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Fate of Anthracene in an Artificial Stream: A Case Study[1]

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The fate of anthracene, a representative polycyclic aromatic hydrocarbon, was followed in a large outdoor stream microcosm. The major nonadvective route for the removal of anthracene was photolytic degradation to anthraquinone (half-life 43 min). The anthraquinone also photolyzed rapidly in this shallow stream system. Excluding the plastic channel liner, the sediment acts as the major sink for anthracene, absorbing 0.2% of the 14-day input dose. The periphyton community was the second most important sink, absorbing 0.04% of the input dose. All other compartments were of significantly less importance on a mass basis. Anthracene (11 μg liter^{-1}) caused photo-induced 100% mortality of the bluegill sunfish in 9 hr in the upstream reach. Fish at the downstream station survived for ~26 hr and all died within 1 hr of each other. Other organisms, clams and dragonfly larvae, started to die off toward the end of the 14-day input period.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants with both natural and anthropogenic sources (Braunstein *et al.*, 1977; Harrison *et al.*, 1975; Suess, 1976; Neff, 1979). PAH are of environmental concern because many of the homologous series are carcinogenic. PAH levels in the aquatic environment, expected to increase as energy sources are shifted from oil to coal and synthetic fuels (Gehrs, 1976), have been implicated in tumor formation in fish and other aquatic organisms (Hodgins *et al.*, 1977; Yevich and Barszez, 1977; Baumann *et al.*, 1981). PAH can be acutely toxic to fish at low concentrations (Bowling *et al.*, 1983) and can bioaccumulate in aquatic organisms that may be a vector of PAH to man (Neff, 1979).

The large number of PAH prohibits detailed fate studies for each individual compound. Therefore, more generalized PAH fate prediction mechanistic simulation models, such as FOAM (Bartell *et al.*, 1981) or EXAMS (Baughman and Lassiter, 1978), may be useful for regulatory decisions. However, before such models can be of general use, parameterization for specific applications and verifications of the predicted results are highly desirable.

We here examine the fate of anthracene in a large artificial stream to provide a case study for a model PAH. We used the data generated for verification of FOAM and other models, such as EXAMS. The data may also prove useful for construction of new models and provide an understanding of the dominant fate processes for

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anthracene in clear shallow streams. The studies followed the advection downstream and uptake by various compartments of anthracene and its major photoproduct, anthraquinone, from a constant infusion of anthracene and elimination of the compounds after the anthracene input was terminated.

Facility Description

The artificial streams used in this study are located on the Department of Energy's (DOE) Savannah River Plant (SRP) near Aiken, South Carolina. The facility is a single pass system consisting of six separate cinderblock channels, each 91.5 m long, 0.61 m wide, and 0.31 m deep (Bowling *et al.,* 1980). The channels and headpools were lined with 0.05-cm-thick, black polyvinyl chloride (PVC) film; 5 cm of washed quartz sand was added for basic substratum.

Water for the channels was pumped from the Tuscaloosa aquifer via a deep well located near the facility. The water was treated to remove CO_2, and hydrated lime was added to produce inorganic water quality similar to that of surface waters (Table 1). Water flows were monitored by v-notch weirs on each headpool. Flow rates of 75.7 liter min^{-1}, maintained manually, resulted in a current velocity of 1.0×10^{-2} m sec^{-1} and a retention time of 2.5 hr. The water depth in each channel was maintained at 20 cm.

Initially, four plastic-lined channels were inoculated with a 50-liter slurry of water, sediment, and biological material (i.e., algae, zooplankton, macroinvertebrates) from seven locations around the SRP site. The inoculation was conducted weekly from March 11, 1979, through June 13, 1979, and daily from June 13, 1979, to July 16, 1979. These channels were allowed 10 weeks of additional colonization prior to the

TABLE 1

MEAN WATER QUALITY OF TREATED WELL WATER

Total alkalinity	17.5 mg l^{-1} as $CaCO_3$
Hardness	21.5 mg l^{-1} as $CaCO_3$
pH	6.6
Specific conductance	31 μmho cm^{-1}
Ionic strength (*I*)	2.5×10^{-4}
DO	8.5 mg l^{-1}
CO_2	3.25 mg l^{-1}
SO_4^{-2}	1.9 mg l^{-1}
Total P	10.1 μg l^{-1}
Nitrogen ($NO_2 + NO_3$)	19.2 μg l^{-1}
Ca	3.17 mg l^{-1}
Cu	3.4 μg l^{-1}
Co	2.5 μg l^{-1}
Cd	0.023 μg l^{-1}
Cr	0.3 μg l^{-1}
Fe	1.7 μg l^{-1}
K	1.1 μg l^{-1}
Mg	246 μg l^{-1}
Mn	7.0 μg l^{-1}
Na	1.8 mg l^{-1}

introduction of anthracene. During the early stages of the inoculation processes, the macrophytic angiosperm *Juncus canadensis* was transplanted to the system.

MATERIALS AND METHODS

First, as a control, anthracene (Aldrich Chemical Co.) was added continuously to a lined, uncolonized artificial stream for 5 days (August 18–22, 1980). Then, it was added to a biologically colonized channel for 14 days (September 22–October 6, 1980). The anthracene solution (624 ± 47 mg ml^{-1}, $\bar{X} \pm SE$, $n = 4$ for the uncolonized experiment and 408 ± 5 mg ml^{-1}, $n = 9$ for the colonized experiment) was introduced via a peristaltic pump in pharmaceutical grade ethanol at 2.12 ± 0.1 ml min^{-1} ($\bar{X}$ ± SE, $n = 4$) and 2.27 ± 0.1 ml min^{-1} ($n = 9$). The nominal anthracene concentration was 18.2 μg $liter^{-1}$ for the uncolonized and 12.2 μg $liter^{-1}$ for the colonized study. Both studies had nominal ethanol concentrations of approximately 28 mg $liter^{-1}$. Fresh stock prepared daily was introduced through stainless-steel tubing (except for 15 cm of silicon rubber tubing within the pump) directly into the turbulent mixing zone at the weir.

