) unless otherwise noted. Blanks of Milli-Q<sup>®</sup> water were < 0.1 mg/L, and hardened Milli-Q<sup>®</sup> water < 0.2 mg/L. Total carbon and inorganic carbon in freshwater were determined using a Beckman Model 915B Carbon Analyzer and DOC was calculated by difference.

### PAH BIOAVAILABILITY

*Daphnia magna* from laboratory cultures (Southeastern Research Laboratory, U.S. EPA, Athens, GA) were exposed for 6 h to PAH in paired 1-L beakers with and without humics (60–70 *Daphnia*). Five samples of 10 animals were removed by pipet from each test water, collected on nylon netting, rinsed quickly in 50 mL deionized water, and poured through a Millipore<sup>®</sup> filter apparatus. Animals were collected on tared 25 mm Millipore<sup>®</sup> Type HA cellulose acetate filters, dried over desiccant for 24–48 h and sample dry weights were determined on a Cahn Model 4700 Electrobalance. Animals and filters were combusted in a Packard Model 306 sample oxidizer, CO<sub>2</sub> and H<sub>2</sub>O collected in suitable trapping agents, and counted by liquid scintillation. Samples were corrected for quench using internal and external standards and the sample channels ratio method. In B[*a*]P studies, *Daphnia* were analyzed for B[*a*]P and transformation products (non-B[*a*]P<sup>14</sup>C) to insure that accumulation was primarily of parent compound (Leversee et al. 1981).

To evaluate the importance of natural particulates and DOC on B[*a*]P bioavailability, similar experiments were conducted using water from Upper Three Runs Creek (UTRC) and Skinfac Pond on the Department of Energy's Savannah River Plant, near Aiken, SC. B[*a*]P uptake was determined in water directly from the creek, in water filtered to remove particles (0.45- $\mu$ m Whatman GFC glass fiber filters, precombusted at



TABLE 1. Radio-labeled PAH used in bioavailability studies.

| Compound   | Label                    | Specific activity (GBq/mmol) | Solvent                   | Radiopurity (%) | Source                                 |
|--|--------------------------|------------------------------|---------------------------|-----------------|--|
| Naphthalene<br>mw 128                                  | 1- <sup>14</sup> C       | 0.325                        | Ether                     | ≥98             | California<br>Bionuclear<br>lot 2545   |
| Anthracene<br>mw 178                                   | 9- <sup>14</sup> C       | 0.122                        | Acetone                   | ≥98             | California<br>Bionuclear<br>lot 770824 |
| Benzo[ <i>a</i> ]pyrene<br>mw 252                      | 7,10- <sup>14</sup> C    | 0.803                        | Toluene                   | ≥98             | Amersham<br>Batch 29                   |
| 1,2,5,6-<br>Dibenzanthracene<br>mw 278                 | <sup>3</sup> H(G)        | 1121.1                       | Benzene                   | ≥97             | New England<br>Nuclear<br>lot 1253-021 |
| 7,12-dimethyl<br>Benzo[ <i>a</i> ]anthracene<br>mw 256 | dimethyl <sup>14</sup> C | 3.589                        | Benzene                   | ≥98             | New England<br>Nuclear<br>lot 1267-176 |
| 3-Methylcholanthrene<br>mw 268                         | 6- <sup>14</sup> C       | 2.109                        | Benzene:<br>ethanol (9:1) | ≥98             | New England<br>Nuclear<br>lot 1231-171 |

TABLE 2. Effect of Aldrich<sup>®</sup> humic acids (2 mg DOC/L) on accumulation of five PAH by *Daphnia magna*.

