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Bioavailability of Benzo(a)pyrene and Dehydroabietic Acid from a Few Lake Waters Containing Varying Dissolved Organic Carbon Concentrations to *Daphnia magna*

A. Oikari and J. Kukkonen

University of Joensuu, Department of Biology, P.O. Box 111,
SF-80101 Joensuu, Finland

Dissolved organic carbon (DOC) in natural waters consists of a great variety of organic molecules. Some of these molecules have been identified but most of them cannot be identified. This unidentified group of heterogeneous organic macromolecules is considered as humic substances (Thurman 1985). The role of humic substances in water chemistry and in aquatic toxicology is receiving increasing attention. A number of studies have demonstrated the binding of metals (Benes et al. 1976; Giesy 1983) or organic pollutants, e.g., DDT, PAHs, PCBs (Carter and Suffet 1982; Hassett and Milicic 1985; McCarthy and Jimenez 1985a), to dissolved humic substances. However, the role of humic substances in the transport and the fate of organic pollutants is still greatly unknown.

The effects of DOC on the bioavailability of organic pollutants have been demonstrated in several studies (McCarthy and Jimenez 1985b; Leversee et al. 1983; Kukkonen et al. 1989). A decreased bioavailability has been demonstrated in most cases. Both the quantity and the quality of DOC are suggested determinants of this apparent "ecotoxicological buffer" of inland waters worldwide.

In this study, we measured the bioaccumulation of benzo(a)pyrene (BaP) and dehydroabietic acid (DHAA) in Daphnia magna using a wide range of naturally occurring DOC levels. Another objective was to associate the reduced bioavailability with the chemical characteristics of water and DOC.

MATERIALS AND METHODS

Radiolabelled (^{14}C) benzo(a)pyrene (BaP; 29.7 mCi/mmol) was purchased from Amersham (UK) and ^{3}H-dehydroabietic acid (DHAA; 2.3 mCi/mmol) was labelled according to Kutney et al. (1981). All other chemicals were from commercial sources at reagent grade purity.

Lake waters were filtered (Nuclepore, Pleasanton, California; 0.22 μm) after sampling and stored (2 months for Lake Baikal sample, ~2 weeks for the others) in the dark at 4 °C until used in the experiments. Table 1 shows the

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Send correspondence to Dr. A. Oikari at the above address.

chemical characteristics of the waters. Artificial humus-free control water was prepared from distilled and deionized water (DOC < 0.3 mgC/L) with the following inorganic salts added: $CaCl_2 \cdot 2H_2O$, 58.8 mg/L; $MgSO_4 \cdot 7H_2O$, 24.7 mg/L; KCl, 1.1 mg/L; and $NaHCO_3$, 13.0 mg/L (Ca+Mg hardness 0.5 mM or 50 mg $CaCO_3$/L, pH adjusted to 6.5).

DOC in the lake waters was fractionated with XAD-8 resin, using the procedure of Leenheer and Huffman (1979). The bed volume of purified XAD-8 was 5 mL, and an acidified (pH<2, conc. H_2SO_4) sample (150 mL) was run through the column at a flow rate of 1.2 mL/min. The organic carbon fraction that came through the columns was defined as hydrophilic compounds. Hydrophobic acids were next eluted from the column with 0.1 N NaOH, and hydrophobic neutrals were calculated by subtracting the concentrations of hydrophilics and hydrophobic acids from the total DOC. In addition, the water samples were fractionated by using Amico's (Danvers, Massachusetts) ultrafiltration cell (8200) and ultrafiltration membranes with nominal molecular weight cut-offs 100000, 10000 and 1000 daltons. The DOC concentrations were measured by the method of Salonen (1979). UV-VIS spectras of water samples were run with Hitachi (Japan) spectrophotometer (100-80A).

An equilibrium dialysis technique (Carter and Suffet 1982) was used to determine the partition coefficient (K_p) between BaP and DOC. Dialysis tubing (Spectra/Por 6, Spectrum Inc., Los Angeles, California) with a molecular weight cut off 1000 was used. At least three replicate determinations were made. In order to confirm that equilibrium was obtained, a parallel experiment with clean water inside the dialysis tubing was performed. At the end of the experiment the water inside and outside the dialysis tubing was analyzed for ^{14}C-activity using a liquid scintillation counter (Packard Tri-Carb 2000CA; UK). The outside concentration (C_o) was considered to represent the free dissolved organic pollutant, while the difference between the inside and outside concentration (C_p) was considered to represent the pollutant bound to dissolved organic material in the sample. K_p was calculated from the following equation:

$$K_p = C_p/(C_o \cdot DOC)$$

where DOC is concentration of dissolved organic carbon (kg org.carbon/L).

