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Octanol-Water Partition Coefficient of Benzo(a)pyrene: Measurement, Calculation, and Environmental Implications

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Benzo(a)pyrene (BaP) is a potent carcinogen produced in significant quantities during pyrolysis of such substances as coal, wood, and cigarettes. It also occurs naturally in plants and plant products such as lettuce, fossil fuels, and natural petroleum. Because BaP has a water solubility of only 3.8×10^{-6} g/L (Mackay and Shiu 1977), it preferentially leaves dilute water solutions and concentrates primarily in lipids of animals and in organic material of soil and sediments. Several researchers have shown that the lipophilic storage and soil sediment accumulation of many organic solutes is proportional to the partitioning between octanol-1 and water (i.e., Neely et al. 1974, Karickhoff et al. 1979). The octanol-water partition coefficient (P) is defined as

$$P = C_O/C_W \quad , \qquad (1)$$

where C_O and C_W are the concentration of the solute in n-octanol and water.

Considerable data are available demonstrating that P values measured in the laboratory can be used to predict the environmental behavior of organic pollutants. It is therefore especially important to have P values for organic chemicals such as BaP that are carcinogenic, toxic, or otherwise potentially dangerous. Literature searches reveal that calculated, but not measured, log P values are reported for BaP. The values range from 5.81 (Zepp and Scholtzhauer 1979) to 6.57 (Mackay et al. 1980). This laboratory study was initiated to define better the log P of BaP.

MATERIALS AND METHODS

Benzo(a)pyrene, "Gold Label, 99+% pure," was obtained from Aldrich (Milwaukee, WI), reagent grade octanol-1 from Baker (Phillipsburg, NJ), and high-purity water from Burdick and Jackson (Muskegon, MI); the purity of the BaP and n-octanol was confirmed by high-performance liquid chromatography (HPLC). Burdick and Jackson "Distilled in Glass, HPLC Grade" methanol and methylene chloride were used as needed.

The UV spectra were measured on a Gilford Instruments 2000 spectrophotometer (Oberlin, OH) fitted with a Hewlett-Packard 7225-B plotter (Palo Alto, CA). The HPLC studies were done on a Hewlett-Packard 1084B fitted with a Hewlett-Packard RP8 column. The results were further analyzed by a Hewlett-Packard 3354 computerized data system. Bottles were shaken on a Eberbach model 6150 rotary shaker (Ann Arbor, MI).

All glassware was washed with chromerage, rinsed 11 times with double-distilled water, and oven dried. Caps were fitted with Teflon liners that had been presoaked in methylene chloride, rinsed three times with methanol, and then three times with double-distilled water. Plastic tips for the micropipetters were rinsed three times with the appropriate solvent before use except those that were used to remove an octanol layer or prepare an extract for analysis. These were used directly to avoid dilution of the samples.

Because BaP is a potent carcinogen, it was weighed, handled, and disposed of with appropriate care. A BaP stock solution of 1.54 g/L in n-octanol was prepared, 0.3 to 0.5 mL added to centrifuge bottles containing 112- or 212-mL water, and the mixture shaken at 150 rev/min at 23°C. Samples 1, 2, 5, and 6 were shaken for 1 d, samples 3 and 4 for a total of 3 d. Samples were centrifuged to facilitate separation of the two layers, and the n-octanol layer was removed for analysis. The n-octanol fraction was diluted 1000:1 for spectrophotometric analysis and 100:1 for HPLC analysis.

The surface of the water was carefully cleaned by gently flooding (washing) with octanol, which was then quickly removed and discarded. Either 100 or 200 mL of the water layer was transferred to a clean bottle, and then extracted with 0.5 mL of n-octanol. The octanol extracts of the water layer were analyzed as collected or diluted 1:2 with water-saturated n-octanol when the volume was insufficient for analysis.

The BaP concentrations in the n-octanol layer and the n-octanol extract of the water layer were determined by comparison to known standards. The UV spectra of the BaP found in the octanol and water layers were confirmed by comparing them with that of a second source and to UV maxima reported in the literature (Fechner and Seifert 1979). Data obtained using the UV spectrophotometer were quantified from the absorbances at 285.0 and 297.5 nm. The HPLC separations were made using a water-methanol gradient; peak areas were used to calculate concentrations by separate analyses at 254, 296, and 365 nm. (To avoid confusion in the discussion section, the UV spectrophotometer maxima at 297.5 will be considered the same as 296 nm for ease of comparison with HPLC values.) The accuracy of the individual measurements on the UV spectrophotometer was $\pm$0.02 and on the HPLC, $\pm$0.03 log units.