| Initial water PAH concentration (µg/L)   | Humic concentration DOC (mg/L) | Bioaccumulation <sup>a</sup> (6 h)      | Bioconcentration <sup>b</sup> factor (6 h) |
|--|--------------------------------|---|--|
| Anthracene (1.96)<br>mw 178              | 0.2<br>2.0                     | 82.3 (19.7) <sup>c</sup><br>43.9 (4.0)  | 607<br>319                                 |
| Benzo[ <i>a</i> ]pyrene (1.15)<br>mw 252 | 0.2<br>2.0                     | 131.6 (7.3) <sup>d</sup><br>99.4 (7.7)  | 2745<br>2158                               |
| Dimethylbenzanthracene (1.57)<br>mw 256  | 0.2<br>2.0                     | 64.2 (23.2)<br>55.8 (30.0)              | 968<br>666                                 |
| Methylcholanthrene (1.29)<br>mw 268      | 0.2<br>2.0                     | 34.0 (7.4) <sup>d</sup><br>105.5 (10.8) | 667<br>2064                                |
| Dibenzanthracene (0.72)<br>mw 278        | 0.2<br>2.0                     | 20.3 (0.8)<br>24.2 (2.4)                | 652<br>773                                 |

<sup>a</sup>Bioaccumulation: nmol/g dry wt *Daphnia*. Dry/wet = 0.06. Mean dry weight for a single sample of 10 *D. magna* = 1.14 mg.

<sup>b</sup>Bioconcentration factor:  $\frac{\text{ng PAH/g wet wt } Daphnia}{\text{ng PAH/mL water}}$

Calculated using final water concentrations.

<sup>c</sup> $\bar{x}$  (SE),  $n = 5$ .

<sup>d</sup> $P < 0.05$  ( $t$  test).

550°C, 8 h), and in filtered water UV photo-oxidized to remove DOC. DOC in 100-mL aliquots of water was removed by exposure for 6 h in a La Jolla Scientific UV Photo-oxidizer with a few drops of hydrogen peroxide as a catalyst. Oxidized water was filtered to remove colloidal iron, hardened by the addition of salts and refiltered before use.

## Results and Discussion

Concentrations of five PAH were determined in *Daphnia magna* after 6-h exposures in water with Aldrich<sup>®</sup> humics (DOC = 2.0 mg/L) and in water without humics (DOC ≤ 0.2 mg/L). *Daphnia* in humic waters had significantly lower concentrations of benzo[*a*]pyrene (B[*a*]P) (-24.5%)

than comparable animals in nonhumic waters (Table 2). On the other hand, humics increased concentrations of methylcholanthrene (MC) in *Daphnia* (+210%). Humics had little effect on dimethylbenzanthracene (DMBA) (-13.1%) or dibenzanthracene (DBA) (-19.1%). While the effect on anthracene (-46.7%) appeared large, it was not significant due to large sample variance. Results reported assume that all radioactivity was parent compound (see below). These results are significant for two reasons: (1) they demonstrate that humics at low concentrations affect bioaccumulation of some PAH; (2) they demonstrate that humics may either increase or decrease bioaccumulation. Mechanisms which may be operating to cause these differences will be considered later, but several questions concerning the relationship between 6 h



TABLE 3. Effect of Aldrich<sup>®</sup> humic acids on PAH accumulation in *Daphnia magna*. Results (ANOVA) were highly significant for B[a]P, not quite significant at  $P < 0.05$  for anthracene, and not significant for naphthalene.

| PAH                                   | Humic concentration<br>(DOC in mg/L) | Bioaccumulation <sup>a</sup><br>(6 h) | Bioconcentration <sup>b</sup><br>factor |
|---------------------------------------|--------------------------------------|---------------------------------------|---|
| Benzo[a]pyrene <sup>c</sup><br>mw 252 | 0.3                                  | 139.2 (14.2)                          | 1716                                    |
|                                       | 1.5                                  | 90.9 (7.6)                            | 979                                     |
|                                       | 5.7                                  | 72.6 (5.7)                            | 838                                     |
|                                       |                                      | $F_{2,12} = 12.19 (P < 0.005)$        |   |
| Anthracene<br>mw 178                  | 0.3                                  | 37.2 (2.0)                            | 389                                     |
|                                       | 1.5                                  | 34.9 (2.0)                            | 362                                     |
|                                       | 5.7                                  | 31.0 (1.1)                            | 340                                     |
|                                       |                                      | $F_{2,12} = 3.16 (NS)$                |   |
| Naphthalene<br>mw 128                 | 0.3                                  | 1.7 (0.2)                             | 61                                      |
|                                       | 1.5                                  | 1.6 (0.3)                             | 74                                      |
|                                       | 5.7                                  | 1.8 (0.1)                             | 57                                      |
|                                       |                                      | $F_{2,6} = 0.95 (NS)$                 |   |

<sup>a</sup>Bioaccumulation: nmoles PAH/g dry wt *Daphnia*. Mean  $\pm$  SE,  $n = 5$ . Naphthalene  $n = 3$ .