For the uptake experiments, water samples were refiltered (Nuclepore, 0.22 μm), and their pH adjusted to 6.5 with 0.1 M NaOH or HCl. Nominal concentrations of BaP and DHAA were 1 and 15 μg/L, respectively. Daphnia magna were obtained from a culture maintained at the University of Joensuu and were fed a culture of green alga Monoraphidium contortum. Before exposures D. magna were allowed to remain 1 hr in the clean artificial control water. Groups of 20 D. magna (age 7-8 days) were transferred to 250 mL of water with BaP or DHAA. For each sample four replicates were made. Beakers were kept in the dark at 20±1 °C. After 24 hr animals were collected on filter paper, quickly washed with 50 mL of distilled water, blotted dry, weighted in groups of 5 animals, and measured for ^{14}C-activity. Each experiment included a parallel control experiment using artificial humus-free water with similar animals. The equation used to calculate bioconcentration factors (BCF) was:

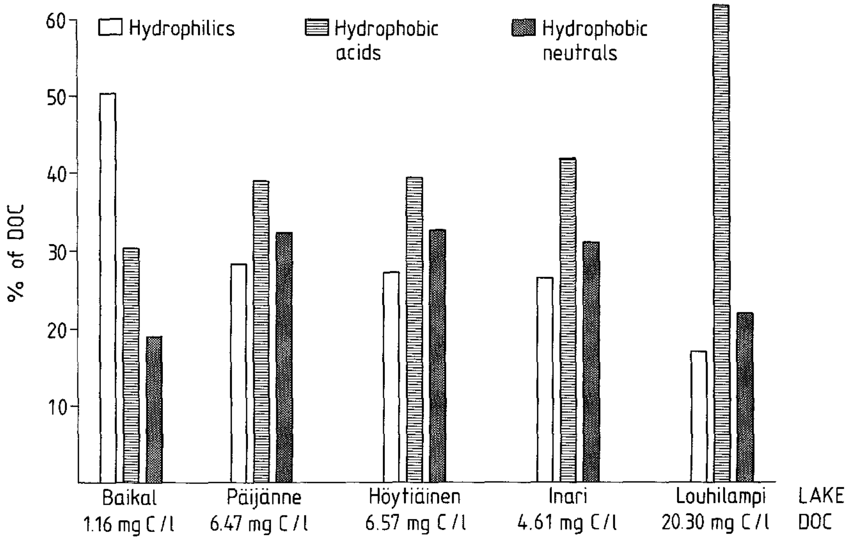

Figure 1. Percentage distribution of dissolved organic carbon between three XAD-8 fractions determined for the five lake waters with different DOC concentrations.

$$BCF = C_a/C_w$$

where C_a and C_w are the concentration of the pollutant in the animals (ng/gwet weight) and in the water (ng/mL) after the experiment, respectively. The concentrations were calculated from the specific activities of the compounds. The predicted BCF values for BaP were calculated using measured K_p values and assuming that the bound fraction of the pollutant cannot be accumulated by the animals. All statistical analyses were performed with using SAS version 5.0 (Statistical Analysis System, Cary, North Carolina).

RESULTS AND DISCUSSION

The percentage distribution of different XAD-8 fractions in Lake Baikal and Lake Louhilampi differed clearly from the three other lakes (Fig. 1). In Lake Baikal 52 % of the DOC was hydrophilic compounds, while in Lake Louhilampi up to 62 % of DOC was hydrophobic acids which are considered as humic material itself (humic and fulvic acids belong to this group), when isolating these materials according the recommended procedures (Thurman and Malcolm, 1981)

The DOC in Lake Baikal water is extremely low and consists of over 50% low molecular weight substances (nominal molecular weight under 1000 daltons) (Table 2). On the other hand, the other four lakes did not differ very much from each others. The absorptivity (ABS = 10^3 x A_{270}/(DOC x cuvette[cm])) of Lake Louhilampi DOC was the highest, whereas the lowest absorptivity was found in Lake Baikal water. The lowest E_2/E_3 ratio (absorbance at 250 nm divided by absorbance at 365 nm) was found in Lake Louhilampi water, possibly reflecting the highest concentration of fulvic acids in this water.

Table 1. General characteristics of waters from the five lakes examined in this study.

