# **Bioaccumulation Kinetics and Bioconcentration Factors for Polycyclic Aromatic Hydrocarbons in Tissues of Rasbora Daniconius**

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## Abstract

Bioaccumulation kinetics and bioconcentration factors (BCF) of the Polycyclic Aromatic Hydrocarbons (PAHs) Naphthalene and Anthracene in tissues of fish Rasbora daniconius were studied in detail using a continuous fed system. The process of bioconcentration is summarized using a first order uptake model. The steady state BCF is calculated on the basis of exposure of fish to the PAHs for 30 days. The rate of bioaccumulation was found to be maximum of  $0.331 \ \mu g \ g^{-1}$  wet weight for Naphthalene in intestine tissue and 3.97  $\ \mu g \ g^{-1}$  wet weight for Anthracene in liver tissue in case of Rasbora daniconius. The regression coefficient ( $R^2$ ) between the PAH concentration and exposure time varied between 0.874 and 0.959, indicating high correlation. Based on actual calculated BCF values, the Octanol water partition coefficient ( $K_{ow}$ ) values were predicted. In order to prove the hydrophobic property of PAH compounds and their affinity towards lipid, the  $K_{ow}$  is predicted. Results showed that the PAH burden differs from one tissue to another and that it is possible to correlate the same with the lipid content of the tissue and exposure time in case of either PAH.

**Keywords:** Bioaccumulation; Bioconcentration factor; Polycyclic Aromatic Hydrocarbons; *Rasbora daniconius*; Organ Distribution.

#### Introduction

Bioaccumulation of various xenobiotic pollutants in aquatic organisms gained public attention as early as 1960s as residues of different compounds began to be discovered in fish and other aquatic organisms. The prediction of the extent to which such xenobiotics achieve concentration in biotic phases such as bodies of fish in comparison to the abiotic medium, such as water, and the comparative order of magnitude of such concentration in the biotic phase becomes important to assess the environmental fate



of these substances. Similar to other xenobiotics, PAHs react with lipids at cellular and subcellular levels and may destroy tissue in the long term. Tissue lipid has been found to be an important factor in determining the PAH concentration in fishes <sup>1</sup>.

The bioaccumulation potential of a chemical in aquatic organisms, in addition to their toxicity and biotic as well as abiotic degradation, is an important indicator for assessing environmental damage <sup>2,3</sup>. Literature indicates that bioaccumulation of PAH in aquatic organisms occurs at different concentrations, which depend on the molecular weight of the compound, different feeding habits, habitat, biotransformation capacities of the organisms in relation to trophic levels <sup>4</sup>. PAH concentrations of up to 44.9  $\mu$ g g<sup>-1</sup> wet weight have been found in tissues of fish inhabiting aquatic habitats of high anthropogenic influence in the Arabian Gulf <sup>5</sup>. In other cases, levels of PAH in fish samples have ranged between 1.91 to 224.03 ng g<sup>-1</sup> wet weight, recorded in China, and between 43 and 195 ng g<sup>-1</sup> wet weight, as recorded in Australia <sup>1,6</sup>.

The BCF is an estimate of a chemical's propensity to accumulate in an aquatic animal. Fish are preferred organisms for BCF assessment, since they are important to man as a source of food, and due to the availability of standardized protocols for such assessment. The establishment of a correlation between the hydrophobicity of a chemical and its BCF forms an important basis for BCF assessment. BCFs for heavy metals such as chromium and lead have been calculated for the fish *Colisa fasciatus* and reported in literature <sup>7,8</sup>. Bioaccumulation of different chlorinated pesticides in different fish tissues was also estimated and reported <sup>9</sup>. Bioaccumulation kinetics and bioconcentration factor of chlorinated pesticides in tissues of Puntius ticto (Ham.) have also been calculated and reported <sup>10</sup>. In addition different proposals for calculation of BCF have also been reviewed and reported <sup>11</sup>.

Any chemical with a BCF value exceeding 100 on a wet-weight basis is considered to have a potential to bioaccumulate and is classified as "dangerous to the environment" in the European Union (EU) because it could impair the health of an organism or that of the organisms feeding upon it. A BCF value exceeding 100 has therefore been recommended as a trigger for classification of the chemical as being hazardous, by the European commission, the administrative directorate of the EU. The USEPA considers values of BCF above 1000 as triggers for high concern for potential effects of bioaccumulation <sup>12</sup>, whereas a guideline value of 0.67 ng g<sup>-1</sup> for human consumption <sup>6</sup>, while Canada considers chemicals with BCF values exceeding 500 as hazardous, whereas those above 5000 to be indicative of bioaccumulation and recommends such chemicals for "virtual elimination" <sup>13</sup>.

