OPEN ACCESS

Int. Res. J. of Science & Engineering, 2017; Vol. 6 (2): 43-53
ISSN: 2322-0015

http://www.irjse.in
UGC Approved Journal No. 63628

RESEARCH ARTICLE

A study on the bioaccumulation kinetics and bioconcentration factors of few polychlorinated biphenyls in freshwater fish *Rasbora daniconius*

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Manuscript Details
Received : 24.03.2018
Accepted: 15.04.2018
Published: 19.04.2018

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Bhagade Advait. A study on the bioaccumulation kinetics and bioconcentration factors of few polychlorinated biphenyls in freshwater fish *Rasbora daniconius*. *Int. Res. Journal of Science & Engineering,* 2018, 6 (2): 43-53.

ABSTRACT

Bioaccumulation kinetics and bioconcentration factors (BCF) of the Polychlorinated Biphenyls (PCBs) PCB 126 and PCB 169 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 PCBs for 30 days. The rate of bioaccumulation was found to be maximum of 64.68 µg g-1 wet weight for PCB 126 and 7.12 µg g-1 wet weight for PCB 169 respectively in gill tissue in Rasbora daniconius. The regression coefficient (R2) between the PCB concentration and exposure time varied between 0.532 and 0.994, indicating good to high correlation. Based on actual calculated BCF values, the Octanol water partition coefficient (Kow) values were predicted. In order to prove the hydrophobic property of PCB compounds and their affinity towards lipid, the Kow is predicted. Results showed that the PCB 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 PCB.

Keywords: Bioaccumulation; Bioconcentration factor; Polychlorinated Biphenyls; 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, PCBs 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 PCB concentration in fishes. [1] PCBs have been shown to have accumulated in tissues of birds, [2] indicating their passage along food chains.

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. [3],[4] Literature indicates that bioaccumulation of PCB 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. [5]

Many pollutant substances are in the form of mixtures of various compounds. Whereas the toxicity of such mixtures has been studied more, lesser studies have been conducted on the toxic effects of individual compounds in them. This has been more so in case of polychlorinated biphenyls (PCBs) in environmental or biological samples, which have, for some time been identified and quantified using **Aroclor®** mixtures to determine total PCBs, while few data have been generated on individual congeners. [6] Similarly, J.C. Duinker et al, [7] Victor A. McFarland and Joan U. Clarke, [8] Shinsuke Tanabe et al, [9] and Satyendera P. Bhavsar, et al, [10] also advocate the use of congener specific analysis rather than the use of mixtures.

The BCF or Bioconcentration factor 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. [11], [12].

Bioaccumulation of different chlorinated pesticides in different fish tissues was also estimated and reported. [13] Bioaccumulation kinetics and bioconcentration factor of chlorinated pesticides in tissues of Puntius ticto (Ham.) have also been calculated and reported. [14] In addition different proposals for calculation of BCF have also been reviewed and reported. [15]

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, [16] whereas a guideline value of 0.67 ng g-1for human consumption, [17] 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". [18]

However, unlike that in developed countries, less work has been carried out on bioconcentration of PCBs 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 PCBs 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 PCB congeners 126 and 169, i.e. 3,3',4,4',5- pentachlorobiphenyl and 3,3',4,4',5,5'-hexachlorobiphenyl, respectively, on the gill, liver, intestine and kidney tissues of freshwater fish *Rasbora daniconius*.

Bioconcentration kinetics

The bioconcentration process in case of nonbiodegradable 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).

$$\frac{dC_F}{dt} = k_1 C_w - k_2 C_F \quad (1)$$

k1 is the uptake rate constant, day-1

k2 is the elimination or depuriation rate constant, day -1

CF is the chemical concentration in fish, mg g-1

CW is the chemical concentration in water, mg g-1.

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

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

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.

METHODOLOGY

Continuous bioassay studies were carried out as per standard practice using an indigenously designed constant dosing device. [19] Schematic diagram of the device is shown in (Figure 1). Fish required for the experiments were procured from local fresh water bodies. The PCB congeners 126 and 169 used were obtained from **Dr. Ehrenstorfer, Germany**. Dichloromethane was used as a solvent. [20] 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. [21]

The estimation of PCB 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 PCBs as stated in the literature [22].