The streams were divided into five 18.3-m-long reaches for sampling purposes. Samples were taken at 4.6-, 9.2-, and 13.7-m points from the beginning of each reach. For the uncolonized study, samples of water from reach 1, 2, and 5 were taken on days 2 and 4 at dawn (~0630) and 1530 to evaluate photolytic loss of anthracene. Samples of water, sand, and plastic liner were taken from the same reaches at noon on days 1 and 3 and at 1030 on day 5. No samples were taken after the 5-day input.

For the colonized study, samples of water, organic sediment, periphyton, fish, clams, and plastic liner were taken from reaches 1, 3, and 5. Water samples were taken at noon on days 1, 2, 3, and 5 and at dawn on days 2, 5, 8, 15, 16, 17, 19, 23, and 29. Sediments and periphyton were sampled on days 2, 3, 5, 8, 15, 16, 17, 19, 23, and 29 at 0830. Clams, fish, and plastic liner were sampled only from reaches 1 and 5 at 0830 on the same days as the sediment and periphyton.

Water

One- or two-liter background water samples were taken at the weir prior to the addition of anthracene. One milliliter of concentrated HCl was added to each sample and control during the colonized study when recoveries for controls fortified with anthracene and anthraquinone in the uncolonized study were found to be low. The water samples were passed through Amberlite XAD-4 resin (Rhom and Haas, Philadelphia, Pa.), and eluted with ether and acetone following the method of Landrum and Giesy (1981).

The solvents were combined, dried over anhydrous sodium sulfate, and reduced to ~200 μl by a combination of rotary flash evaporation and evaporation under a stream of nitrogen. The chrysene internal standard was added to each sample in 2.0 ml acetonitrile and the samples were analyzed by high-pressure liquid chromatography (HPLC). (See Quantitation.)

In the uncolonized study, recoveries for the controls were 67.5 ± 9.7% ($\bar{X}$ ± SE, $n = 7$) for anthracene and 89 ± 5% ($n = 6$) for anthraquinone. These values were considerably lower than those for samples fortified in the laboratory, 81.1 ± 0.9% ($n = 3$) for anthracene and 97.6 ± 2.2 ($n = 3$) for anthraquinone. The additional handling and transport time (30 min) at high temperature (~34°C) from the channel's mi-

crocosms to the laboratory may have caused the low recoveries. The recoveries from the fortified samples for the colonized study were improved by the addition of HCl to 89.2 ± 2% ($n = 14$) for anthracene and 105 ± 3.4% ($n = 14$) for anthraquinone.

Sediment

Crucibles (3.3 cm deep) containing sand sediments were placed in the sand and sampled in the uncolonized study. One crucible was removed from each station at each sampling time. The interstitial water was removed by vacuum filtration. Samples were extracted in a Soxhlet apparatus for 18 hr with benzene:acetonitrile (65:33 v/v). The sample extracts were dried over sodium sulfate and prepared for HPLC analysis as previously described for water extracts. Prior to the addition of anthracene, blanks and control samples were removed from the stream, filtered, and stored frozen under acetonitrile. Before analysis, controls were fortified with anthracene and anthraquinone.

Organic sediments (~20% organic carbon by weight) for the colonized study were collected at Box Landing, Upper Three Runs Creek, on the SRP. The sediment was sieved through 5-mm stainless-steel screen, mixed, and placed in 15 × 60-mm glass petri dishes. Twelve of the sediment-filled petri dishes were placed at each sampling station in reaches 1, 3, and 5. One petri dish from each sampling station was removed at each sampling time. Three similar sediment control samples from an adjacent channel not receiving anthracene were also taken. The interstitial water was removed by vacuum filtration. The sediment was weighed and a measured subsample (10 g) taken for analysis. Dry weight was determined for the remainder of the sample by drying at 50°C for 1–2 weeks. Each subsample for extraction was mixed with 10 g of granular anhydrous Na_2SO_4, transferred to a Whatman 22 × 80-cm cellulose extraction thimble, and stored under 35 ml of acetonitrile at −20°C. The three control samples were treated identically, except that one sediment was fortified with anthracene and anthraquinone in acetonitrile prior to addition of sodium sulfate.

The sediments and acetonitrile for each sample set were Soxhlet extracted with 65 ml of benzene for 18 hr. The crude extracts were rotary flash evaporated to 2 ml, passed through a 7-cm column of Florisil, and eluted with 3-ml aliquots of benzene and 10 ml of 90% benzene:methylene chloride (9:1 v/v). The samples were prepared for HPLC analysis as previously described for water samples except that the samples were placed in a freezer overnight and precipitates that formed overnight were removed by centrifugation. Anthraquinone could not be quantified from sediments due to an interfering compound.

Periphyton

A constant area of 64.8 cm^2 of periphyton was scraped from the walls of the channel at each sampling station. Three controls and one blank were taken from a nonexposed channel. Water was removed by filtration and a wet weight determined. The dry to wet weight ratio for periphyton was 0.034 ± 0.016 ($n = 11$, $\bar{X} \pm$ SD). A weighted subsample was stored under ethyl acetate:acetone (4:1 v/v) at −20°C. The controls were fortified with anthracene and anthraquinone in 10 μl acetonitrile.

