International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 01-12-2023, Accepted: 17-12-2023, Published: 02-01-2024
Volume 9, Issue 1, 2024, Page No. 1-6

A comparative account of chronic environmental Impact of PCB 169 on the tissues of two freshwater fish *Rasbora daniconius* and *Puntius ticto*

Advait Bhagade
Department of Zoology, St. Francis de Sales College, Seminary Hills, Nagpur, Maharashtra, India

Abstract
Freshwater fish *Rasbora daniconius* and *Puntius ticto* were subjected to chronic toxicity assay to determine the degree of bioaccumulation of PCB 169 in gill, liver, kidney and intestine tissues. GC-MS was used to analyse bioaccumulation in the tissues.
Maximum bioaccumulation of the PCB congener 169 over a period of 30 days' exposure was observed at 30.61 µg g-1 wet weight in the gill tissue of *Puntius ticto*, as compared to 7.12 µg g-1 wet weight in the gill tissue of *Rasbora daniconius* over the same period, clearly indicating its tendency to accumulate preferentially in gill tissue as compared to other tissues. Further, greater accumulation was detected in the fish *Puntius ticto* as compared to *Rasbora daniconius*.

Keywords: Bioaccumulation, GC-MS, PCB 169, Toxicity

Introduction
Due to their tendency to persist in the environment, PCDDs, PCDFs and PCBs are part of the so-called persistent organic pollutants group of compounds that also include some chlorinated pesticides. Since PCBs have a high lipophilicity and resist transformation, they bio-accumulate in animal and human adipose tissues [1]. Being lipophilic, they are found to bioaccumulate more at colder temperatures, and thus pose a serious threat in temperate and polar environments [2-4].
Significant uptake of PCBs by fish from aquatic systems, increasing from 0.06 to 118.3 µg g -1 over an exposure period of 28 days, has been documented [5, 6]. PCB 126 has been shown to be toxic to fish medaka [7] (Youngchul Kim and Keith R. Cooper, 1998) and to have high levels of bioaccumulation and biomagnification in Rainbow Trout [8]. It is considered the most toxic of the non-ortho coplanar congeners [9]. This congener has also been found in man where populations are predominantly dependent on fish and sea food for nutritional requirements [10]. PCBs have also found their way into tissues of farmed marine fish [11, 12] and in other foodstuff [13].
Study of the bioaccumulation potential of two highly toxic coplanar PCB isomers 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB 126) and 3, 3', 4, 4', 5, 5'-hexachlorobiphenyl (PCB 169), in relation to kinetic parameters of coplanar PCBs based on lipid weight-related data [14, 15] and the degree of bioaccumulation based on the proportion of coplanar PCBs in total PCBs have been shown to clearly indicate that coplanar PCBs are highly bioaccumulative in lower organisms, and that these highly toxic and persistent PCB congeners are concentrated by all aquatic organisms, and may reach higher consumers (including humans) in quantities of toxicological concern [16]. Biomagnification of pollutants in food chains has also been observed [17].
Only few references are available for bioassays using the pollutant substance for direct exposure of the freshwater dwelling test organisms through their environment. Bioassays to determine acute toxicity after 96 hour exposures to determine LC50 values [18, 19], and use of continuous flow through bioassays [20] are some of the methods followed for such study.
The use of GC and GC-MS to estimate the degree of bioconcentration has been widely accepted [21, 22].
The present study has attempted to analyse the impact of the PCB congener 169 on aquatic organisms in terms of bioaccumulation in some of their tissues by means of initial acute bioassays of 96 hour duration to determine LC50 values, and subsequently, based on such values, a chronic bioassay of 30 days using a continuous flow through method, using the fresh water fish *Rasbora daniconius* and *Puntius ticto* as test organisms.