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. PCBs, along with other organic compounds, are co-extracted. To prevent the interference of such other organic compounds in the estimation of PCBs, a suitable clean-up procedure becomes necessary for their removal.

Table 1: Characteristics of Dilution Water

Parameters	Values*
Temperature ^{o} C	25-27
pH	7.5-8.2
Total Alkalinity as CaCO3	156-190
Total Hardness as CaCO3	142-172
Ca Hardness as CaCO3	80-94
Mg Hardness as CaCO3	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

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

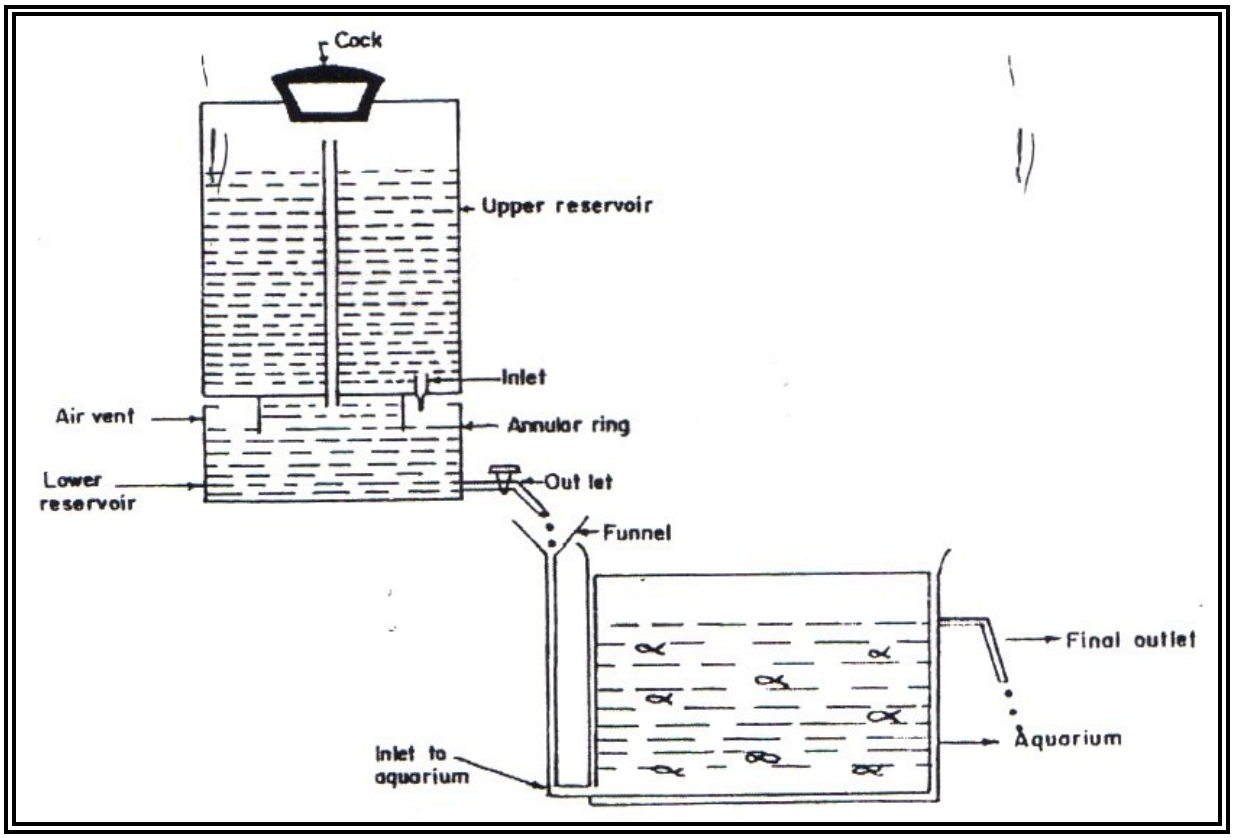

Fig. 1. Schematic diagram of the dosing unit

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. [23]