The samples were extracted twice by homogenization in a TenBroeck tissue grinder with 12 ml ethyl acetate:acetone (4:1 v/v) and twice with 12 ml cyclohexane. The extracts were combined and prepared for HPLC analysis as previously described for

water extracts, except that the internal standard was added either in 2 ml acetonitrile or in 1 ml ethanol.

Fish

Juvenile bluegills, *Lepomis machrochirus*, of mean length 5.0 ± 0.5 cm ($\bar{X}$ ± SE, n = 58), mean wet wt 1.2 ± 0.1 g (n = 58), and dry to wet weight ratio of 0.222 (n = 5), were obtained from the National Fish Hatcheries at Millen, Georgia, and Orangeburg, South Carolina. The fish were acclimated for 48 hr in a control channel and then transferred to glass and stainless-steel mesh cages in the experimental channel 12 hr before the infusion of anthracene. Fish were fed Tetra Min staple food flakes daily.

Fish were collected by dip net, weighed, and stored in vials containing ethyl acetate:acetone (4:1 v/v) at −20°C. Fish were homogenized twice with 12 ml ethyl acetate:acetone (4:1 v/v) and twice with 23 ml cyclohexane in a TenBroeck glass tissue grinder. Extracts were combined and prepared for HPLC analysis as previously described for water extracts. Control fish were fortified with anthracene and anthraquinone in 5 μl of acetonitrile.

Clams

Papershell clams, *Anodonta imbecillis*, were collected from Par pond on the SRP. The clams were placed in the channel 13 days before anthracene addition. Individual clams were sampled from each station and weighed. The tissue and all fluid from the animal were blended and a homogenous subsample was taken for analysis. The subsample and shell were weighed. The sample was added to 40–60 ml of anhydrous Na_2SO_4 for desiccation and stored under cyclohexane or cyclohexane:ethyl acetate (1:1 v/v) at −20°C. Three controls fortified with anthracene and anthraquinone and two blanks were prepared in the same manner from an unexposed population.

The sodium sulfate coated with desiccated clam was extracted by shaking twice with 50 ml cyclohexane:ethyl acetate (1:1 v/v) and twice with 50 ml cyclohexane. The solvents were combined and the samples prepared for HPLC analysis as previously described for water extracts.

Plastic Liner

Plastic strips from the middle of reaches 1, 2, and 5 (uncolonized study) and 1, 3, and 5 (colonized study) were sampled with a cork borer that yielded plugs of 4.45 cm^2. Six plugs from each reach were taken at each sampling time and frozen (−20°C) until analysis. The plastic was extracted by twice shaking 5 min in 20 ml ethyl acetate:acetone (4:1 v/v). The samples were prepared for HPLC analysis as previously described for water extracts.

Weather and Sunlight

Air temperature, water temperature, and general weather conditions were recorded at each sampling time. Solar radiation was determined with a Belfort Instrument Company Pyranograph Cat. No. 5-3850A located approximately 5 miles southwest of the stream site.

Quantitation

Varian 5000 HPLC with a 254 fixed-wavelength uv detector was used to analyze for anthracene and anthraquinone. Separations were made with a 30-cm Micro-pak MCH-10 reverse-phase column equipped with a guard column of Whatman Co-Pel C_{18}ODS on 35-μm particles. The separation gradient was from 45% acetonitrile in water to 90% acetonitrile with a flow rate of 1 ml min^{-1}. Acetonitrile (90%) was pumped for an additional 5 min to elute late-eluting interferences before recreating initial conditions. The sediments required a gradient to 100% acetonitrile to remove late-eluting compounds.

The recovery and precision for each of the matrices were determined by applying the proposed method to fortified samples (Table 2). Many of these studies were performed with [^{14}C]anthracene. The storage stability was checked by storing fortified samples the same length of time as the samples.

The background determinations were made from samples analyzed at the same time as the environmental samples. The limit of detection was defined as 3σ + background (Table 2). The linearity of the analysis was determined for each sample set by generating a standard curve.

The accuracy of the analysis was determined by analyzing a standard PAH mixture, Alltech (PAH-I), which contained anthracene. The relative amount of anthracene determined over several months was 96.5 ± 8% (n = 10). All data reported are corrected for background but not for extraction efficiency.

Statistical Methods and Models

Rate constants for accumulation and elimination and steady-state concentrations from each geological and biological matrix were estimated by least-squares fits of the data to first-order, donor-dependent models. Each data set was fit independently by the methods of Giesy *et al.* (1980) and Leversee *et al.* (1982) with a Marquardt procedure (NLIN of the Statistical Analysis System; Barr *et al.,* 1979).

Kinetics of anthracene in each component were calculated by fitting data to equations with the general form

$$\frac{dC_a}{dt} = K_U C_w - K_D C_a, \tag{1}$$

where

K_U = uptake rate constant,

K_D = depuration rate constant,

C_w = concentration of anthracene in the water, and

C_a = concentration of anthracene in the component.

The uptake flux into each component was described as

$$J = C_w K_U. \tag{2}$$

By assuming that the amount of anthracene in the component of interest is small during the initial uptake period, the uptake rate constant was estimated by taking a tangent to the uptake curve (J) and dividing by C_w.