Parameter		Baikal	Inari	Päijänne	Höytiäinen	Louhilampi
Country		USSR	Finland	Finland	Finland	Finland
Location: N lat.,		54°00'	69°00'	61°30'	62°45'	62°50'
E long.		107°30'	28°00'	25°30'	29°50'	29°15'
Sampling month (1987)		August	October	September	September	September
Sampling depth (m)		150	30	30	3	2
DOC	mgC/L	1.2	4.6	6.5	6.6	20.3
Color	mgPt/L	5	35	25	40	250
pH		7.7	7.0	6.9	6.7	4.9
Conductivity	mS/m	9.5	2.5	5.1	4.2	3.0
Hardness Ca+Mg	mM/L	0.45	0.13	0.20	0.23	0.10
Calcium	mg/L	13.0	2.7	4.3	5.4	1.9
Magnesium	mg/L	3.1	1.6	2.2	2.2	1.4
Potassium	mg/L	0.8	0.3	1.1	1.2	0.3
Zinc	mg/L	0.04	0.07	0.06	0.05	0.07
Iron	mg/L	N.D.	0.10	0.06	0.07	0.78

N.D. = not determined

Table 2. Spectral characterization and percentage distribution of DOC in ultrafiltration fractions (nominal molecular weight in daltons of used filters) in five lake waters.

	ABSORBANCE			ULTRAFILTRATION (% of DOC)			
Lake	ABS	E_2/E_3	E_4/E_6	$>10^5$	10^5-10^4	10^4-10^3	$<10^3$
Baikal	16.3	4.4	2.0	na	na	na	50.9
Inari	39.9	4.3	4.3	4.4	54.9	21.0	19.7
Päijänne	31.1	5.8	3.7	0.3	47.8	40.2	11.7
Höytiäinen	36.5	5.3	4.0	2.5	58.1	30.3	9.1
Louhilampi	55.1	4.1	4.9	3.6	58.0	30.6	7.8

ABS = absorptivity at 270 nm (units of $L/mgC \times cm \times 10^3$), E_2/E_3 = ratio of absorbances at 250 to 365 nm, E_4/E_6 = ratio of absorbances at 465 to 665 nm, na = not analyzed

According to de Haan (1983), E_2/E_3 values close to four indicate fulvic acids. Lake Baikal water has the lowest E_4/E_6 ratio (absorbance at 465 nm divided by absorbance at 665 nm) and Lake Louhilampi has the highest ratio. According to Chen et al. (1977), a high E_4/E_6 ratio can be related to high molecular weight. Schnitzer (1977) found that the E_4/E_6 value for humic acids and fulvic acids extracted from soils formed under widely differing conditions are in the range of 3.8 to 5.8 and 7.6 to 11.5, respectively. These spectral results suggest the same that can be seen in the molecular weight distribution; DOC in Lake Baikal is composed of low molecular weight compounds, while the other lakes have more high molecular weight compounds, which are typically classified as fulvic acids.

The measured K_p values of BaP to DOC for the five lake waters studied are shown in Table 3. The average K_p value for Lake Päijänne is lower than the others and it differs significantly ($P<0.05$) from the other Scandinavian lakes. The K_p value for Lake Baikal, however, has a large standard deviation, possibly due to very low DOC concentration accompanied by a low molecular weight distribution. In general, measured K_p values fall nicely within the range of K_p values for BaP given in the literature (Landrum et al. 1984; McCarthy and Jimenez 1985a; Kukkonen et al. 1989). Within these few lake waters, K_p was significantly correlated ($P<0.05$) with E_2/E_3 ratio (Table 4), possibly reflecting the importance of fulvic acids in binding organic pollutants.

The bioaccumulation of BaP by D. magna from the five lake waters was inversely correlated with the DOC concentration of the waters (Fig. 2, Table 4). The largest reduction was 84% in Lake Louhilampi water compared to the control. Using the K_p values of lake waters (Table 3), it is possible to predict the accumulation of freely dissolved BaP from water to D. magna. The measured BCF values agreed well with the predicted BCF values (Fig. 3). This implies that BaP bound to DOC was not available to waterfleas.