Materials and Methods
The acronym PCB (PolyChlorinated Biphenyl) is used for a group of 209 organo-chlorine chemicals existing in the form of molecules termed as congeners. They are a class of non-polar chlorinated hydrocarbons with a biphenyl nucleus of which one or more of the 10 Hydrogen atoms are substituted with chlorine. They have a general formula $C_{12}H_{10-n}Cl_n$ where n may range from 1 to 10. E.g. 3, 3', 4, 4', 5, 5'-hexachlorobiphenyl has n=6.
The chemical formula of PCB 169 (3, 3', 4, 4', 5, 5'-hexachlorobiphenyl) is $C_{12}H_4Cl_6$. The commercially available congener of Dr. Ehrenstorfer make, Germany, was used for the present work.
Fish were obtained from local freshwater lakes and rivers. *Rasbora daniconius* specimens used for the present work were of average length 7-10 cm and weighed 20 gm while *Puntius ticto* were of average length 5-7 cm and weight 25g.

a. Acute Toxicity study
Both types of fish, on exposure to PCB solution remained at the bottom, showed quick opercular movements and a temporary disorientation, visible as loss of balance while swimming. However, they recovered after some time and started normal movement. Moreover, they did not show any lethal effect upto concentrations as high as 50 µl l^{-1}. Exploratory tests with different concentrations of PCB 169 were carried out but no mortality was observed till 96 hours exposure. This observation is in concurrence with literature

available [23, 24]. Sublethal dose of the PCB congener was therefore arbitrarily fixed and chronic toxicity study was carried out. Very high dosages were not attempted since the same was not economically feasible.

b. Chronic toxicity study

A continuous flow through method was used for determination of chronic toxicity and bioaccumulation of PCB 169. A glass aquarium of 20 l capacity, with an overflow arrangement exceeding that capacity, was used. A daily dose of 25 µl l^{-1} and 20 µl l^{-1} was administered to 20 experimental fish *Rasbora daniconius* and *Puntius ticto* respectively for a period of 30 days adjusting the flow rate from the dosing unit to 7 ml min^{-1}. The solution containing the PCB 169 was delivered to the bottom of the aquarium, with excess water continuously flowing out of the outlet. Investigations for each concentration were carried out separately for increasing durations of 5, 10, 15, 20, 25 and 30 days. Appropriate controls were maintained using dechlorinated tap water. It may be noted here that no mortality was observed either in the test organisms or the control organisms group during their period within the aquarium.

Feeding and replacements of fresh solutions were followed as per protocols followed in literature [25].

Few live fish from fish in the aquarium were removed on intervals of every 5 days. They were sacrificed and their gill, liver, intestine and kidney tissues were harvested and fixed in Buoin’s fixative to further analyse the bioaccumulation of PCB 169 in them.

c. Bioaccumulation study

Tissues were subjected to thorough clean-up using Celite 545 columns and extracted using Dichloromethane of MERCK make for analysis of bioaccumulation. This was carried out using a GC-MS of Thermo Scientific (USA) make. GC-MS is a widely accepted method for analysis of the extent of bioaccumulation, as can be seen from various sources.

Mass spectra and standard chromatograms of PCB 169 were first obtained, and the tissue samples were subsequently analysed in order to determine the degree of bioaccumulation of PCB 169 in them.

Mass spectra and standard Chromatograms of PCB 169 are shown in Fig 1.and 2.