Because C_w is relatively constant for a given period of exposure, the kinetics of anthracene in a given component can be simplified to

TABLE 2

RECOVERY, BACKGROUND, AND LIMIT OF DETECTION OF ANTHRACENE AND ANTHRAQUINONE FROM VARIOUS MATRICES

	Recoveries (%)		Background		Limit of detection	
Matrix	Anthracene[a]	Anthraquinone	Anthracene[b]	Anthraquinone	Anthracene[a]	Anthraquinone
Sediments						
(A) Sand	15.5 ± 2	I[c]	ND	ND[d]		
(B) Organic	71.2 ± 12	I	57 ± 26 ng g^{-1}	ND	134 ng g^{-1}	
Fish	87.7 ± 6	92.3 ± 8	47.6 ng $fish^{-1}$	ND	116.9 ng $fish^{-1}$	
Periphyton	78.5 ± 6	82.2 ± 3	0.68 ± 0.64 ng cm^{-2}	ND	2.6 ng cm^{-2}	
Clams	78.9 ± 2	93.2 ± 3	103 ± 70 ng g^{-1} dry wt	ND	315.75 ng g^{-1} dry wt	
Water	81.0 ± 2	97.6 ± 4				
Input phase			56 ± 44 ng l^{-1}[b]	322 ± 336 ng hr^{-1}	188 ng l^{-1}	1330 ng l^{-1}
Depuration phase			3.8 ± 8.5 ng l^{-1}	ND	29.3 ng l^{-1}	—
Plastic liner	91.1 ± 7	Not determined	ND	ND		

[a] $\bar{X} \pm 1$ SD.
[b] High background is probably due to volatility of anthracene at the input.
[c] I = Not determined owing to chromatographic interferences.
[d] ND = Not detected. Instrument detection limit 0.26 ng = signal × 2 (noise level).

$$C_a = \frac{K_U}{K_D}(C_w)(1 - e^{-K_D t}), \quad (3)$$

where t = time.

C_w approaches steady state as $t \rightarrow \infty$. Thus, at steady state

$$\text{BCF} = \frac{C_a}{C_w} = \frac{K_U}{K_D}. \quad (4)$$

Equation (4) gives the relationship between the bioconcentration factor (BCF) or steady-state distribution between the water and the animal component of interest. Also, from Eq. (3), the steady-state concentration can be predicted when C_w is constant:

$$C_a = C_{ss}(1 - e^{-K_D t}), \quad (5)$$

where C_{ss} = steady-state concentration in the component.

To test for biases in fitting the equation to estimate KD during only the uptake period, the concentration of anthracene in each component was followed during the depuration period, when no anthracene was added to the channels. Estimations of multiple depuration rate constants were made by fitting the observed data to

$$C_{A_t} = \sum_{n=1}^{n} C_{ai} \cdot e^{(-K_{D_i} t)}, \quad (6)$$

where

C_{A_t} = concentration in component at time t,

C_{ai} = initial concentration in ith component,

i = individual depuration component,

K_{D_i} = depuration rate constant for ith component, and

n = number of independent depuration components.

RESULTS

Weather

The weather during the uncolonized experiment was generally clear, with water temperature ranging from 22 to 25°C in reach 1 and from 23 to 29°C in reach 5. The winds were generally light (<4.5 m $\sec^{-1}$). The sunlight was very intense during the day and reached a maximum of 1.2 g cal cm^{-2} min^{-1}. The length of measurable daylight averaged approximately 12 hr.

The weather during the colonized experiment varied more because the experiment was longer. Morning water temperature ranged from 20 to 23°C in reach 1 and from 18 to 23°C in reach 5. Afternoon water temperature ranged from 20 to 22°C in reach 1 and from 22 to 27°C in reach 5. The winds were generally light (<4.5 m $\sec^{-1}$). The sunlight ranged from 0.2 to 1.1 g cal cm^{-2} min^{-1}, with an average maximum most days of 0.9 g cal cm^{-2} min^{-1}. The length of measurable daylight was approximately 11 hr.

Plastic Liner

Accumulation of anthracene by the PVC liner was linear with time during the anthracene input (Fig. 1). After inputs ceased, anthracene desorbed slowly. The uptake

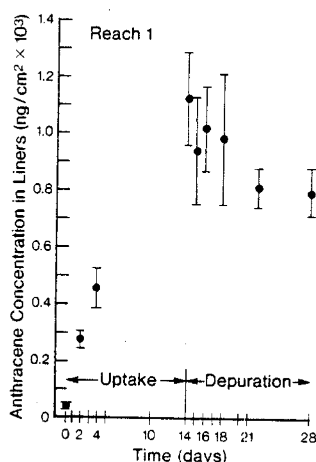

FIG. 1. Concentrations ($\bar{X}$ ± SD) of anthracene associated with plastic liner during the two microcosm studies (n = 6).

rate constant (0.28 ml cm^{-2} hr^{-1}) was estimated with a constant infusion model [Eq. (2)] for the PVC based on the average dawn water concentration corrected for recovery. A first-order decay model was used to estimate desorption of anthracene from the PVC as having a half-life of 1155 ± 231 hr ($\bar{X}$ ± SE, n = 3). No anthraquinone was detected in the plastic liner.

Because the possible loss to the PVC liner was estimated by exposing uncolonized PVC strips, these estimates are conservative and represent a maximum expected loss to the liner. At the end of the 5-day uncolonized study, the plastic had absorbed approximately 0.183 g (2.3%) of the total 5-day anthracene input (7.96 g). The plastic liner likely contributed even less in the colonized study because the surface was covered by periphyton. In either case, sorption to plastic could not be considered an important loss pathway, on a mass basis, for removal of anthracene from water under a flowing water constant input regime. More importantly, the plastic liner should not interfere significantly with the other removal pathways for dissolved anthracene.