<sup>b</sup>Bioconcentration factor:  $\frac{\text{ng PAH/g wet wt } Daphnia}{\text{ng PAH/mL water}}$

<sup>c</sup>Initial B[a]P water concentration was 1.5  $\mu\text{g/L}$  (6.0 nmol); Anthracene was 1.3  $\mu\text{g/L}$  (7.2 nmol); Naphthalene was 0.8  $\mu\text{g/L}$  (6.6 nmol).

TABLE 4. Effect of Aldrich<sup>®</sup> humic acids on methylcholanthracene accumulation by *D. magna*.

| Humic concentration<br>(DOC mg/L) | Bioaccumulation <sup>a</sup><br>(6 h) | Bioconcentration factor <sup>b</sup><br>(6 h) |
|-----------------------------------|---------------------------------------|---|
| 0                                 | 142.1(15.2) <sup>c</sup>              | $\frac{2401}{3.1} = 775$                      |
| 2.0                               | 309.3(66.1)                           | $\frac{5288}{2.9} = 1823$                     |
| 4.0                               | 311.2(47.1)                           | $\frac{5260}{2.8} = 1879$                     |
| 6.0                               | 376.9(71.6)                           | $\frac{6370}{2.9} = 2197$                     |
| 8.0                               | 260.1(40.8)                           | $\frac{4396}{3.0} = 1465$                     |
| 10.0                              | 233.6(12.5)                           | $\frac{3945}{3.6} = 1096$                     |

<sup>a</sup>Bioaccumulation: nmol methylcholanthrene/g dry weight *D. magna*.

<sup>b</sup>Bioconcentration factor:  
 $\frac{\text{ng methylcholanthrene/g wet weight } D. magna}{\text{ng methylcholanthrene/mL water}}$

<sup>c</sup> $\bar{x}$  (SE),  $n = 5$ .

<sup>d</sup>Water methylcholanthrene concentration at 6 h used for BCF. Initial mean water concentration was 3.5 (0.04)  $\mu\text{g/L}$ .

uptake and steady state bioconcentration of unaltered PAH must be addressed.

Evidence from this and other studies suggests that results reported for the 6 h uptake tests were not transient differences but were representative of steady state differences for all PAH tested. In preliminary 24-h uptake studies, steady state radioactivity in *D. magna* was reached within 6 h for anthracene, and B[a]P. Approximately 60–80% of steady state was

reached after 6 h with DMBA, DBA and MC. In no case did the relative difference between *Daphnia* PAH in humic and nonhumic waters change after 6 h. In *Daphnia pulex*, Southworth et al. (1978) reported that 6-h exposures produced steady state concentrations of naphthalene and anthracene, while up to 24 h were required for benzantracene, results consistent with our observations. The values reported do not necessarily represent maximum bioaccumulation, since depletion of water PAH concentrations can occur in static tests. It will be noted that results for *Daphnia* accumulation of anthracene and methylcholanthrene are not identical in Tables 2, 3, and 4 at comparable humic concentrations. These differences are due to different initial concentrations of PAH, as well as to inherent variance due to individual experimental procedures over several months. Trends within replicate experiments have always been consistent. Previous studies have shown that *Daphnia* have slow PAH biotransformation rates relative to accumulation rates (Herbes and Risi 1978; Leversee et al. 1981). In this study with <sup>14</sup>C-B[a]P, after 6 h, parent compound represented 91.3  $\pm$  2.1% of test water activity and 92.8  $\pm$  0.8% of *D. magna* activity, respectively. Biotransformation may be even less than 10% since some degradation of B[a]P on TLC plates has been observed (by P. F. Landrum). Active biotransformation and excretion of PAH reported for some invertebrates and fish are probably not important factors in PAH accumulation by *D. magna*.