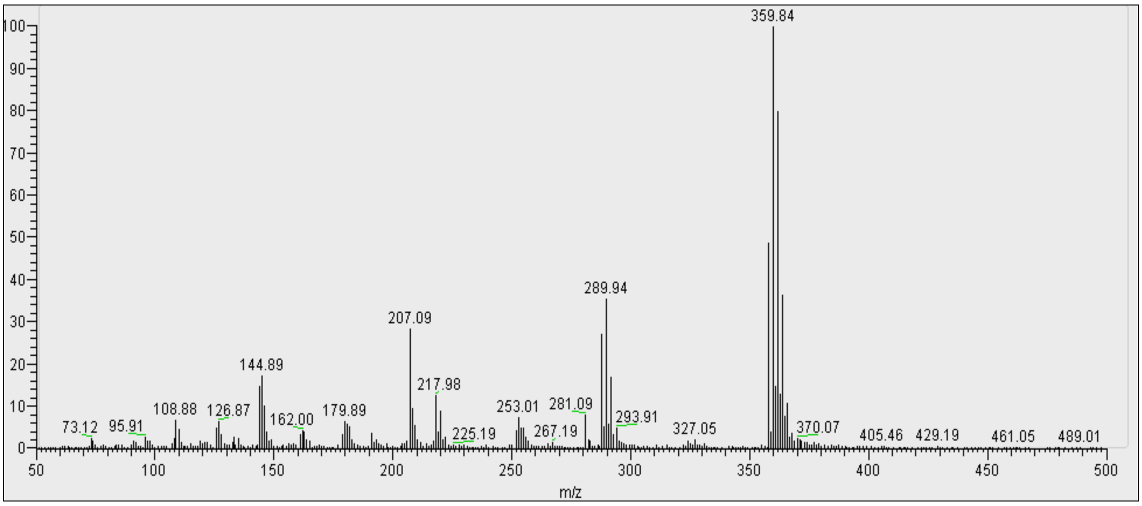

Fig 1: Mass spectra of PCB-169 (3, 3’, 4, 4’, 5, 5’-Hexachlorobiphenyl)

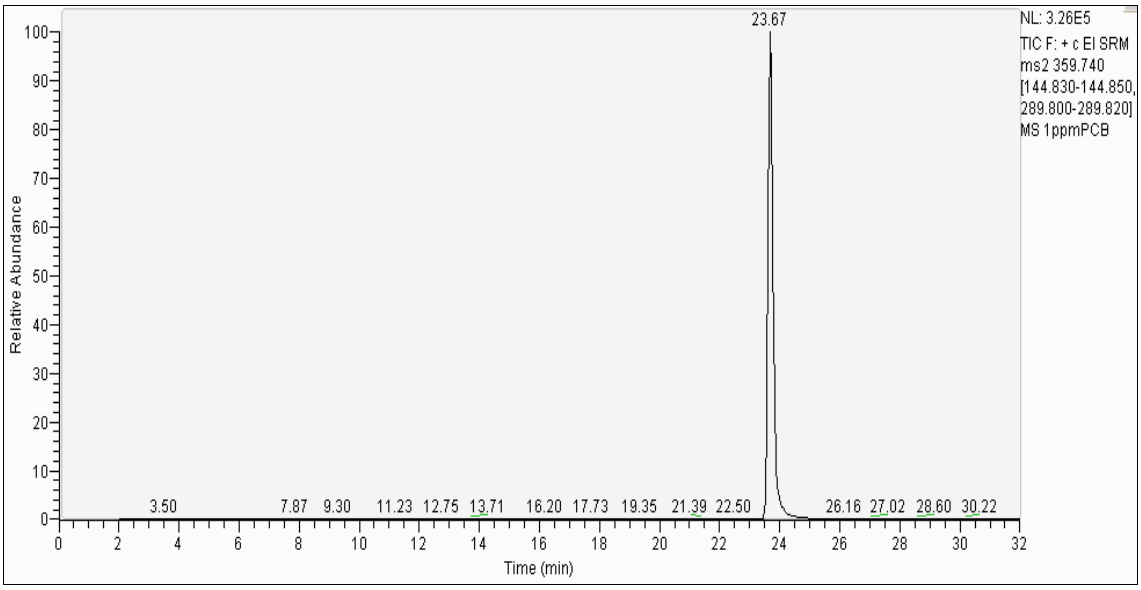

Fig 2: Standard Chromatogram of PCB-169 (3, 3’, 4, 4’, 5, 5’-Hexachlorobiphenyl)

Results

Bioaccumulation was determined by means of chromatograms obtained after analysis of fish tissue extracts on GC-MS. Some chromatograms showing bioaccumulation are shown in Fig. 3 to Fig 6.