Water

Nominal anthracene concentrations in water were not obtained. After correcting for recovery, some loss of anthracene not accounted for by the sum of the anthracene and anthraquinone (on a molar basis) occurred between the input and the first sampling station. The average accountability, based on the number of moles input compared to anthracene measured in reach 1, was 76 ± 5.3% and ranged from 48 to 125%. Part of this loss may have been due to volatilization occurring in the turbulent mixing zone as indicated by the high background samples (Table 2). The average anthracene concentration measured at reach 1, station 1, below the input in both colonized and uncolonized studies, was 11.7 ± 2.5 μg $liter^{-1}$ ($\bar{X}$ ± SD, n = 13). In the absence of photolysis, the anthracene concentration in the water was at steady state within 24 hr in both the colonized and uncolonized studies (Tables 3 and 4). In reaches 2, 3, and 5, samples taken at noon and 1530 hr had lower concentrations of anthracene than samples taken in the early morning (Tables 3 and 4). The loss rate constant for

TABLE 3

CONCENTRATION OF ANTHRACENE AND ANTHRAQUINONE IN WATER FOR UNCOLONIZED STUDY

Day	Time	Reach 1		Reach 2		Reach 5	
		Anthracene ($\mu g\ l^{-1}$)	Anthraquinone ($\mu g\ l^{-1}$)	Anthracene ($\mu g\ l^{-1}$)	Anthraquinone ($\mu g\ l^{-1}$)	Anthracene ($\mu g\ l^{-1}$)	Anthraquinone ($\mu g\ l^{-1}$)
1	12:00 PM	7.7 ± 0.3	2.4 ± 0.3	3.1 ± 0.1	3.9 ± 2	1.8 ± 0.1	2.4 ± 0.7
2	8:00 AM	7.7 ± 0.4	0.09 ± 0.05	8.2 ± 0.3	0.08 ± 0.03	7.9 ± 0.2	0.08 ± 0.05
2	3:00 PM	5.3 ± 0.8	0.8 ± 0.4	3.4 ± 0.3	2.1 ± 0.5	1.7 ± 0.3	1.7 ± 0.3
3	12:00 PM	4.9 ± 1.0	3.0 ± 0.4	3.4 ± 1.0	3.1 ± 0.4	1.3 ± 0.1	2.7 ± 0.3
4	8:00 AM	15.8 ± 13.3	0.8 ± 0.5	13.1 ± 0.3	0.6 ± 0.3	11.7 ± 1.3	0.3 ± 0.08
4	3:00 PM	14.1 ± 12.2	3.6 ± 1.2	4.7 ± 0.6	4.6 ± 1.4	0.9 ± 0.2	3.0 ± 0.9
5	10:30 AM[a]	5.2 ± 3.4	1.5 ± 0.6	9.9 ± 1.5	4.9 ± 1.5	9.0 ± 0.2	4.8 ± 1.2

[a] All values are means ± SD of three sampling stations in each reach. Corrected for background only.

TABLE 4

CONCENTRATION OF ANTHRACENE AND ANTHRAQUINONE IN WATER FOR COLONIZED STUDY

Day	Sample time	Reach 1 Anthracene ($\mu g\ l^{-1}$)	Reach 1 Anthraquinone ($\mu g\ l^{-1}$)	Reach 3 Anthracene ($\mu g\ l^{-1}$)	Reach 3 Anthraquinone ($\mu g\ l^{-1}$)	Reach 5 Anthracene ($\mu g\ l^{-1}$)	Reach 5 Anthraquinone ($\mu g\ l^{-1}$)
1	None	11.1 ± 1	1.4 ± 0.7	6.6[a]	6.4[a]	3.9 ± 0.3	3.6 ± 0.3
2	Dawn	11.6 ± 1	1.4 ± 0.3	10.8 ± 1	2.0 ± 0.2	10.0 ± 0.5	3.2 ± 1
2	Noon	9.9 ± 1	1.8 ± 1	5.3 ± 0.6	4.2 ± 1.7	3.4 ± 0.1	2.9 ± 0.2
3	Dawn	8.8 ± 0.6	0.1 ± 6	8.2 ± 0.3	0.1[a]	8.2 ± 0.4	0.3 ± 0.2
5	Dawn	12.1 ± 0.6	0.2[b]	14.4 ± 0.7	0.8 ± 0.7	13.7 ± 0.5	1.0 ± 0.1
5	Noon	12.1 ± 1.7	1.5 ± 0.2	7.6 ± 0.7	3.7 ± 0.2	3.7 ± 0.4	3.1 ± 0.8
8	Dawn	10.8 ± 1.2	0.2[b]	9.9 ± 1.0	0.4[a]	9.3 ± 1.1	0.2[b]
8	Noon	10.8 ± 1.9	0.6 ± 0.4	8.5 ± 0.3	1.6 ± 0.3	7.0 ± 0.5	1.6 ± 0.4
15	Dawn[c]	9.8 ± 0.3	0.03[a]	9.2 ± 0.1	0.04[a]	8.9 ± 0.2	0.01[a]
16	Dawn[d]	0.007 ± 0.005	0.2[b]	0.01 ± 0.008	0.02	0.07 ± 0.02	0.2 ± 0.08
17	Dawn[d]	0.06 ± 0.02	0.2 ± 0.07	0.04 ± 0.007	0.24[a]	0.01 ± 0.01	0.2 ± 0.05
18	Dawn	0.03[b]	0.01[b]	0.02[b]	ND	0.01[a]	0.01[b]
22	Dawn[d]	ND	ND	ND	ND	ND	ND
28	Dawn	ND	0.5[b]	ND	0.07[b]	ND	0.2[b]

Note. All values are means ± SD of three sampling stations in each reach corrected for background only.
[a] Compound detected at only two sampling stations or one sample lost.
[b] Compound detected at only one sampling station.
[c] Input terminated after sampling.
[d] No anthracene input.

all samples taken at noon and in the afternoon (for the uncolonized study) was 0.016 ± 0.006 ($\bar{X}$ ± SD, n = 4) min^{-1}, translating to a half-life of 43.3 ± 12 min ($\bar{X}$ ± SD, n = 4), similar to the rate measured by Zepp and Schlotzhauer (1979) for the photolysis of anthracene.