The effect of varying Aldrich<sup>®</sup> humic acid concentrations (0.3–5.7 mg DOC/L) on accumulation by *Daphnia* of B[a]P, anthracene, and naphthalene was determined. Humics significantly reduced the accumulation of B[a]P but results were not significant for anthracene and naphthalene (Table 3). In this simple unsubstituted PAH series, the effect of humics was greatest for B[a]P > anthracene > naphthalene, which is consistent with the order of reported octanol–water partition coefficients. This suggests that hydrophobic PAH may be partitioned competitively between *Daphnia* lipids and humics. Although the effect of humics on B[a]P and anthracene





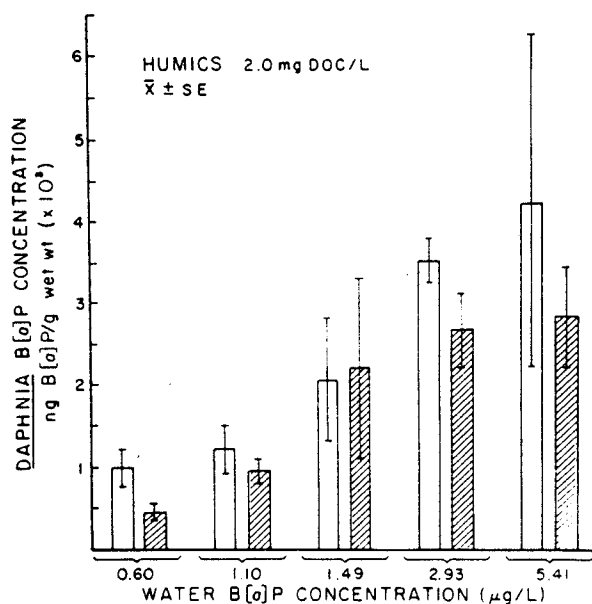


FIG. 1. Effect of Aldrich<sup>®</sup> humic acids (2.0 mg DOC/L) on *Daphnia magna* accumulation of B[a]P at five B[a]P concentrations. Shaded bars represent water with humics, open bars water without humics.

was greatest at highest humic concentrations, there was not a simple linear relation between humic concentration and *Daphnia* PAH concentration. The greatest effect was found between no humic acids and 1–2 mg DOC/L. Additional data at several concentrations of PAH and humics will be necessary before determining the possibility that predictive sorption isotherms can be developed for PAH like B[a]P.

The effect of varying humic concentrations over a wide range (0–10 mg DOC/L) while holding initial PAH concentration constant was determined using methylcholanthrene (3.5 µg/L). At all humic concentrations, *Daphnia* accumulated significantly more MC than animals in nonhumic water (Table 4). Among various humic concentrations (2–10 mg DOC/L), there were no significant differences in *Daphnia* MC concentration. The lack of a simple predictive relation between humic concentration and *Daphnia* PAH concentration was apparent for MC, as it was for anthracene and B[a]P.

The effect of varying PAH concentration (B[a]P = 0.6–5.4 µg/L) while holding humic concentration constant (2.0 mg DOC/L) was also examined. In general, results confirmed our previous conclusions that humics reduced B[a]P accumulation in *Daphnia* (Fig. 1). The large variance inherent in working with small samples of *Daphnia* and PAH concentrations of 1–10 µg/L resulted in a significant difference between *Daphnia* in humic and nonhumic waters only at 2.9 µg B[a]P/L (*t*-test,  $P < 0.05$ ) (Fig. 1). It has been suggested that marine DOM increased uptake of the aromatic fraction of No. 2 fuel oil by *Mercenaria mercenaria* at high aromatic concentrations by a process of co-solubilization (Boehm and Quinn 1976). This study suggests that no apparent major difference in the effects of humics occurred when

B[a]P concentration exceeded the reported limits of B[a]P water solubility (1.1–1.2 µg/L). Also, it did not appear that increasing B[a]P concentrations in the range examined saturated the humic effect. The mean values for *Daphnia* B[a]P concentrations were still reduced in the presence of humics, even at initial B[a]P concentrations in water of 2.9 and 5.4 µg/L.