RESULTS AND DISCUSSION

The log P values calculated from the spectrophotometer measurements ranged from 5.85 to 6.12 and those from HPLC measurements on fresh samples from 5.88 to 6.04 (Table 1). Water layer concentrations were always lower than the BaP solubility limit of 3.8×10^{-6} g/L determined by Mackay and Shiu (1977). There were differences in water layer concentrations because the relative volumes of water, n-octanol, and stock solution were not constant. Samples 1 and 2 were analyzed by HPLC after they were exposed to UV irradiation in the spectrophotometer. An increase in the peak height at 266.5 nm and decreases in peak heights at 285.0 and 297.5 nm were observed in the water-layer octanol extract with UV irradiation (Fig. 1). Also, the concentrations in the water layer measured by spectrophotometry were roughly double that measured by HPLC for samples 1 and 2 (Table 1). Therefore, these values are reported, but not included in the average.

The possibility that other UV-absorbing compounds were present and affecting the log P values obtained was evaluated by comparing the log P values calculated using the BaP peak area on the HPLC spectrum with that obtained using the sum of all the peak areas. The amount of BaP found in the BaP peak area in an analysis performed at the 296-nm wavelength accounted for >98% of the sum of all peak areas. For example, for sample 4, a value of 6.04 is obtained using only the BaP peak areas, whereas a value of 6.05 is obtained using the total peak areas. These data indicate that little or no interference from contaminants is occurring at 296 nm.

Because preferential absorbance of BaP is maximum at 296 nm, log P calculated from data obtained at this portion of the spectrum should be the most accurate. Excluding the values obtained by HPLC for samples 1 and 2 because of possible degradation, we obtained an average log P of 5.99 with a standard deviation of ± 0.08 at 296 nm from HPLC and UV spectrophotometer-derived concentrations. We rounded this value to 6.0 ± 0.1.

We calculated log P values for BaP using formulas and data in the literature to compare with those reported previously (Zepp and Scholtzhauer 1979, Mackay et al. 1980, Yalkowsky and Valvani 1979). (See Table 2.) Many of the calculated values are much higher than our measured values. Chiou et al. (1982) report similar findings for two pesticides with high octanol-water Ps. They report a measured value of log P for DDT of 6.36 in contrast to that calculated by Mackay et al. (1980) of 7.48. Chiou et al. (1982) suggest that the solubility of DDT is increased in water by the presence of octanol dissolved in water. They report the same trend in measurements with hexachlorobenzene (HCB), which has a measured log P of 5.5. Because the solubility and log P of BaP are also in the same range as DDT and HCB, we may expect the same trends for BaP.

Table 1. Measured log P results at 23°C.

Method and date	Sample number	Concentration water layer (µg/L)	Values at 285.0 mn	Values at 297.5 nm	Number days shaken
UV analysis					
8-82	1	1.5	5.96	6.06	1
	2	2.2	6.00	6.05	1
9-82	3	3.2	5.85	5.92	3
	4	3.2	5.91	5.91	3
10-82	5	1.5	6.05	6.12	1
	6	2.1	5.88	5.93	1
Average			5.94	6.00	

HPLC	Sample number	Concentration water layer (µg/L)	Values at 254 nm	Values at 296 nm	Values at 316 nm	Previous UV exposure
8-82	1	0.79	6.19	6.22	--	yes
	2	1.16	6.15	--	--	yes
9-82	3	3.06	5.89	5.91	5.88	no
	4	2.33	5.97	6.04	5.99	no
Average samples 3 & 4			5.93	5.98	5.94	

NOTE: Average log P at 296 or 297.5 nm = 5.99 (previous UV-exposed samples excluded). Average log P at all wavelengths = 5.96 (previous UV-exposed samples excluded).

Environmental implications of the log P of BaP are illustrated in its correlation with other properties related to environmental behavior. Because BaP is a potent carcinogen, models describing its environmental fate can be made using the octanol-water partition coefficient to estimate the bioconcentration factor (BCF) and soil-sediment absorption coefficient corrected for the organic content (K_{om} or K_{oc}). Because the BCF and K_{om} can be considered as organic solvent-water partition coefficients, any pair of these are related by a log-log relationship between partition coefficients (i.e., Neely et al. 1974, Karickoff et al. 1979). According to Briggs (1981), these relationships allow predictions of unknown partitioning functions within an order of magnitude.

Bioconcentration is a measure of the concentration of a compound in the protein and lipid material in contrast to the surrounding aqueous medium. Briggs (1981) predicts that for nonhydrogen-bonding compounds, the lipids are better solvents and act essentially the same as octanol as concentrating agents. He predicts a smaller log BCF (4.18) for proteins because they have water dissolved in them.