Anthracene and anthraquinone concentrations were estimated for the day 1 noon sample for the uncolonized study (Table 5), assuming that anthraquinone was the only photoproduct produced from anthracene photolysis, using the simple first-order decay rate constant for apparent anthracene photolysis found in the streams experiment and the predicted photolytic rate constant from FOAM (Bartell *et al.,* 1981) for anthraquinone. These estimates suggest that the variability in the anthraquinone concentration down the channel during the daylight was probably due to photolysis of the anthraquinone (Tables 3 and 4).

Sediments

The rate constants estimated for uptake and depuration of anthracene from sediments were not significantly different among the three reaches (Tables 6 and 7 and Fig. 2). Mean concentrations of anthracene in the sediments varied proportionately with time-averaged concentrations in the water. This behavior supports our assumption of first-order kinetics in estimation of K_U and K_D for sediments. Since this is a diffusion process, it is not surprising that the behavior of anthracene is described by first-order kinetics.

Removal from the sediments also appeared to be first order with respect to concentration in the sediments (Tables 6 and 7 and Fig. 2). Because the accumulation rate should be a function of the rate of diffusion into the sediments, we normalized the concentration of anthracene in the sediments on both a weight (Table 6) and an areal basis (Table 7). The relative variabilities of the K_U, K_D, and C_{ss} values from the three reaches were slightly less when normalized to area (Tables 6 and 7).

Periphyton

Concentration of anthracene in the periphyton community, normalized to both area and wet weight, showed similar trends (Table 8). As with the sediments, the variation was generally less when concentrations were reported on an areal basis.

TABLE 5

COMPARISON OF ANTHRACENE AND ANTHRAQUINONE PREDICTED CONCENTRATIONS VERSUS MEASURED CONCENTRATIONS FOR PHOTOLYTIC LOSS DOWN THE CHANNEL

	Anthracene[a] (μg l^{-1})		Anthraquinone[b] (μg l^{-1})	
Reach	Predicted	Measured	Predicted	Measured
1	10.3	12.4	2.1	4.1
2	6.4	7.3	3.7	6.3
5	1.5	4.3	3.0	3.6

[a] Anthracene initial concentration 13.1 μg l^{-1} for day 1 noon sample; rate constant 0.016 min^{-1}.

[b] Anthraquinone rate constant 0.018 min^{-1} step calculation.

TABLE 6

FIRST-ORDER RATE CONSTANTS FOR UPTAKE AND RELEASE OF ANTHRACENE FROM ORGANIC SEDIMENTS DURING THE CHANNELS MICROCOSM EXPERIMENT BASED ON DRY WEIGHT OF SEDIMENT

	Reach 1	Reach 3	Reach 5
$K_U{}^a$	1.3 ± 0.093	1.4 ± 0.13	1.4 ± 0.11
$K_D{}^b$	0.0048 ± 0.0007	0.0049 ± 0.0009	0.0040 ± 0.0007
$K_D{}^c$	0.0022 ± 0.0003	0.0022 ± 0.0004	0.0025 ± 0.0003
$C_{ss}{}^d$	3265 ± 266	2980 ± 298	2917 ± 306

Note. Data were fit by the Marquardt iterative least-squares procedure; $\bar{X}$ ± SE, $n = 3$, $P_F < 0.001$ for all regressions.
[a] Expressed as ml g^{-1} hr^{-1}.
[b] Expressed hr^{-1}, assuming density of sediment is near 1, estimated from data collected during anthracene input.
[c] Expressed hr^{-1}, estimated from data collected during period of no anthracene input.
[d] Expressed as ng anthracene · g sediment, dry wt.

Concentrations generally increased during the first 48 hr of exposure. Periphyton concentrations in reach 1 were greater than those in reach 3 and approximately four times greater than those in reach 5. The concentration gradient remained consistent at all sampling times during anthracene exposure. Uptake of anthracene was rapid, and reached apparent steady state within 168 hr.

Desorption of anthracene was also rapid (Table 8). The anthracene concentration was not significantly different from background after 48 hr of depuration. Thus, the half-time for elimination was less than 1 day. The rapid elimination rate was consistent with laboratory results for two natural periphyton communities (Bruno *et al.*, 1982). Because of the variability of anthracene concentrations in periphyton, no rate constants were calculated.

Removal of anthracene from the water column by periphyton was not significant on a mass basis. After days 4 and 14, 0.09 and 0.04%, respectively, of the total anthracene added to the channels had been removed by periphyton. While this is

TABLE 7

FIRST-ORDER RATE CONSTANTS FOR UPTAKE AND RELEASE OF ANTHRACENE FROM ORGANIC SEDIMENTS BASED ON AREA OF SEDIMENT

	Reach 1	Reach 3	Reach 5
$K_U{}^a$	0.22 ± 0.014	0.27 ± 0.016	0.26 ± 0.019
$K_D{}^b$	0.0034 ± 0.00056	0.0039 ± 0.0005	0.0032 ± 0.0006
$K_D{}^c$	0.002 ± 0.0003	0.0023 ± 0.00033	0.0027 ± 0.00028
$C_{ss}{}^d$	800 ± 86	685 ± 57	680 ± 87

Note. Data were fit by the Marquardt iterative least squares procedure. $\bar{X}$ ± SE, $n = 3$, $PF < 0.0001$ for all regressions.
[a] Expressed as ml cm^{-2} hr^{-1}.
[b] Expressed hr^{-1}, estimated from data collected during period of anthracene exposure.
[c] Expressed hr^{-1}, estimated during period of no anthracene input.
[d] Expressed as ng anthracene · cm^2 sediment.