The effect of natural organics and particles on *Daphnia* B[a]P accumulation was examined to determine whether results would be comparable to those with Aldrich<sup>®</sup> humics. Upper Three Runs Creek and Skinface Pond, typical coastal plain surface waters, are soft (hardness = 10) mg/L as CaCO<sub>3</sub> acidic (pH 6.1) and rich in humic and fulvic acids (10–12 mg TOC/L). In both waters, *Daphnia* B[a]P concentrations were significantly reduced by the presence of particles and DOC (Table 5). Removal of particles and dissolved organics from Skinface Pond water caused a threefold increase in *Daphnia* B[a]P concentrations, while in Upper Three Runs Creek water, a 60% increase occurred. Dissolved organics caused about 40% of the observed effect in both waters, i.e. particles and DOC were about of equal importance. In addition to direct effects, dissolved refractory organics are important in formation of organic films on clay particles (Hunter 1980). Over 90% of DOC in Skinface Pond and Upper Three Runs Creek waters is in small ultrafilter size fraction  $0.0015 \leq F \leq 0.0009 \mu\text{m}$  (nominal mw < 500) (Giesy and Briese 1978) and the effect observed may be due to this fraction. The humic acids of these waters have been well characterized in terms of seasonal changes in DOC concentration, molecular size fractions and effects on metal speciation, metal binding, and metal toxicity (Giesy and Briese 1978; Giesy et al. 1982).

In summary, results of these *Daphnia* PAH uptake studies suggest a complex effect of humic acids on PAH bioavailability. In general, humics reduce the partitioning into *Daphnia* of single unsubstituted PAH, with the greatest reduction observed in PAH having the largest octanol–water partition coefficient (e.g. B[a]P > anthracene > naphthalene). Methylcholanthrene accumulation by *Daphnia* was increased by humics in a manner which is not clear. This apparent anomaly was found in many replicate studies in our laboratory but should be confirmed by other workers. If correct, this unexpected result could have significant implications for models of PAH bioaccumulation.

In the test system used, significant interactions may occur among any or all components (PAH–humics–*Daphnia*). Partitioning into internal lipids is usually assumed, but Crosby and Tucker (1971) found that up to 50% of DDT in *Daphnia magna* was on the carapace, probably by simple surface sorption. Little is known concerning humic–organism interactions. Other studies in our laboratory have demonstrated that humics reduce B[a]P uptake in both bluegill sunfish (*Lepomis macrochirus*) and chironomid larvae (*Chironomus riparius*). Thus we feel that the results observed are not unique to *Daphnia* and probably do involve significant humic–PAH interactions. Questions concerning the nature of these interactions are better answered using chemical techniques such as affinity or gel permeation chromatography, ultrafiltration, or solvent extraction. Our purpose was to demonstrate effects of humics on bioaccumulation using a range of PAH.



TABLE 5. Effect of Skifface Pond and Upper Three Runs Creek organics and particles on B[a]P accumulation by *Daphnia magna*.

| Water source and treatment | Organic carbon (mg/L) | Bioaccumulation <sup>a</sup> (6 h) | Bioconcentration <sup>b</sup> factor |
|----------------------------|-----------------------|------------------------------------|--------------------------------------|
| Upper Three Runs Creek     |                       |                                    |                                      |
| Untreated                  | 10.0                  | 227.4 (12.0)                       | 2571                                 |
| Filtered (0.45 µm)         | 5.5                   | 304.4 ( 7.1)                       | 3581                                 |
| Filtered-oxidized          | 0.2                   | 367.2 (13.9)                       | 4656                                 |
|                            |                       | $F_{2,12} = 37.87^c$               |                                      |
| Skifface Pond              |                       |                                    |                                      |
| Untreated                  | 12.1                  | 70.4 ( 2.6)                        | 903                                  |
| Filtered (0.45 µm)         | —                     | 156.2 ( 5.6)                       | 2312                                 |
| Filtered-oxidized          | 0.2                   | 206.7 (13.1)                       | 3292                                 |
|                            |                       | $F_{2,12} = 67.84^c$               |                                      |

<sup>a</sup> $\bar{x}$  (SE),  $n = 5$ . Values = nmol/g dry weight.

<sup>b</sup> $\frac{\text{ng B[a]P/g wet weight } Daphnia}{\text{ng B[a]P/mL water}}$

<sup>c</sup>ANOVA,  $P < 0.001$ .