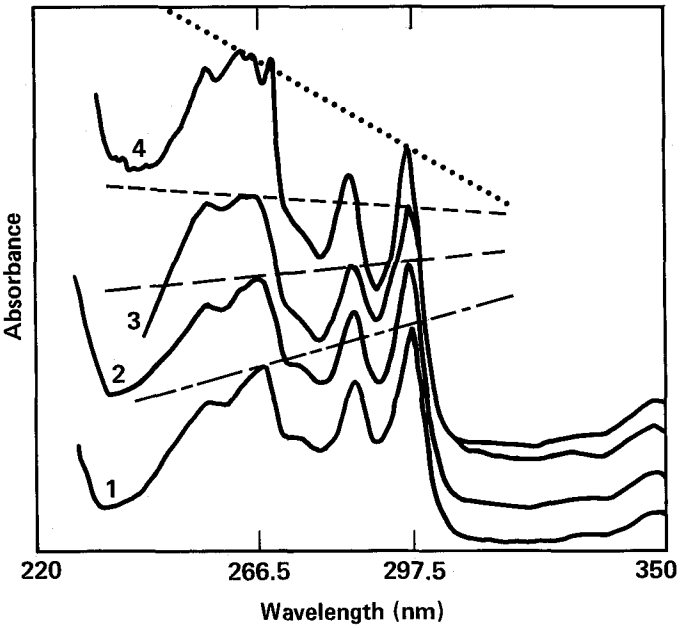

Figure 1. Changes in BaP UV spectra: (1) octanol layer, (2) water layer extracted after 1 d, (3) water layer extracted after 3 d, and (4) water layer extract that was UV exposed the previous day. Note increase in peak height at 266.5 nm with exposure relative to 297.5 nm. These two peaks for each layer are connected by broken lines.

Because organisms have both proteins and lipids as well as other matter, empirically determined formulas for whole organisms are varied. Using the equilibrium BCF formula by Neely et al. (1974) for trout muscle, we calculate a BCF of 3.4 for BaP. After 1-d exposure, a log BCF of 2.37 can be calculated from the data of Neff and Anderson (1975) for BaP uptake in whole clams. They found the highest concentrations in the clam viscera throughout the uptake and depuration studies. Different parts of the clams would, therefore, have different BCFs. Some BCFs for BaP are questionable because of the method used to measure BaP concentrations. Because BaP metabolizes at different rates in different species, the concentration of ^{14}C, for example, may represent different proportions of BaP and its metabolites. Leversee et al. (1981) measured BCFs of ^{14}C-labeled BaP for bluegills, midge larva, and periphyton. They found steady-state log BCFs for ^{14}C to be 2.07, 2.78, and 3.50, respectively, whereas for BaP alone, the log BCFs were 1.09, 2.22, and 3.45, respectively.

Table 2. Observed, published, and calculated values for log P of BaP.

Log P	Method	Reference
6.00	UV spectrophotometric analysis	This paper
5.98	HPLC analysis	This paper
5.81	Published calculation	Zepp and Scholtzhauer (1979)
6.50	Published calculation	Yalkowsky and Valvani (1979)
6.57	Published calculation	Mackay et al. (1980)
5.99	Calculated by fragment method with pyrene as a model	Hansch and Leo (1979)
5.78	Calculated by fragment method from single carbon fragments	Hansch and Leo (1979)
6.32 to 6.62	Calculated from comparison formula	Mackay et al. (1980)
5.82	Calculated from formula	Karickhoff et al. (1979)
6.22	Calculated from formula	Chiou et al. (1982)
5.93	Calculated from formula	Briggs (1981)
6.14 to 6.56	Calculated from formula	Chiou et al. (1977)

The K_{om} or K_{oc} is similar to BCF with uptake being proportional to the organic content of the soil (K_{om} = 100 x K_d/percent organic material). (The K_{oc} is the same on an organic carbon basis.) We determined from equations listed by Briggs (1981), that the log K_{om} would equal 3.36 to 4.16 for BaP.

Equations unrelated to log P are still needed to predict degradation and metobolism before a complete model is constructed. Several authors have suggested equations to model distribution of a chemical as the result of a spill or constant discharge, which require the parameters discussed above (i.e., McCall et al. 1983).

Even with its low solubility of 3.8 µg/L (Mackay and Shiu 1977), BaP is capable of environmental damage. Eastern mudminnows sustained chromosomal damage when exposed to BaP concentrations of 0.1 µg/L (Hooftman and Vink 1981). Environmental BaP levels of this magnitude are present. The National Research Council, Safe Drinking Water Committee (1980) has indicated that polycyclic aromatic hydrocarbons leach from tar and asphalt linings of water pipes. They reveal studies showing BaP levels from <0.1 to 2.1 µg/L in 15 U.S. cities. Discharges from oil shale and coke by-products were listed as up to 12 µg/L at the discharge point and still up to 1 µg/L, 3500 m downstream (Hooftman and Vink 1981). With BaP levels of this magnitude, the importance of log P in predicting its movements and persistence is apparent.

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