However, unlike that in developed countries, less work has been carried out on bioconcentration of PAHs and their fate in the Indian environment. Though a thorough analysis of the edible portions of fish is desirable before human consumption, whole fish are also consumed in many cases by fishing communities, people from lower economic strata and by other predatory animals including birds. Thus, the investigation of bioaccumulation of PAHs in whole fish is important from an environmental point of view. An attempt has therefore been made to assess the organ distribution and bioconcentration kinetics of PAHs Naphthalene and Anthracene, on the gill, liver, intestine and kidney tissues of freshwater fish *Rasbora daniconius*.

# **Bioconcentration kinetics**

The bioconcentration process in case of non-biodegradable chemicals can generally be interpreted as a passive partitioning process between body lipids and the water surrounding the organism, so that the process can be aptly described as a first-order two-compartment (water and aquatic organism) model. The conventional equation describing the uptake and elimination of a persistent chemical by aquatic organisms such as fish is given by Equation (1).

$$\frac{dC_F}{dt} = k_1 C_w \cdot k_2 C_F \tag{1}$$

 $k_1$  is the uptake rate constant, day<sup>-1</sup>

 $k_2$  is the elimination or depuriation rate constant, day <sup>-1</sup>

 $C_F$  is the chemical concentration in fish, mg g<sup>-1</sup>

 $C_W$  is the chemical concentration in water, mg g<sup>-1</sup>.

At steady state,  $\frac{dC_F}{dz} = 0$ , and the BCF value can be calculated by using Equation (2).

$$BCF = \frac{k_1}{k_2} = \frac{C_F}{C_W}$$
(2).

The BCF can be estimated by exposure of the fish for an appropriate period to a constant chemical concentration in water by use of a flow through system till the attaining of a steady state by the fish. However, practically, for many hydrophobic chemicals, such an ideal steady state cannot be reached in an appropriate time, and a 'real' BCF value can be estimated using the only available method- the kinetic approach.

# **Materials and Methods**

Continuous bioassay studies were carried out as per standard practice using an indigenously designed constant dosing device <sup>14</sup>. Schematic diagram of the device is shown in Figure 1. Fish required for the experiments were procured from local fresh water bodies. The PAHs Naphthalene and Anthracene used were supplied by Merck, India. The purity of the PAHs was determined in the laboratory using GC-MS, and was found to be 98-99%. Dichloromethane was used as a solvent <sup>15</sup>. The characteristics of the de-chlorinated dilution water are given in Table 1.

The analysis of physico-chemical parameters was carried out as per Standard Methods <sup>16</sup>. The estimation of PAH concentration, a thermo trace ultra Gas Chromatograph (GC) (Thermo Fisher Scientific Instruments, San Jose, CA95134, USA) with electronic flow control (EFC) fitted with a Thermo Fisher Scientific TSQ Quantum GC triple quadrupole Mass Spectrometer (MS) was used. Standard chromatographic procedures were followed for analysis of PAHs as stated in the literature <sup>17</sup>.



Fig. 1. Schematic diagram of the dosing unit





Parameters	Values *
Temperature ° C	25-27
рН	7.5-8.2
Total Alkalinity as CaCO <sub>3</sub>	156-190
Total Hardness as CaCO <sub>3</sub>	142-172
Ca Hardness as CaCO <sub>3</sub>	80-94
Mg Hardness as CaCO <sub>3</sub>	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride as Cl	126

## Table 1: Characteristics of Dilution Water

All the values are expressed as mg/L except temperature and pH.

#### **Tissue Extraction**

Weighed samples of gills, liver, intestine and kidney from the fish were extracted individually as per standard procedure using dichloromethane as a solvent. PAHs, along with other organic compounds, are co-extracted. To prevent the interference of such other organic compounds in the estimation of PAHs,

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a suitable clean-up procedure becomes necessary for their removal. In such a procedure, a glass column is packed with suitable filter aids such as activated charcoal, silicic acid, celite, florisil, alumina, etc. Celite 545 filter aid from Koch Laboratories, U.K. was used for clean-up in the present study <sup>18</sup>.