Glass columns of size 300 × 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_2SO_4 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 PCBs 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 PCBs in the extract were eluted successfully. This method is known for being capable of recovering 98-99% PCBs 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 PCB concentration has also been followed by other workers, as indicated in literature. [24] Expression of residue concentration on the basis of both wet weight and lipid weight has been recommended in literature. [25]

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 PCB was added to it and the volume made up to 20L. The flow from the reservoir was adjusted so that 20 litres of PCB solution

flowed through the aquarium in 24 hours. 20 fishes were introduced in each aquarium at the commencement of the experiment. Concentration of PCB 126 for the experiment was 17.5 µl l-1 while that of PCB 169 was 25 µl l-1. Feeding and replacements of fresh solution were carried out as per details mentioned in the literature. [26] Fish were removed alive from the aquarium at intervals of 5,10,15,20, 25 and 30 days for each species and PCB 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. [27] 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 PCB 126 and 169 in different tissues of *Rasbora daniconius* are presented in Table 2.

It was seen that maximum accumulation of PCB 126 in tissue of *Rasbora daniconius* was 64.68 µg g-1 wet weight in the gill tissue, followed by 23.95 µg g-1, 17.14 µg g-1 and 0.78 µg g-1 wet weight in intestine, liver and kidney respectively, as compared to its aqueous concentration of 17.5 µl l-1 .

On the other hand, maximum accumulation of PCB 169 was 7.12 µg g-1 wet weight in gill tissue, followed by 4.27 µg g-1, 2.69 µg g-1 and 0.41 µg g-1 wet weight in intestine, kidney and liver tissues respectively, as compared to its aqueous concentration of 25 µl l-1.

From the above observations, it is seen that PCB 126 shows higher accumulation in the gill, liver and kidney tissue of *Rasbora daniconius* as compared to PCB 169.

The liver tissue accumulated more of PCB 126 as compared to the kidney tissue, but a reverse trend was observed for PCB 169, which accumulated more in the kidney than in the liver tissue. This substantiates reports in literature that rates of bioaccumulation depend upon several variable factors. [5] 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. Also, Khan [28] 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 Kow for *Rasbora daniconius* have been evaluated. After exposure for 5 days, accumulation of PCB 126 was seen to be below detectable limits for intestine and kidney tissues, whereas that of PCB 169 was below detectable limits in the liver tissue for the same exposure period.

Maximum BCF and log BCF for both PCB 126 and PCB 169 showed maximum toxicity in gill tissue of *R. daniconius* after 30 days' exposure.

Table 2: Concentration of PCB in tissues of *Rasbora Daniconus*

PCB	Exposure time, days	Concentration of PCB, µg/g wet weight			
		GILL	INTESTINE	KIDNEY	LIVER
PCB 126	5	52.68	0.00	0.00	0.00
	10	59.52	5.08	0.47	1.64
	15	60.66	11.00	0.54	8.45
	20	61.95	15.79	0.62	15.63
	25	63.84	19.42	0.68	16.24
	30	64.68	23.95	0.78	17.14
PCB 169	5	1.88	1.54	0.84	0.00
	10	2.14	3.97	1.61	0.20
	15	2.28	4.03	1.98	0.32
	20	2.44	4.11	2.19	0.38
	25	4.83	4.20	2.42	0.39
	30	7.12	4.27	2.69	0.41

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 [29] and those of the ASTM (American Society for Testing and Materials) (European Centre for Ecotoxicology and Toxicology of Chemicals, 1996) [30] 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)

$$\log BCF = a \log Kow + b, \quad (3)$$

Where a and b are empirically determined constants and Kow is the n-octanol/water partition coefficient.

Fathead minnows, Green Sunfish and Rainbow trout were exposed for 32 days in continuous-flow water and the extent of bioconcentration from water was calculated. [31] The relationship between log BCF and log Kow was expressed as per Equation (4)

$$\log BCF = 0.85 \log Kow - 0.70, R2 = 0.897 \quad (4)$$

The above equation has been applied for developing a correlation between BCF and Kow tissues of the fish species studied. Accumulation in fish tissues indicates a build-up of the PCB, 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 Kow and linear relationships have been established on log scale. [32], [33], [34] & [35].