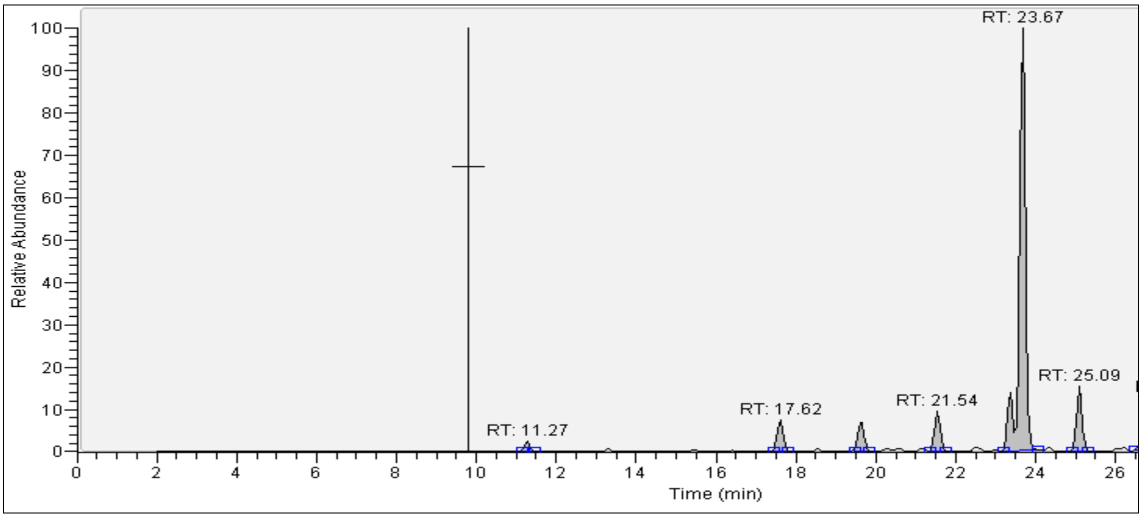

Fig 3: Chromatogram showing PCB 169 in *Rasbora* Gill (10 Days)

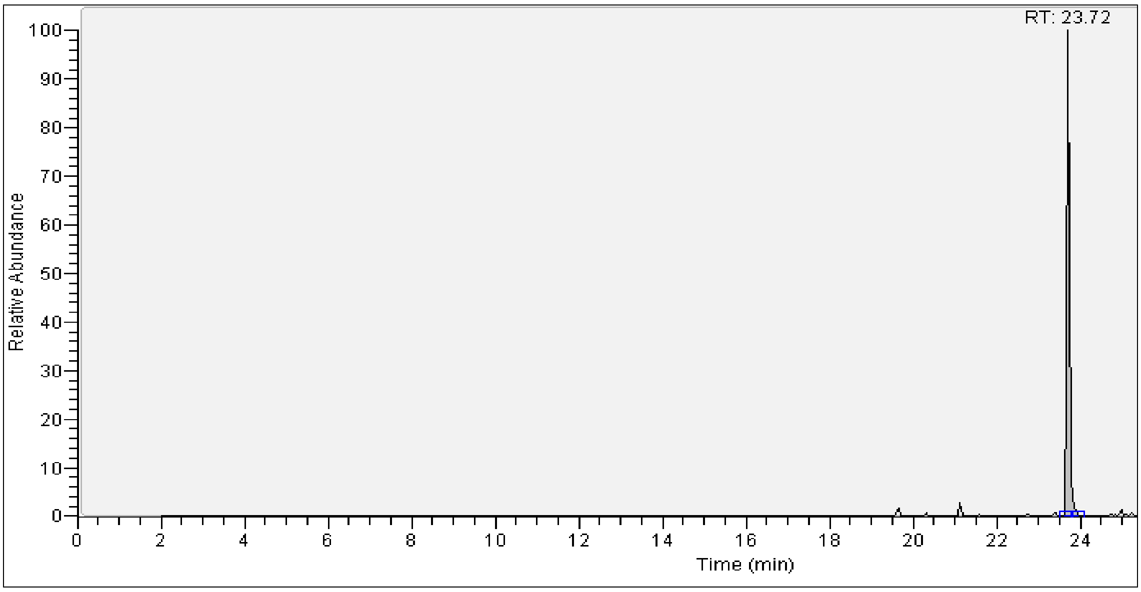

Fig 4: Chromatogram showing PCB 169 in *Rasbora* Kidney (30 Days)