Glass columns of size  $300 \times 10$  mm, with stop cocks at one end were selected. For each column, 2 g of Celite was first taken in a glass beaker and acidified using 1.2 ml of 0.5 M H<sub>2</sub>SO<sub>4</sub> with constant stirring till all the Celite was moistened. 20 ml of a mixture of Acetone and Hexane in a proportion of 10:90 was added to the moistened Celite and stirred for 2-3 hours. A small wad of glass wool was first washed in acetone and then pressed into the end of the column. Following this, the Celite slurry as prepared above was packed tightly a little at a time above the glass wool by pressing with the help of a glass rod flattened at one end, till the column was filled up to an appropriate height. The column of Celite so prepared was topped by 1g of anhydrous sodium sulphate to remove any traces of moisture from the tissue extract.

After packing, the columns were washed with n-hexane to remove traces of acetone. A fresh column was used for every tissue extract. Tissue extracts were obtained in dichloromethane and transferred into the column slowly by adjusting the flow by stopcock. The Celite 545 filter aid column retains all interfering compounds, including lipids, and only the PAHs are eluted. The eluate was collected in clean glass KD tubes. The column was washed at least 3-4 times after collecting the eluate so that all PAHs in the extract were eluted successfully. This method is known for being capable of recovering 98-99% PAHs from the tissue samples. The eluate was allowed to dry and the same was then diluted with a known quantity of dichloromethane for direct injection into the GC-MS. This method of determination of PAH concentration has also been followed by other workers, as indicated in literature <sup>19</sup>. Philips <sup>20</sup> has recommended expression of residue concentration on the basis of both wet weight and lipid weight.

# **Experimental details**

Experiments were carried out using 20L glass aquariums, for a continuous period of 30 days. The reservoir of the dosing unit was filled with dilution water and the required concentration of PAH was added to it and the volume made up to 20L. The flow from the reservoir was adjusted so that 20 litres of PAH solution flowed through the aquarium in 24 hours. 20 fishes were introduced in each aquarium at the commencement of the experiment. Concentration of Naphthalene in case of *Rasbora daniconius* for the experiment was  $0.6 \text{ mg I}^{-1}$  while that of Anthracene was  $0.016 \text{ mg I}^{-1}$ . These concentrations for the doses were determined on the basis of LC<sub>50</sub> values arrived at after performing acute toxicity tests. Feeding and replacements of fresh solution were carried out as per details mentioned in the literature <sup>21</sup>. Fish were removed alive from the aquarium at intervals of 5,10,15,20, 25 and 30 days for each species and PAH used. Fish were dissected and their tissues- gills, liver, intestine and kidney, were removed and preserved



in aqueous Buoin's fixative at 4°C in the refrigerator. Literature reports that tissue can be preserved in either 4% formalin or Buoin's fixative prior to sample extraction and cleanup <sup>22</sup>. Fixed tissues were thoroughly washed, weighed and blended in a closed vessel using dichloromethane. The extract was then subjected to cleanup through the Celite column.

## **Results and Discussion**

## **Bioconcentration and organ distribution**

The results for the concentration of Naphthalene and Anthracene in different tissues of *Rasbora daniconius* are presented in Table 2.

It was seen that maximum accumulation of Naphthalene in tissue of *Rasbora daniconius* was 0.331  $\mu$ g g<sup>-1</sup> wet weight in the intestine, followed by 0.098  $\mu$ g g<sup>-1</sup>, 0.083  $\mu$ g g<sup>-1</sup> and 0.043  $\mu$ g g<sup>-1</sup> wet weight in kidney, gill and liver respectively, as compared to its aqueous concentration of 0.6 mg l<sup>-1</sup>.

РАН	Exposure	Concentration of PAH, µg/g wet weight								
	time, days	GILL	INTESTINE	KIDNEY	LIVER					
Naphthalene	5	0.010	ND	ND	ND					
	10	0.020	0.001	0.011	0.013					
	15	0.050	0.023	0.042	0.022					
	20	0.070	0.099	0.088	0.033					
	25	0.076	0.201	0.092	0.040					
	30	0.083	0.331	0.098	0.043					
Anthracene	5	ND	ND	ND	ND					
	10	0.440	ND	0.360	0.964					
	15	0.628	0.012	0.610	1.220					
	20	0.771	0.018	0.720	2.236					
	25	0.894	0.026	0.840	2.990					
	30	0.983	0.029	0.920	3.970					