Most models for humic interactions with both metals and trace organics assume covalent or other binding to humics (Parris 1980) which competes for binding to biota. It has been suggested, however, that natural organics may increase or decrease bioavailability and biological effects of metals depending on organic molecular size class, number and type of binding sites and conditional stability constants (Giesy et al. 1977; Giesy et al. 1982). The same may be true of humic complexed PAH. Natural organic matter in river water reportedly binds cholesterol, reducing efficiency of solvent extraction. Gel permeation studies suggest that the cholesterol is bound to high molecular weight organics (Hassett and Anderson 1979). Whatever the nature of the effect observed (increased or decreased bioavailability), studies with B[a]P and MC suggest that it is reasonably consistent over a range of both humic and PAH concentrations. However, the results reported here suggest that a simple linear relation between humic concentration and PAH bioconcentration does not exist. Additional data are needed over a wider range of humic concentrations and different humic types.

DOC is the most abundant form of reduced carbon in freshwater (Hobbie and Likens 1973; Wetzel and Manny 1977; Naiman and Sibert 1978; Rich and Wetzel 1978), estuaries (Head 1976), and oceans (Williams 1975). Given an average river organic carbon concentration of 10 mg C/L, the annual input to oceans can be calculated at  $3 \times 10^{14}$  g C (Handa 1977), of which 60–90% is DOC (Dahm 1981). Estimates of labile organics (amino acids, lipids, etc.) in river systems vary widely but are in the range of 20–40% of DOC (Larsen 1978), the remainder being refractory humic and fulvic acids.

Over 230 000 t of PAH enter the oceans and surface waters of the world each year (Suess 1976). A significant portion of that is from industrial and urban runoff. These amounts can be expected to increase due to increased coal use through direct combustion, or from processing into synfuels (Gehrs 1976) and from petroleum spills. Formation of PAH–humic complexes may alter partitioning of PAH into biota and organic sediments and thereby alter downstream transport. An understanding of the factors affecting this transport will be im-

portant in developing models for PAH input into estuarine and marine systems.

### Acknowledgments

Thanks to Garfield Keaton, John Bowling, Sarah Gerould, and Pam Tolbert for technical assistance in various aspects of this study. Dr James Alberts materially assisted this study through many long discussions and critical comments. This research was supported by Interagency Agreement EPA-78-D-X0290 between the U.S. Environmental Protection Agency and U.S. Department of Energy and Contract DE-AC09-76SR00819 between the U.S. Department of Energy and the University of Georgia.

- BOEHM, P. D., AND J. G. QUINN. 1974. The solubility behavior of No. 2 fuel oil in sea water. *Mar. Pollut. Bull.* 5: 101–105.
1976. The effect of dissolved organic matter in sea water on the uptake of mixed individual hydrocarbons and Number 2 fuel oil by a marine bivalve (*Mercenaria mercenaria*). *Estuarine Coastal Mar. Sci.* 4: 93–105.
- CROSBY, D. G., AND R. K. TUCKER. 1971. Accumulation of DDT by *Daphnia magna*. *Environ. Sci. Technol.* 5: 714–716.
- DAHM, C. N. 1981. Pathways and mechanisms for removal of dissolved organic carbon from leaf leachate in streams. *Can. J. Fish. Aquat. Sci.* 38: 68–76.
- GARDNER, W. S., AND D. W. MENZEL. 1974. Phenolic aldehydes as indicators of terrestrially derived organic matter in the sea. *Geochim. Cosmochim. Acta* 38: 813–822.
- GEHRS, C. W. 1976. Coal conversion, description of technologies and necessary biomedical environmental research. Oak Ridge National Laboratory Publication No. 5192.
- GIESY, J. P., AND L. A. BRIESE. 1978. Trace metal transport by particulates and organic carbon in two South Carolina streams. *Verh. Int. Verein. Limnol.* 20: 1401–1417.
- GIESY, J. P., G. J. LEVERSEE, AND D. R. WILLIAMS. 1977. Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphniidae) and *Gambusia affinis* (Poeciliidae). *Water Res.* 11: 1013–1020.
- GIESY, J. P., A. NEWELL, AND G. J. LEVERSEE. 1982. Copper speciation in soft, acid, humic waters: effects on copper bioaccumulation by and toxicity to *Simocephalus serrulatus* (Daphniidae). *Biological Availability of Trace Metals. Twenty-first*