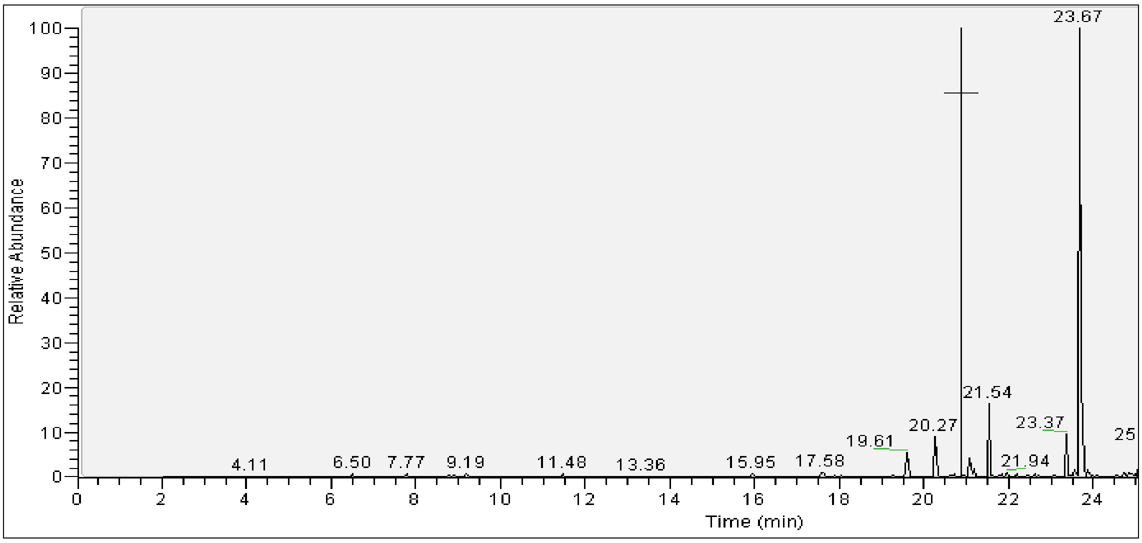

Fig 5: Chromatogram showing PCB 169 in *Puntius* Kidney (10 Days)

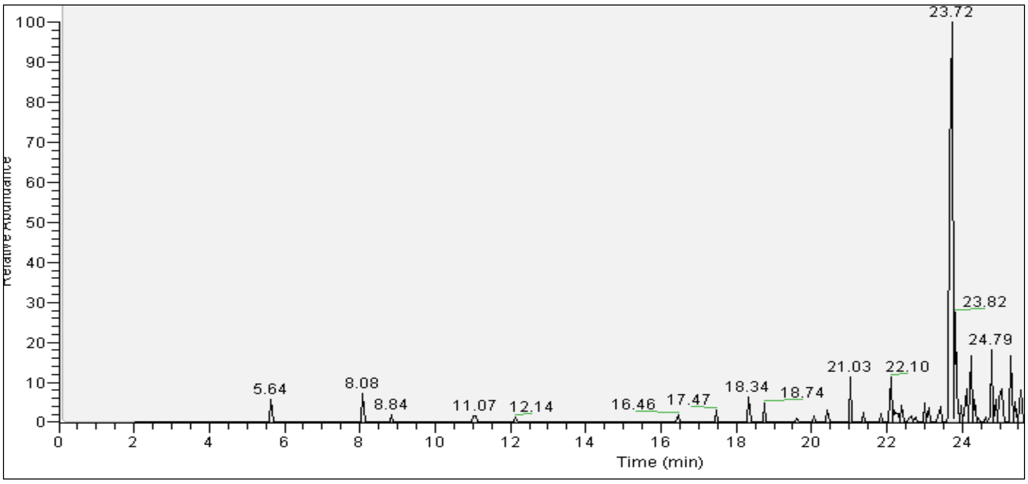

Fig 6: Chromatogram showing PCB 169 in *Puntius* Gill (20 Days)