TABLE 2: Concentration of PAH in tissues of Rasbora Daniconus

On the other hand, maximum accumulation of Anthracene was  $3.97 \ \mu g \ g^{-1}$  wet weight in liver, followed by  $0.983 \ \mu g \ g^{-1}$ ,  $0.92 \ \mu g \ g^{-1}$  and  $0.029 \ \mu g \ g^{-1}$  wet weight in gill, kidney and intestine respectively, as compared to its aqueous concentration of  $0.016 \ m g \ l^{-1}$ . From the above observations, it is seen that Anthracene shows higher accumulation in the gill, liver and kidney tissue of *Rasbora daniconius* as



compared to Naphthalene. However, Naphthalene shows higher accumulation in intestine tissue of *Rasbora daniconius*. This substantiates reports in literature that rates of bioaccumulation depend upon several variable factors <sup>4</sup>.

This also clearly indicates that the same compound can accumulate at different rates among different tissues, and that the same tissue accumulates different compounds at different concentrations. However, Khan <sup>23</sup> is of the opinion that accumulation in tissues involves factors other than just the lipophilicity of the compound and the fat content of the concerned tissue. Relations between BCF, log BCF, and calculated log  $K_{ow}$  for *Rasbora daniconius* have been evaluated. After exposure for 5 days, accumulation of Anthracene was seen to be below detectable limits for most tissues.

Maximum BCF and log BCF for Naphthalene showed maximum toxicity in intestine tissue of *R*. *daniconius* after 30 days' exposure, whereas the same for Anthracene was seen only in the liver tissue.

#### **Bioconcentration kinetics**

The BCF is an estimate of the propensity of a chemical to accumulate in an aquatic animal. The phenomenon of bioaccumulation, and the ability of a chemical to build up in living tissue, is recognized as important by the USEPA for establishing effluent standards for toxic pollutants, and for establishing criteria for use by effluent treatment plants. The recent fish and oyster BCF test guidelines by the USEPA <sup>24</sup> and those of the ASTM (American Society for Testing and Materials) <sup>25</sup> reflect the importance of BCF. BCF is usually calculated with the help of the regression equation, and is given by the general formula as per Equation (3)

$$\log BCF = a \log K_{ow} + b, \tag{3}$$

Where a and b are empirically determined constants and  $K_{ow}$  is the n-octanol/water partition coefficient. Veith <sup>26</sup> exposed Fathead minnows, Green Sunfish and Rainbow trout for 32 days in continuous-flow water and calculated the extent of bioconcentration from water. The relationship between log BCF and log  $K_{ow}$  was expressed as per Equation (4)

$$\log BCF = 0.85 \log K_{ow} - 0.70, R^2 = 0.897$$
(4)

The above equation has been applied for developing a correlation between BCF and  $K_{ow}$  tissues of the fish species studied. Accumulation in fish tissues indicates a build-up of the PAH, which follow an uppish trend, divided into two phases. The first phase, of about 15 days from commencement of exposure, shows a rather slow build-up, whereas the second phase shows a rapid build-up. This indicates complexities in the kinetics of the build-up process. It is difficult to delineate such an observation with mathematical equations, taking into consideration the complexities of calculation involved.

BCF have frequently been found to correlate well with hydrophobicity expressed by  $K_{ow}$  and linear relationships have been established on log scale <sup>27, 28, 29, 30</sup>. According to Neely, Branson and Blau <sup>31</sup>, the partition coefficient of a pollutant would be the most logical parameter among different properties

Naphthale

Anthracene

ear (Naphth

of a compound to examine the extent of its bioconcentration by aquatic organisms. Literature reports different trends of bio-concentration <sup>32, 33, 34</sup>. Linear graphs for regression representing the relationship between PAH concentration and exposure time for the complete period of 30 days with respect to both PAHs in gills, intestines, liver and kidney tissues of the fish are shown in Tables 3 and 4 and presented in Figures 2 to 5, respectively.

3.5

3

2.5

2

1.5







LIVER

0.7698x - 0.797

0.9845

daniconius.



Fig.5:Comparative study between exposure time and PAH concentration in Kidney of Rasbora daniconius.