- Hanford Life Sciences Symposium, Richland, WA. (In press)
- HANDA, N. 1977. Land sources of marine organic matter. *Mar. Chem.* 5: 341-359.
- HASSETT, J. P., AND M. A. ANDERSON. 1979. Association of hydrophobic organic compounds with dissolved organic matter in aquatic systems. *Environ. Sci. Technol.* 13: 1526-1529.
- HEAD, P. C. 1976. Organic processes in estuaries, p. 53-91. *In* J. D. Burton and P. S. Liss [ed.] *Estuarine chemistry*. Academic Press, London.
- HERBES, S. E., AND G. F. RISI. 1978. Metabolic alteration and excretion of anthracene by *Daphnia pulex*. *Bull. Environ. Contam. Toxicol.* 19: 147-155.
- HOBBIE, J. E., AND G. E. LIKENS. 1973. Output of phosphorus, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. *Limnol. Oceanogr.* 18: 734-742.
- HUNTER, K. A. 1980. Microelectrophoretic properties of natural surface-active organic matter in coastal seawater. *Limnol. Oceanogr.* 25: 807-822.
- LARSON, R. A. 1978. Dissolved organic matter in a low coloured stream. *Freshwater Biol.* 8: 91-104.
- LEVERSEE, G. J., J. P. GIESY JR., P. F. LANDRUM, S. BARTELL, S. GEROULD, M. BRUNO, A. SPACIE, J. BOWLING, J. HADDOCK AND T. FANNIN. 1981. Disposition of benzo(a)pyrene in aquatic systems components: periphyton, chironomids, daphnia, fish, p. 357-366. *In* M. Cooke and A. Dennis [ed.] *Polynuclear aromatic hydrocarbons*. Battelle Press, Columbus, OH.
- LYTLE, C. R., AND E. M. PERDUE. 1981. Free, proteinaceous, and humic-bound amino acids in river water containing high concentrations of aquatic humus. *Environ. Sci. Technol.* 15(2): 224-228.
- NAIMAN, R. J., AND J. R. SIBERT. 1978. Transport of nutrients and carbon from the Nanaimo River to its estuary. *Limnol. Oceanogr.* 21: 60-73.
- NEELY, W. B., D. R. BRANSON, AND G. E. BLAU. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. *Environ. Sci. Technol.* 8: 1113-1115.
- OGNER, G., AND M. SCHNITZER. 1970. Humic substances: fulvic acid-dialkyl phthalate complexes and their role in pollution. *Science* 170: 317-318.
- PARRIS, G. E. 1980. Covalent binding of aromatic amines to humates. I. Reactions with carbonyls and quinones. *Environ. Sci. Technol.* 1099-1108.
- RICH, P. H., AND R. G. WETZEL. 1978. Detritus in the lake ecosystem. *Am. Nat.* 112: 57-71.
- SOUTHWORTH, G. R., J. J. BEAUCHAMP, AND P. K. SCHMIEDER. 1978. Bioaccumulation potential of polycyclic aromatic hydrocarbons in *Daphnia pulex*. *Water Res.* 12: 973-977.
- SUESS, M. J. 1976. The environmental load and cycle of polycyclic aromatic hydrocarbons. *Sci. Total Environ.* 6: 239-250.
- WERSHAW, R. L., P. J. BURCAR, AND M. C. GOLDBERG. 1969. Interaction of pesticides and natural organic material. *Environ. Sci. Technol.* 3: 271-273.
- WETZEL, R. G., AND B. A. MANNY. 1977. Seasonal changes in particulate and dissolved organic carbon and nitrogen in a hard-water stream. *Arch. Hydrobiol.* 80: 20-39.
- WILLIAMS, P. J. leB. 1975. Biological and chemical aspects of dissolved organic material in sea water, p. 301-363. *In* J. P. Riley and G. Skirrow [ed.] *Chemical oceanography*. Vol. 2. 2nd ed. Academic Press, London.