The extent of bioaccumulation found in the four different tissues of either fish over increasing 5 days' periods of chronic exposure is detailed in Tables 1 and 2. The graphical comparison of the same has been shown in Fig.7.

Table 1: Bioaccumulation of PCB 169 in *Rasbora daniconius*.

Exposure time, in days	Values in µg g^{-1} wet weight of tissue			
	Gill	Intestine	Kidney	Liver
5	1.88	1.54	0.84	0
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

Table 2: Bioaccumulation of PCB 169 in *Puntius ticto*.

Exposure time, in days	Values in µg g^{-1} wet weight of the tissue			
	Gill	Intestine	Kidney	Liver
5	14.88	6.89	0.00	0.68
10	25.57	9.84	0.57	1.79
15	27.02	10.02	4.78	2.03
20	28.04	10.26	7.69	2.14
25	29.54	11.98	8.89	3.14
30	30.61	13.23	12.50	4.73

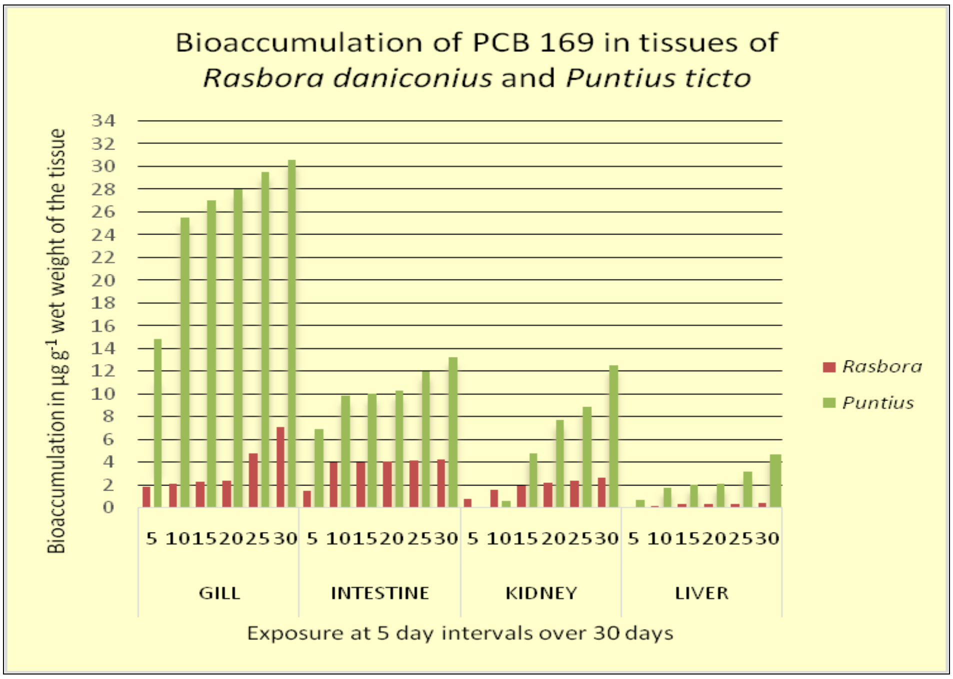

Fig 7: Graphical comparison of bioaccumulation of PCB 169 in tissues of fish *Rasbora daniconius* and *Puntius ticto.*

Accumulation of PCB 169 in the gill tissue, seen to be at a maximum, was 30.61 µg g^{-1} and 7.12 µg g^{-1} wet weight for *Puntius* and *Rasbora*, respectively.