Standard chromatograms for Anthracene and Naphthalene, and few significant chromatograms for bioaccumulation analysis are shown in (Figures 6 and 7) and (Figures 8 and 9), respectively. The rate of bioaccumulation of Naphthalene was found to be maximum of 0.066 µgg<sup>-1</sup> in the intestine, and that of Anthracene a maximum of 0.984  $\mu$ gg<sup>-1</sup> for liver in case of *R. daniconius*. As evidenced by increasing values of BCF, the uptake of PAHs also increased with increase in exposure time. The R<sup>2</sup> values in all cases were greater than 0.9 for both the PAHs studied, other than the exception was Naphthalene, for which the value was 0.874 for intestine indicating high correlation between PAH concentration and exposure time. Actual results obtained from the present study confirm the hydrophobicity of PAH compounds. Moreover, the regression models encompass a large range of BCF and show a high correlation with Kow.











Fig.8: Chromatogram for Naphthalene in Gill tissue of Rasbora on exposure for 20 days



Fig.9: Chromatogram of Anthracene accumulated in intestine tissue of *Rasbora* after exposure for 30 days



	Statistical	GILL	INTESTINE	KIDNEY	LIVER				
РАН	constants	Exposure time 30 days							
Naphthalene	Rate of	0.015	0.066	0.022	0.008				
	bioaccumulation*								
	Intercept	-0.003	-0.123	-0.022	-0.005				
	$\mathbb{R}^2$	0.939	0.874	0.918	0.964				
Anthracene	Rate of	0.813	0.006	0.175	0.769				
	bioaccumulation*								
	Intercept		-0.008	0.04	-0.797				
	$R^2$	0.91	0.959	0.918	0.984				

# Table3: Bioaccumulation kinetics constants for tissues of fish Rasbora daniconius

 $\mu g g^{-1}$  wet weight of the tissue

## Table4: Calculated BCF, log BCF and predicted log $K_{\text{ow}}$ values for tissues of Rasbora

PAH		GILL					INTESTINE			KIDNEY		LIVER			
Naphthalene	Exposur e time, davs	BCF	lc B	og SCF	log Kow	BCF	lo	g BCF	log Kow	BCF	log BCF	log Kow	BCF	log BCF	log Kow
	5	16.67		ID	ND	ND	N	D	ND	ND	ND	ND	ND	ND	ND
	10	33.34		.523	2.61	5 1.67	0.	222	1.085	18.34	1.263	2.309	21.66667	1.336	2.395
	15	83.34	1	.921	3.084	4 38.34	l 1.	584	2.687	70.00	1.845	2.994	36.66667	1.564	2.664
	20	116.6	72	.067	3.25	5 165.0	)0 2.	217	3.432	146.67	2.166	3.372	56.33333	1.751	2.884
	25	126.6	72	.103	3.298	335.0	)0 2.	525	3.794	153.34	2.186	3.395	66.66667	1.824	2.969
	30	138.3	4 2	.141	3.342	2 551.6	57 2.	742	4.049	163.34	2.213	3.427	71.66667	1.855	3.006
PAH			GILL				INTESTINE			KIDNEY			LIVER		
Anthracene	Exposure days	time,	BCF	log BC	lo F	og Kow	BCF	log BCF	log Kow	BCF	log BCF	log Kow	BCF	log BCF	log Kow
	5	ND		ND	Ν	ID	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10	27500		4.4	39 6	.046	ND	ND	ND	22500	4.352	5.944	60250	4.78	6.447
	15 3925		39250	4.5	94 6	.228	750	2.875	4.206	38125	4.581	6.213	76250	4.882	6.567
	20		48187.	.5 4.6	83 6	.333	1125	3.051	4.413	45000	4.653	6.298	139750	5.145	6.876
	25		55875	4.7	47 6	.408	1625	3.211	4.601	52500	4.720	6.376	186875	5.272	7.026
	30		61437.	.5 4.7	88 6	.456	1812.5	3.258	4.656	57500	4.760	6.424	248125	5.395	7.171

# Conclusion

An important feature apparent from the present study is that bioaccumulation was relatively a slow process for all the PAH concentrations tested, and the degree of accumulation varied from tissue to tissue. Both species of fish tested accumulate PAHs under chronic bioassay conditions, and bioconcentration is a function of time and the sublethal concentration in the exposed medium. Anthracene was seen to be significantly more toxic as compared to Naphthalene, as is evident from the concentrations

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accumulated over a period of 30 days. PAHs even at minimum concentrations in the aquatic environment tend to accumulate in fish tissues over long exposure periods, indicating the onset of chronic toxicity.

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