Table 3: Bioaccumulation kinetic constants for fish tissues of *Rasbora daniconius*

PCB	Statistical constants	GILL	INTESTINE	KIDNEY	LIVER
PCB-126	Rate of bioaccumulation*	2.12	4.787	0.339	3.904
	Intercept	53.13	-3.126	0.767	-3.816
	R2	0.847	0.994	0.928	0.908
PCB-169	Rate of bioaccumulation*	0.984	0.412	0.131	0.076
	Intercept	0.002	2.242	0.053	0.014
	R2	0.771	0.532	0.807	0.825

* µgg-1 wet weight of the tissue

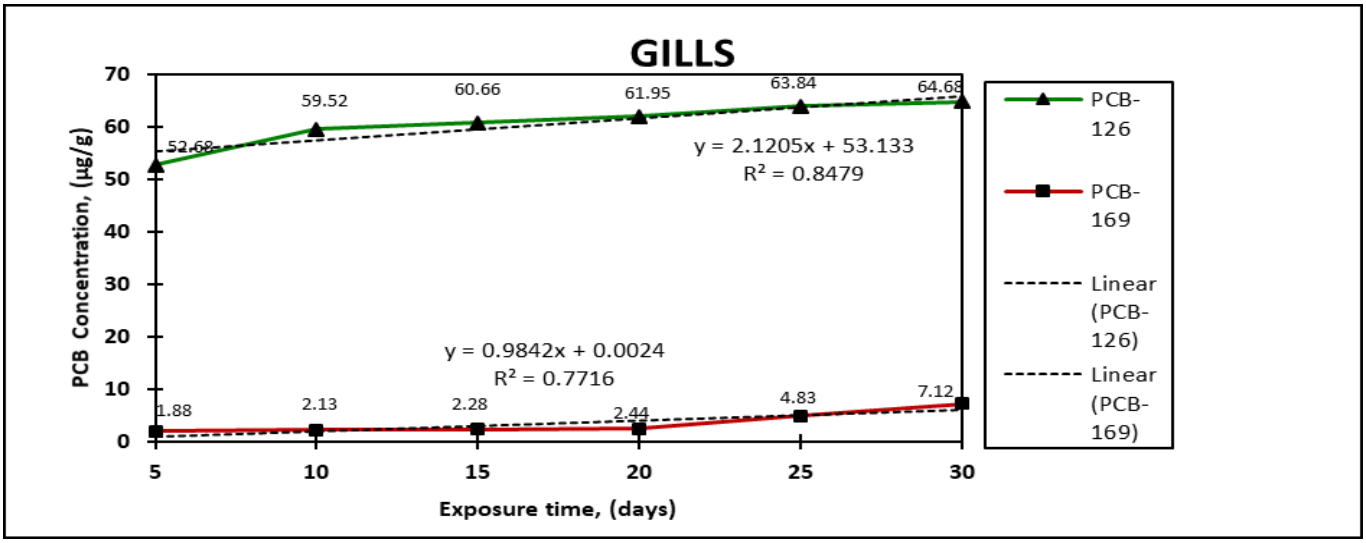

Fig 2: Comparative study between exposure time and PCB concentration in Gills of *Rasbora daniconius*.