Discussion

The kidney tissue is expected to excrete biotransformed PCB 169; yet the PCB is seen to accumulate in the tissue over the exposure period (12.50 µg g^{-1} and 2.69 µg g^{-1} wet weight for *Puntius* and *Rasbora*, respectively, in the complete 30 days' exposure). This clearly indicates that PCB 169 has a tendency to accumulate in the kidneys, as well as other tissues.

The phenomenon can be better understood if the path of PCB within the fish body can be traced. Physiologically, the gill is the first tissue/ organ to come into contact with the PCB, through respiration. Here, most of the PCB is seen to accumulate, and only a lesser quantity (13.23 µg g^{-1} and 4.27 µg g^{-1} wet weight for *Puntius* and *Rasbora*, respectively, in the complete 30 days' exposure) is accumulated in the intestine, through ingestion of the dosage water along with food.

Blood, on passing through the liver, should ideally offload any xenobiotic for the process of biotransformation at the site of the liver, and such a biotransformed substance is then expected to be excreted through the kidney. In this study, the lowest accumulation is seen in the liver tissue (4.73 µg g^{-1} and 0.41 µg g^{-1} wet weight for *Puntius* and *Rasbora*, respectively, in the complete 30 days' exposure). This indicates that the process of biotransformation in the liver has been an efficient one, and that least accumulation was observed in the liver tissue.

In kidney tissue, bioaccumulation was higher than the liver, indicating that the kidney could not excrete the PCB 169 that found its way into the tissue through blood.

Conclusion

The results of the experiment clearly indicate that PCB 169 has the maximum propensity for accumulation in the Gill tissue as compared to the other tissues studied; *viz*. intestine, kidney and liver. Further, over the complete exposure period, the build up of PCB 169 is initially slow, and becomes more rapid as time in the number of days of exposure increases. *Rasbora daniconius* is seen to accumulate PCB 169 in all its tissues to a much greater extent as compared to *Puntius ticto*.

There is a need to further study bioaccumulation and biomagnification of PCB 169 through freshwater trophic systems, in order to analyse its presence and resultant threat to the health of top consumers including man.