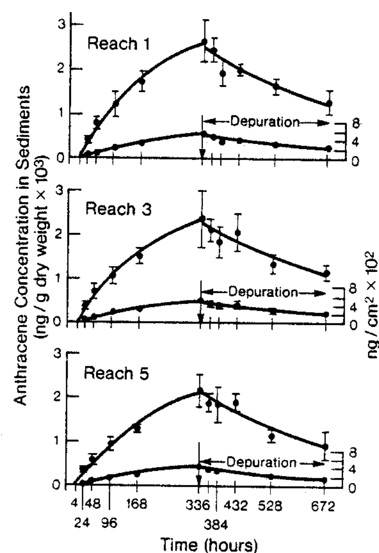

FIG. 2. Uptake and depuration of anthracene by organic sediments on both a weight (upper curve) and areal (lower curve) basis in petri dishes in the channel microcosm.

not a significant reduction of the mass of anthracene added to the water, the periphyton component may be much more important for food web accumulation.

Clams

After 336 hr of exposure to anthracene, papershell clams, *A. imbecillis*, had attained 96% of the projected steady-state concentration of 17,580 ng (g dry wt of soft tissue)$^{-1}$ (Fig. 3). The first-order uptake and depuration rate constants estimated simultaneously during the 336-hr exposure period were 13.99 ± 1.7 ml (g dry wt)$^{-1}$ hr^{-1} (asymptotic

TABLE 8

CONCENTRATION OF ANTHRACENE IN PERIPHYTON[a]

Day	Reach 1		Reach 3		Reach 5	
	ng g^{-1}	ng cm^{-2}	ng g^{-1}	ng cm^{-2}	ng g^{-1}	ng cm^{-2}
1	964 ± 593	3.2 ± 2.0	78 ± 88	1.4 ± 0.2	82 ± 51	2.5 ± 1.4
2	1044 ± 67	13.7 ± 5.0	267 ± 50	3.8 ± 2.1	160 ± 25	3.2 ± 3.1
3	469 ± 242	4.8 ± 1.9	102 ± 20	2.6 ± 1.2	180 ± 98	3.4 ± 2.4
5	589 ± 306	6.1 ± 2.8	173 ± 30	3.7 ± 1.6	62 ± 49	1.6 ± 0.4
7	1057 ± 58	12.2 ± 5.3	565 ± 222	0.8 ± 11	123 ± 88	3.5 ± 0.8
15	622 ± 272	11.4 ± 6.2	165 ± 38	4.6 ± 3.3	299 (n = 1)	9.3 (n = 1)
16	168 ± 69	4.1 ± 1.0	109 ± 73	4.1 ± 2.2		
17[b]						

[a] Data expressed as $\bar{X}$ ± SD.
[b] Samples below limit of detection.

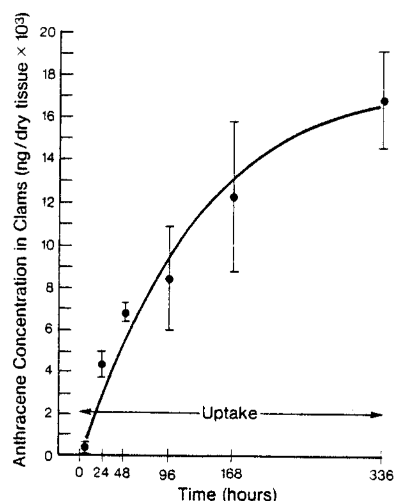

FIG. 3. Mean concentration of anthracene in soft tissues of clams during the period of anthracene input to the channels (n = 6, ±2 SE).

standard error) and 0.008 ± 0.0015 hr^{-1}, respectively. This depuration rate constant corresponds to an elimination half-time of approximately 88 hr. Laboratory studies suggested that the uptake and depuration rate constants should be 83.5 ± 9.6 ml (g dry wt)$^{-1}$ hr^{-1} and 0.21 hr^{-1}, respectively (Giesy *et al.*, 1982). These values would lead to a projected concentration of 4294 ng (g dry wt)$^{-1}$ at 336 hr if the average water concentration of 10.8 ± 1 ng ml^{-1} in reach 1 is used. While projected steady-state concentrations are only different by approximately a factor of 4, the observed uptake curve (Fig. 3) suggests that the kinetics are very different in the microcosm compared to the laboratory studies.

Unlike the other matrices discussed, tissue concentrations did not appear to vary between animals in reaches 1 and 5 even though the downstream animals were exposed to a weighted average anthracene concentration 29.3 ± 8% (weighted mean ± SD) lower than upstream animals. This suggests that the clams may preferentially filter during the night and early morning when water concentrations are at or near steady state, resulting in equal exposure. This is supported by observations that the clams tended to close during periods of most intense light.

Clams succumbed after about 336 hr; this may have been due to nutritional deficiencies, disease, or anthracene toxicity. (See discussion under Fish.) However, in previous studies using these streams, clams were also difficult to maintain; low calcium concentration was the suggested cause (Giesy *et al.,* 1979).

Fish

During the study, the bluegill sunfish died. The first mortality was observed as early as 2 hr after start of the anthracene infusion. After 9 hr of exposure, all of the fish in reach 1 of the experimental streams were dead. In reach 5, farthest from the input, no fish died during the first day of the experiment. All of the fish in this reach were alive at 0800 hr of day 2 of the experiment, but began to die at 1000 hr and

all were dead by 1100 hr. Laboratory studies with bluegill exposed to nearly twice the concentration did not indicate that acute toxicity would be a problem (Spacie *et al.,* 1983).