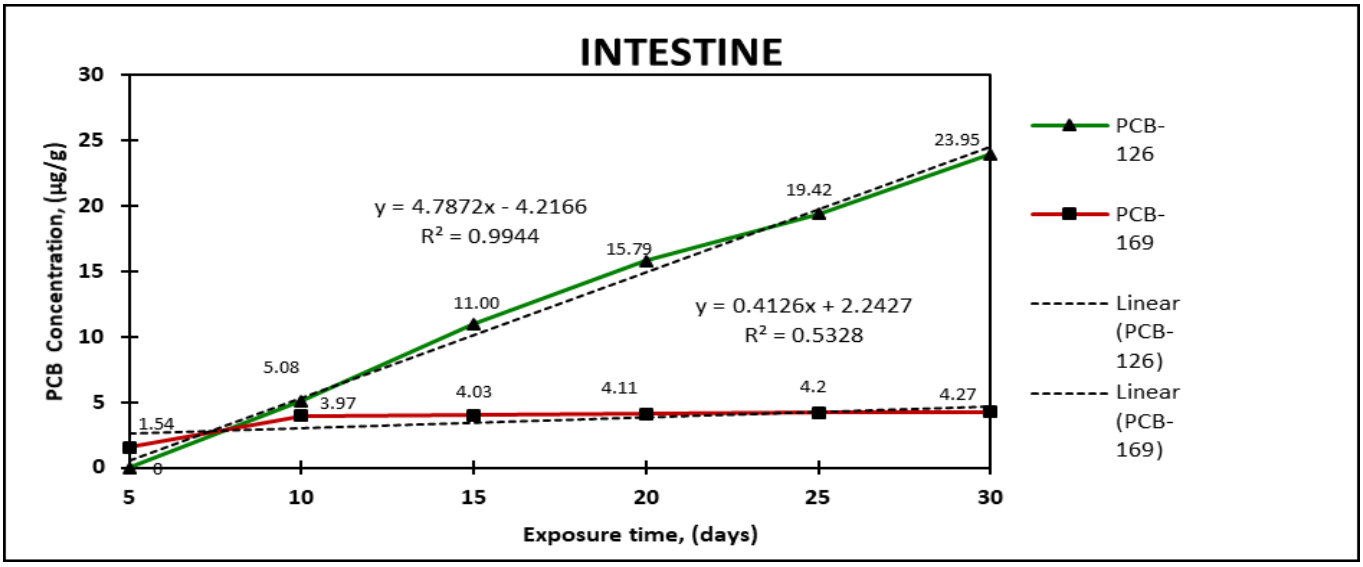

Fig.3: Comparative study between exposure time and PCB concentration in Intestine of *Rasbora daniconius*.