References

1. Srogi K. Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review. Environ Chem Lett,2008:6(1):1-28.
2. Muijs B, Jonker MTO. Temperature-Dependent Bioaccumulation of Polycyclic Aromatic Hydrocarbons. Environ Sci Technol,2009:43(12):4517-4523.
3. Unyimadu JP, Osibanjo O, Babayemi JO. Polychlorinated Biphenyls in Brackish Water Fish in the River Niger. Nigeria J Health Pollut,2018:8(17):31-42.
4. Wangboje OM, Obotha-Adigo O. Residual Levels of Polychlorinated biphenyls (PCBs) in the Atlantic Mackerel (Scomber scombrus, Linnaeus, 1758) Marketed within a Niger Delta Community. Eur J Agric Food Sci,2020:2(4):1-6.
5. Meadows JC, Echols KR, Huckins JN, Borsuk FA, Carline RF, Tillitt DE. Estimation of Uptake Rate Constants for PCB Congeners accumulated by Semipermeable Membrane Devices and Brown Trout (Salmo trutta). Environ Sci Technol,1998:32(12):1847-1852.
6. Mariottini M, Corsi I, Focardi S. PCB levels in European eel (Anguilla anguilla) from two coastal lagoons of the Mediterranean. J Environ Monit Assess,2006:117(1-3):519-528.
7. Youngchul K, Cooper KR. Interactions of 2,3,7,8-tetrachlorodibenzo-P-dioxin (TCDD) and 3,3'4,4'5-pentachlorobihenyl (PCB 126) for producing lethal and sublethal effects in the Japanese medaka embryos and larvae. Chemosphere,1998:36(2):409-418.
8. Brown SB, Fisk AT, Brown M, Villella M, Muir DCG, Evans RE, Lockhart WL, Metner DA, Cooley HM. Dietary accumulation and biochemical responses of juvenile rainbow trout (Oncorhynchus mykiss) to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126). Aquat Toxicol,2002:59(3-4):139-152.
9. Storelli MM, Marcotrigiano GO. Levels and congener pattern of polychlorinated biphenyls in the blubber of the Mediterranean bottlenose dolphins Tursiops truncates. Environ Int,2003:28(7):559-565.
10. Dewailly E, Laliberte JJ, Yan C, Bruneau S, Weber J, Gingras S, Carrier G. Exposure of Remote Maritime Populations to Coplanar PCBs. Environ Health Perspect Suppl,1994:102(1):205-209.
11. Easton MDL, Luszniak D, Von der Geest E. Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. Chemosphere,2002:46(7):1053-1074.
12. Shaw SD, Brenner D, Berger ML, Carpenter DO, Hong C, Kannan K. PCBs, PCDD/Fs, and Organochlorine Pesticides in Farmed Atlantic Salmon from Maine, Eastern Canada, and Norway, and Wild Salmon from Alaska. Environ Sci Technol,2006:40(17):5347-5354.
13. Eljarrat E, Monjonell Caixach AJ, Rivera J. Toxic Potency of Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans, and Polychlorinated Biphenyls in Food Samples from Catalonia (Spain). J Agric Food Chem,2002:50(5):1161-1167.
14. Hernout B, Leleux J, Lynch J, Ramaswamy K, Faulkner P, Matich P, *et al*. The integration of fatty acid biomarkers of trophic ecology with pollutant body-burdens of PAHs and PCBs in four species of fish from Sabine Lake, Texas. Environ Adv,2020:1:100001.
15. Huang Z, Qadeer A, Zheng S, Ge F, Zhang K, Yin D, Zheng B, Zhao X. Fatty acid profile as an efficient bioindicator of PCB bioaccumulation in a freshwater lake food web: A stable isotope guided investigation. J Hazard Mater,2022:423(B):127121.
16. Kannan N, Tanabe S, Tatsukawa R, Phillips DJH. Persistency of highly toxic coplanar PCBs in aquatic ecosystems: Uptake and release kinetics of coplanar PCBs in green-lipped mussels (Perna viridis Linnaeus). Environ Pollut,1989:56(1):65-76.

17. Zhou S, Zhu H, Huang S, Zhou J, Zhang S, Wang C. Biomagnification and risk assessment of polychlorinated biphenyls in food web components from Zhoushan fishing ground, China. Mar Pollut Bull, 2019:142:613-619.
18. Vieira LR, Gravato C, Soares A, Morgado F, Guilhermino L. Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: Linking biomarkers to behaviour. Chemosphere,2009:76(10):1416-1427.
19. Mhadhbi L, Boumaiza M, Beiras R. A standard ecotoxicological bioassay using early life stages of the marine fish Psetta maxima. Aquat Living Resour,2010:23:209-216.
20. Brenniman G, Hartung R, Weber WJ Jr. A continuous flow bioassay method to evaluate the effects of outboard motor exhausts and selected aromatic toxicants on fish. Water Res,1976:10(2):165-169.
21. Maack L, Sonzogni WC. Analysis of polychlorobiphenyl congeners in Wisconsin fish. Arch Environ Contam Toxicol,1988:17(6):711-719.
22. Dmitrovic J, Chan SC, Chan SHY. Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. Toxicol Lett,2002:134(1-3):253-258.
23. Hansen DJ, *et al*. Aroclor 1016: Toxicity to and uptake by estuarine animals. Environ Res,1974:7(3):363-373.
24. Couillard CM, Légaré B, Bernier A, Dionne Z. Embryonic exposure to environmentally relevant concentrations of PCB126 affect prey capture ability of Fundulus heteroclitus larvae. Mar Environ Res,2011:71(4):257-265.
25. Murty AS. Toxicity of pesticides to fish Vol. I. CRC Press Inc. Boca Raton, Florida, 1986, 1-165.