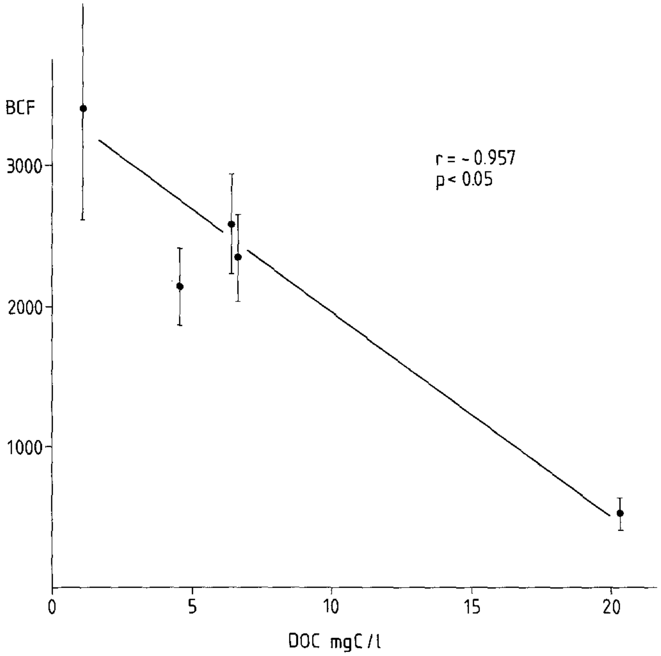

Figure 2. Correlation between accumulation of BaP into D. magna and concentration of DOC. Each point represents the mean of four replicates. Bars are standard deviation of the mean value.

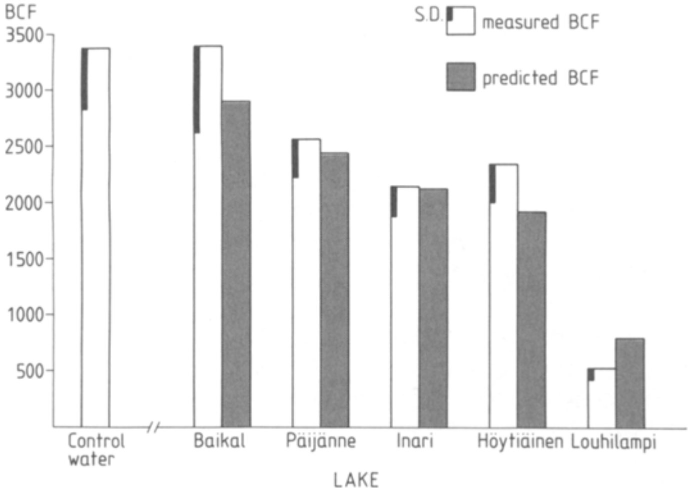

Figure 3. The bioconcentration factors (BCF) in D. magna for BaP in artificial humus-free control water and in five lake waters.

The accumulation of BaP by D. magna was significantly correlated with the parameters describing the quality of DOC in the natural waters (Table 4), including the percentage of hydrophobic acids, absorptivity and E_4/E_6 ratio. All of these correlations suggest that the bioavailability of BaP decreases in waters with DOC having more high-molecular weight hydrophobic acids. Accumulation of DHAA by D. magna was reduced in all five lake waters ranging from 23 % (Lake Inari) to 45 % (Lake Louhilampi), with Lake Baikal

Table 3. Partition coefficients (K_p) of BaP to DOC of five lake waters. The mean values ± S.D. of four replicates are given.

Lake	K_p ± S.D. x 10^4
Baikal	12.7 ± 5.2
Inari	12.7 ± 3.1
Päijänne	5.9 ± 2.1
Höytiäinen	11.3 ± 3.8
Louhilampi	15.3 ± 3.0

Table 4. Correlations between K_p and BCF values for BaP and some parameters which describe the quality of DOC in the lake waters. For details see the text.

	HBA%	ABS	E_2/E_3	E_4/E_6	BCF	K_p
DOC	0.976^{**}	0.891^{*}	-0.260	0.755	-0.957^{**}	0.431
HBA%		0.948^{*}	-0.353	0.843^{o}	-0.995^{***}	0.488
ABS			-0.194	0.969^{**}	-0.971^{**}	0.357
E_2/E_3				-0.084	0.349	-0.921^{*}
E_4/E_6					-0.885^{*}	0.231
BCF						-0.499

Significance: o = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

being intermediate (-31%). This confirms our earlier observations using Finnish waters with high concentrations of humic compounds (Kukkonen and Oikari 1987). The large reduction in BCF obtained in Lake Baikal water may be associated with higher concentrations of water hardness compounds, particularly calcium (Table 1).

We conclude that changes in bioavailability can be predicted from the binding of BaP to DOC in natural lake waters. Although the quality of DOC, in terms of its capacity to bind BaP, was relatively similar in different lakes, there were differences between the lakes with respect to the parameters describing the DOC (Table 2).

Although the quantity of DOC certainly is the most important factor affecting the availability of BaP from natural waters, the proportion of hydrophobic acids in DOC seems to correlate with the bioavailability. In conclusion, the DOC-factor of natural waters is ecotoxicologically very important. Animals in waters with low DOC concentrations are predicted to be more vulnerable to direct water-borne toxicity due to BaP type of environmental chemicals than in waters having high DOC concentrations.

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