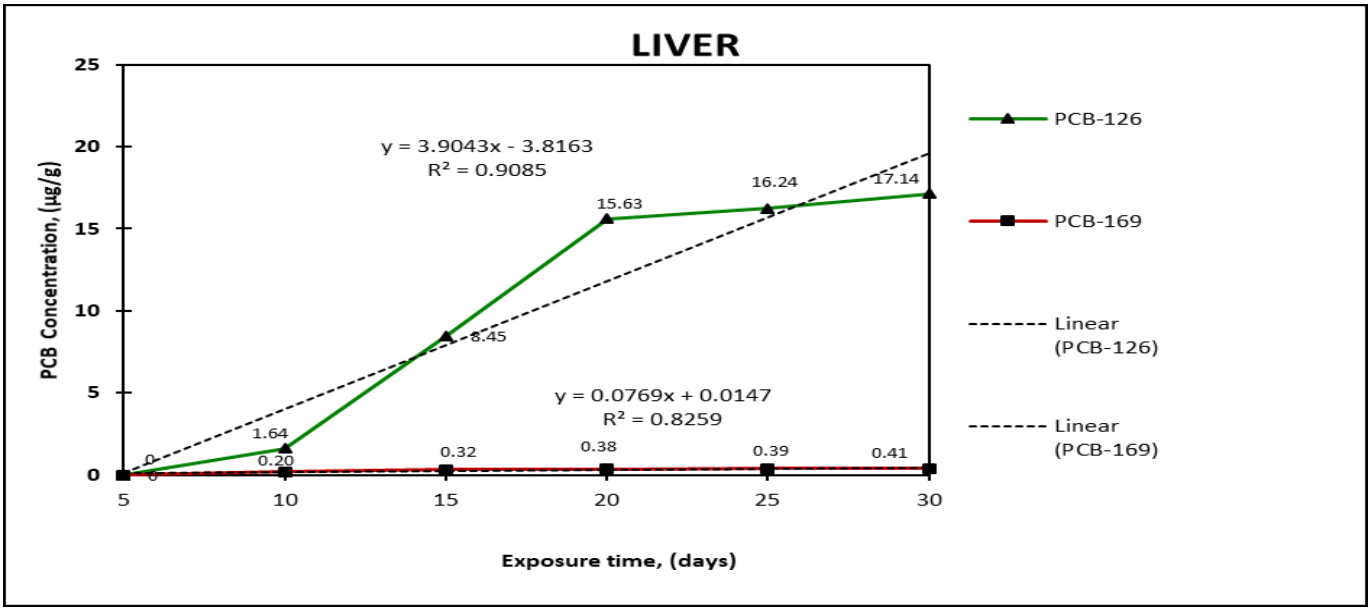

Fig. 4: Comparative study between exposure time and PCB concentration in Liver of *Rasbora daniconius*.

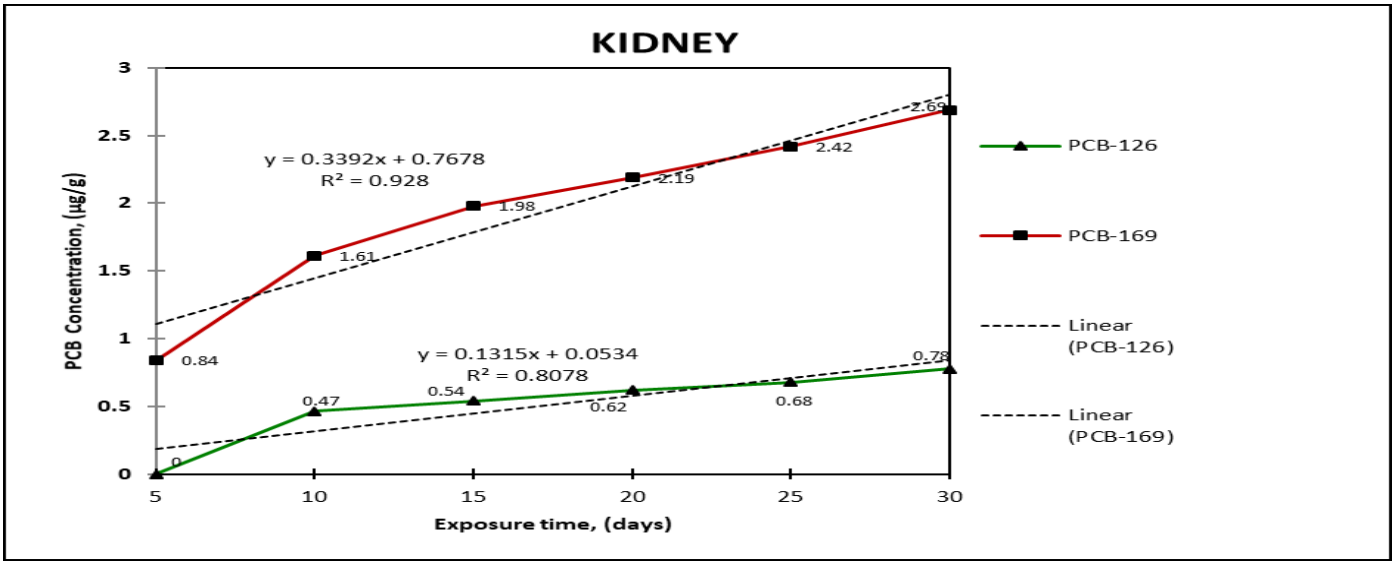

Fig.5:Comparative study between exposure time and PCB concentration in Kidney of *Rasbora daniconius*.