On the first day in reach 1, bluegills were collected just after death. In reach 5, they were collected after a 4-hr exposure; they were all still alive at that time. Fish were also collected from reach 5 at 0800 hr on day 2 (24 hr after anthracene infusion was initiated and prior to any observed mortality). Subsequent studies showed that the mortality was not due to the ethanol carrier. The total anthracene concentrations in fish in reach 1 after 4 hr was 2389 ng (g wet wt)$^{-1}$ (SD = 275, n = 3). Anthracene concentrations in reach 5 were 457 ng (g wet wt)$^{-1}$ (SD = 182, n = 3) and 7763 ng (g wet wt)$^{-1}$ (SD = 1325, n = 3) after 4 and 24 hr exposure (fish taken prior to any mortality), respectively. The amount of anthracene found in fish was consistent with that predicted from the laboratory kinetics and the water concentration in the streams [uptake constant 41 $\pm$ 3 ml (g wet wt)$^{-1}$ hr^{-1} and depuration rate constant 0.04 hr^{-1}] (Spacie *et al.,* 1983).

From the anthracene concentrations in the fish measured at the times when they died compared to the concentrations in the live fish in reach 5 at the start of day 2, we suspected a photo-induced mortality of anthracene-contaminated fish. Further studies (Bowling *et al.,* 1983) confirmed that light makes anthracene more toxic to bluegills. Additionally, after about 240 hr of anthracene input, the dragonfly larvae in the channels were also dying and, as previously noted, clams succumbed after approximately 336 hr.

DISCUSSION

Overall photolytic degradation appeared to be the major nonadvective process for reducing the anthracene concentration during daylight. Photolysis can account for 100% of the anthracene loss down the stream in daylight, with the other matrices falling within the experimental error of the measured water concentration. Similarly, photolytic degradation will explain the variability in the anthraquinone concentrations downstream. The measured rate constants for these processes are very similar to those predicted by the photolytic submodel in FOAM (Bartell *et al.,* 1981).

Although the major nonadvective sorption processes fell within the error of the measured water concentration, it was possible to estimate their importance. Plastic liner sorption in the uncolonized study accounted for less than 3% of the total anthracene. The periphyton mat and organic sediments (assuming 100% sediment coverage) in the colonized study accounted for only 0.04 and 0.2%, respectively. Therefore, the sediments would be the most important sink for anthracene for a similar natural system on a mass basis.

Losses due to volatilization can be compared to sediment uptake in importance, but since volatilization was not measured, its importance can only be stated to be less than the average error in determination of the water concentration, 7%, less that attributed to the other pathways.

Comparing the actual results with those predicted by FOAM (Bartell *et al.,* 1981) indicated that photolysis was the most important pathway and volatilization less important. The influence of sediments was underestimated by the model by a factor of from 3 to 5, while that of periphyton was overestimated by a factor of 4. However, all simulated concentrations agree within approximately 1 order of magnitude of those measured.

The fate of anthracene in a clear, shallow water stream can be extrapolated from the modeling efforts of Southworth (1979), parameterizing his model to our stream. It appears from his calculations that volatilization would not be very important and that photolysis would dominate the loss of anthracene. Additionally, Southworth (1979) indicates that microbial degradation in the water column should contribute significantly as a loss pathway, approaching the same rate as photolysis. However, this pathway was not considered important for our system, since the loss rate was very close to the photolytic rate determined by Zepp and Schlotzhauer (1979); the same photolytic rate was observed in both the colonized and uncolonized studies and there was no significant preexposure to metabolism-inducing pollutants to enrich the microbial population in PAH degraders. Finally, adsorption to sediments appears to be of the same order of importance in both our findings and those of Southworth (1979).

Likewise, Giddings *et al.* (1979) found photolysis to be the major route of loss, with sorption to sediments the next most important process in a pond microcosm. Therefore, the fate of anthracene appears dominated by photolysis in slow, shallow, clear-water streams, with sorption to bed sediments the second most important non-advective pathway on a mass basis. Sorption to suspended particulates would be expected to dominate under storm events or where there was considerable particulate load from resuspension and runoff input. While such storm events are relatively rare, they should have a profound effect on anthracene distribution.

Anthracene elimination from the various components of the stream was relatively fast. Periphyton required less than 2 days to reach background levels. This rapid elimination would cause the periphyton to track the water concentration of anthracene and could have accounted for some of our experimental variability. Most of the variability may have been due to the collection of varying thicknesses of periphyton mat. Since the anthracene should be transported into the inner cells of a thick mat, slowly by diffusion, but sorb rapidly to the surface, analysis of varying mat thickness prior to steady state would result in dilution of the surface sorbed concentration with increasing mat thickness. This would result in considerable variability in concentration among samples taken at the same time but of varying thickness and may also account for some of the variability in our data. This effect on accumulation was confirmed in laboratory studies (Bruno *et al.*, 1982).

Similarly, stream sediments released anthracene relatively quickly. Such rapid removal of anthracene from a free-flowing environment indicates that anthracene should not remain a long-term hazard in streams. Rather the hazard is displaced to the receiving body of water.

The most surprising result was the acute toxicity of anthracene at microgram per liter exposure levels. Confirmation of our results and investigation of the mechanism was performed by Bowling *et al.* (1983). It is clear, however, that the outdoor microcosm experiment was instrumental in demonstrating the acute toxicity of PAH at a level several orders of magnitude lower than that previously reported (Neff, 1979). However, more study is required to understand the potential of this and other PAH to cause acute and subacute mortality at environmental levels under environmental conditions.

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