Table 4: Calculated BCF, log BCF and predicted log Kow values for tissues of Rasbora

PCB	Exposure time, days	GILLS			INTESTINE			KIDNEY			LIVER		
		BCF	log BCF	log Kow	BCF	log BCF	log Kow	BCF	log BCF	log Kow	BCF	log BCF	log Kow
PCB 126	5	5.27 x 10-9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10	5.95 x 10-9	-8.23	-8.85	5.08 x 10-10	-9.29	-10.11	4.65 x 10-11	-10.3	-11.33	1.64 x 10-10	-9.79	-10.69
	15	6.07 x 10-9	-8.22	-8.84	1.10 x 10-9	-8.96	-9.72	5.40 x 10-11	-10.3	-11.26	8.45 x 10-10	-9.07	-9.85
	20	6.19 x 10-9	-8.21	-8.83	1.58 x 10-9	-8.80	-9.53	6.21 x 10-11	-10.2	-11.19	1.56 x 10-9	-8.81	-9.54
	25	6.38 x 10-9	-8.20	-8.82	1.94 x 10-9	-8.71	-9.43	6.80 x 10-11	-10.2	-11.14	1.62 x 10-9	-8.79	-9.52
	30	6.47 x 10-9	-8.19	-8.81	2.39 x 10-9	-8.62	-9.32	7.75 x 10-11	-10.2	-11.07	1.71 x 10-9	-8.77	-9.49
PCB 169	5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10	2.13 x 10-10	-9.67	-10.55	ND	ND	ND	1.61 x 10-10	-9.79	-10.70	2.02 x 10-11	-10.69	-11.76
	15	2.28 x 10-10	-9.64	-10.52	4.03 x 10-10	-9.40	-10.23	1.98 x 10-10	-9.70	-10.59	3.20 x 10-11	-10.50	-11.52
	20	2.44 x 10-10	-9.61	-10.49	4.11 x 10-10	-9.39	-10.22	2.19 x 10-10	-9.66	-10.54	3.75 x 10-11	-10.43	-11.44
	25	4.83 x 10-10	-9.32	-10.14	4.20 x 10-10	-9.38	-10.21	2.42 x 10-10	-9.62	-10.49	3.90 x 10-11	-10.41	-11.42
	30	7.12 x 10-10	-9.15	-9.94	4.27 x 10-10	-9.37	-10.20	2.69 x 10-10	-9.57	-10.44	4.14 x 10-11	-10.38	-11.39

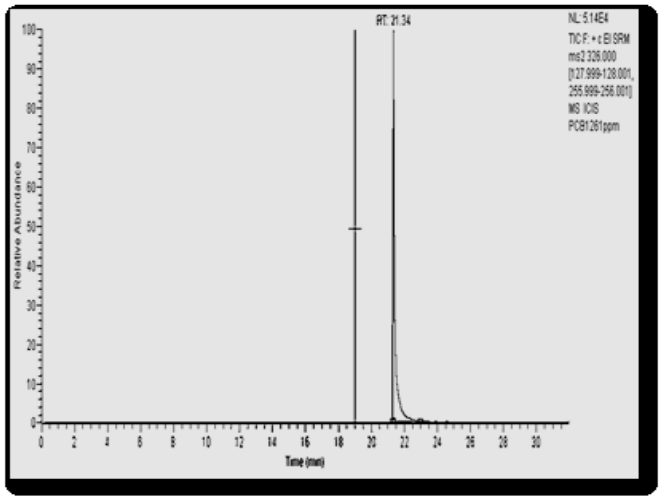

Fig. 6: Standard chromatogram of PCB 126

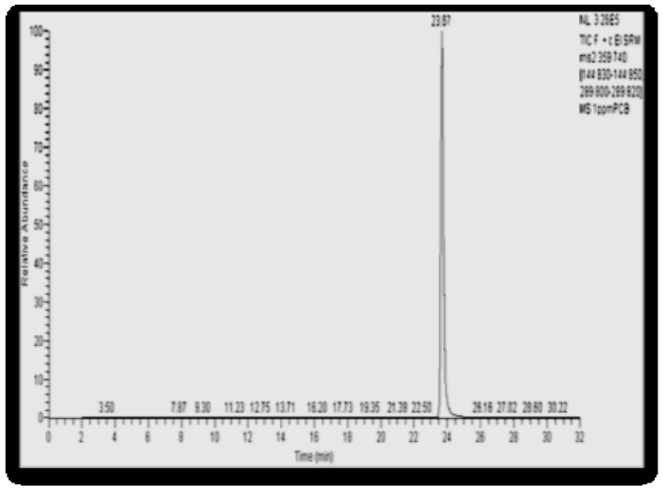

Fig. 7: Standard chromatogram of PCB 169

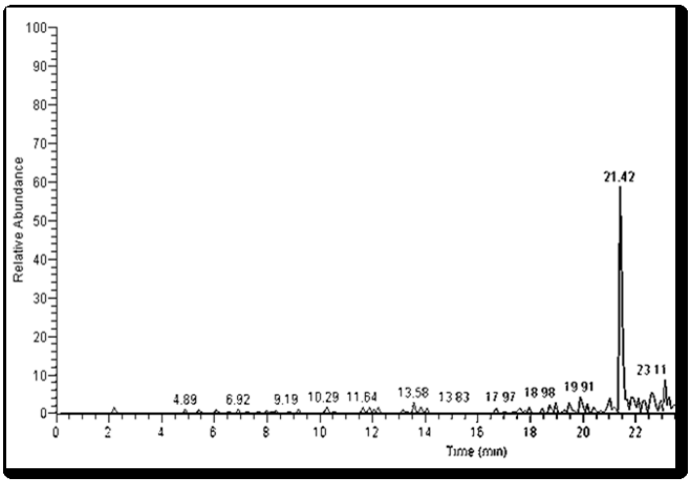

Fig.8: Chromatogram for PCB 126 in Gill tissue of *Rasbora* on exposure for 30 days

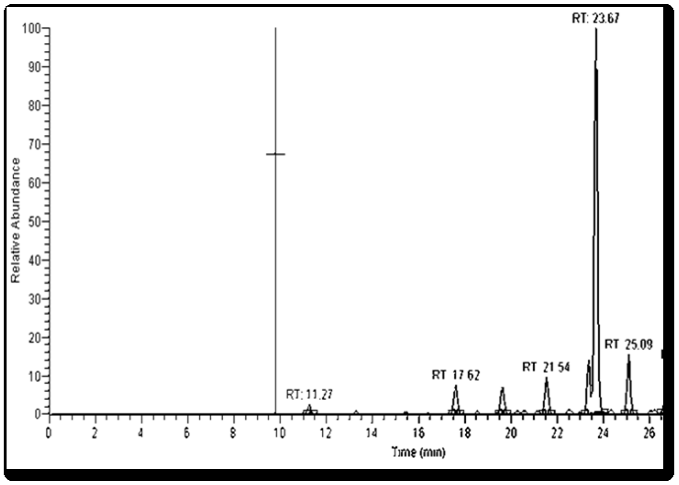

Fig.9: Chromatogram of PCB 169 accumulated in gill tissue of *Rasbora* after exposure for 30 days

According to Neely, Branson and Blau, [36] the partition coefficient of a pollutant would be the most logical parameter among different properties of a compound to examine the extent of its bioconcentration by aquatic organisms. Literature reports different trends of bioconcentration. [37], [38], & [39] Linear graphs for regression representing the relationship between PCB concentration and exposure time for the complete period of 30 days with respect to both PCBs 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.

Standard chromatograms for PCB congeners 126 and 169, 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 PCB 126 was found to be maximum of 64.68 µgg-1, and that of PCB 169 a maximum of 7.12 µgg-1 in gill tissue of *R. daniconius*. As evidenced by increasing values of BCF, the uptake of PCBs also increased with increase in exposure time. The R2 values in most cases were greater than 0.9 for PCB 126, whereas these values vary between 0.532 and 0.825 for PCB 169, indicating fair to very high correlation between PCB concentration and exposure time. Actual results obtained from the present study confirm the hydrophobicity of PCB compounds. Moreover, the regression models encompass a large range of BCF and show a high correlation with Kow.

CONCLUSION

An important feature apparent from the present study is that bioaccumulation was relatively a slow process for all the PCB concentrations tested, and the degree of accumulation varied from tissue to tissue. Both species of fish tested accumulate PCBs under chronic bioassay conditions, and bioconcentration is a function of time and the sublethal concentration in the exposed medium. PCB 126 was seen to be significantly more toxic as compared to PCB 169, as is evident from the concentrations accumulated over a period of 30 days. PCBs 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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