RESEARCH ARTICLE

Tissue-specific tendencies of bioaccumulation of PCB 169 in freshwater fish *Rasbora daniconius*

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ABSTRACT

Chronic exposure of the freshwater fish *Rasbora daniconius* to a sub-lethal concentration of the Poly Chlorinated Biphenyl Congener No. 169 for a continued period of 30 days showed its bioaccumulation in the fish tissues. Collection of samples of some tissues, viz. the gills, kidney, intestine and liver, on every 5th day of exposure, has provided the data for a comparative study of the extent of bioaccumulation in them. Maximum bioaccumulation is seen in the gills, while the least in the liver of the fish.

Keywords: *Bioaccumulation, Chronic toxicity, POPs, GC-MS, Rasbora*.

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INTRODUCTION

All living organisms depend upon water, typically in the form of a solution containing a range of inorganic and organic substances, many of which are integral to their metabolic processes. The degree of such intake may show extreme variations according to the type of environment in which an organism lives. Aquatic ecosystems, comprising water from natural sources, additionally also receive wastewater produced as a result of diverse human activities. Among these substances in this wastewater are xenobiotics, which are not naturally involved in metabolic processes and are often of human origin. These xenobiotics can pervade water sources extensively.

Agricultural or industrial applications of such substances, especially those comprising complex organic compounds, can adversely impact fish populations. Many of these compounds exhibit prolonged persistence in both abiotic and biotic components of the environment, breaking down only slowly in water and adhering to particulate matter, primarily in sediments. Consequently, sediments serve as the primary reservoir for these contaminants in aquatic ecosystems. They have a tendency to bind preferentially to carbon particles within sediments, hence termed persistent organic pollutants (POPs). Additionally, POPs tend to bioaccumulate in living organisms and undergo biomagnification along food chains. However, some literature suggests that fish can excrete POPs through bile.[1-3] Polychlorinated biphenyls (PCBs) represent a category of POPs comprising 209 distinct compounds referred to as congeners. These are chlorinated hydrocarbons with a biphenyl structure, wherein one or more of the ten hydrogen atoms are substituted with chlorine. PCBs are found to have extensive applications as dielectric and coolant fluids, notably in devices such as transformers, capacitors, and electric motors.

PCBs exhibit lipophilic characteristics and have been demonstrated to induce oxidative stress and apoptosis in fish[4]. Both PCB exposure and DNA damage have been identified as contributors to environmental stress in fish.[5]

PCBs have the capacity to accumulate in the epigeal portions of plants and agricultural produce. They are also absorbed by small organisms and fish, consequently posing a risk of exposure to bioaccumulated PCBs for individuals consuming fish. Research suggests that PCBs can be detrimental to the developmental stages of birds, highlighting the potential hazards associated with their transfer up the food chain from fish.[6] Moreover, PCB contamination has been detected in groundwater affected by abandoned mining equipment.[7] These contaminants are observed to accumulate in sediment feeders like *Daphnia magna* and are subsequently transferred up the food chain through predatory fish such as *Pimephales promelas*[8]. Extensive investigations into the subacute and chronic toxic effects of PCBs on mammals and birds, particularly concerning reproduction, have been conducted.[9] Studies indicate that certain vulnerable populations, including specific ethnic groups, the elderly, pregnant women, children, fetuses, and nursing infants, remain exposed to PCBs through the consumption of fish and wildlife.[10]

According to available literature,[11-13] Persistent Organic Pollutants (POPs) predominantly accumulate within sediment layers in aquatic environments, with uptake of these contaminants occurring through the sediment.[14]

While most PCBs exhibit bioaccumulation in fish, not all demonstrate significant effects. Exposure of fish to PCB

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congeners 52, 101, 138, 153, and 180 revealed minimal adverse impacts on fish survival, growth, and reproduction, despite substantial bioaccumulation of these congeners[15]. However, PCB Congener Nos. 77, 126, and 169 are reported to be the most toxic, primarily originating from commercial PCBs and surpassing dioxins and furans in toxicity[16]. Additionally, studies indicate that their uptake as well as clearance from aquatic organisms are slow, suggesting significant bioaccumulation.[17,18]

It is thus known from the available literature that the toxicity of various PCB congeners to living organisms is variable, where some fail to bioaccumulate and others have the propensity to bioaccumulate with deleterious effects. However, the total number of congeners is 209. PCB 169 is the only congener with a non-ortho coplanar molecule with a total of six chlorine substituents at the meta- and para-positions of the biphenyl ring. Since literature indicates toxicity as a function of the degree of substitution, 3,3′,4,4′,5,5′-Hexachlorobiphenyl was selected for this study.

The earlier work on the effect of PCBs on aquatic organisms has focused mainly on the intake of PCBs through sediments or through eating sediment-contaminated forms. The current work has focused on a novel approach of direct exposure to PCB, i.e., through the surrounding medium. This would provide insights to the bioaccumulation of PCB in fish directly from the surrounding water, as in case of leakages or spills of PCB in water.

The current investigation aims to explore any variations in the degree of bioaccumulation of PCB Congener 169 $C_{12}H_6Cl_6$ (3, 3′, 4, 4′, 5, 5′- hexachlorobiphenyl) on various tissues of the freshwater fish, Rasbora daniconius, under chronic exposure conditions. This would enable the correlation of the route followed by the PCB Congener with known systemic pathways in the body of the fish, based on the tendencies of the congener to get accumulate at different concentrations in different tissues. Rasbora, often referred to as a “trash fish,” is commonly caught alongside larger food fish in freshwater bodies and is frequently consumed by economically disadvantaged communities.

MATERIALS AND METHODS

Specimens of *R. daniconius* utilized in this study measured an average length of 7 to 10 cm and weighed 20 g. PCB 169 from Dr. Ehrenstorfer, Germany, was employed.

The fish underwent a 10-day acclimatization period in aquaria filled with dechlorinated tap water at room temperature and were fed with commercially available floating fish food. In case of suspected injury or infection, they were given a dip in a 1% solution of Potassium permanganate.

Table 1 details the characteristics of the dilution water utilized in the experiment. Acute toxicity tests conducted in 10 L aquaria yielded LC0, LC100, and LC50 values, determining a sublethal dose of PCB 169 as 25.0 µg l-1, suitable for a 30-day exposure period.

Table 1: Characteristics of dilution water

Parameters	*Values**
Temperature °C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	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	126

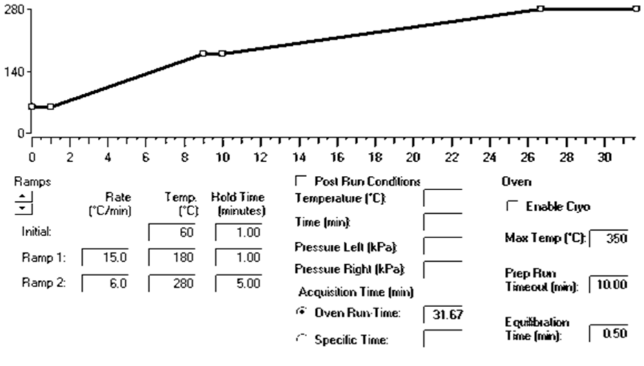
*All the values except temperature and pH are expressed as mg/L

A specially fabricated 20 L capacity aquarium with flow-through capability was filled with dechlorinated tap water containing the sublethal dose of PCB 169 dissolved in dichloromethane. A dosing unit subsequently introduced 20 L of water with the sublethal dose of PCB 169 dissolved in dichloromethane every 24 hours. About 20 healthy fish were utilized, and feeding and solution replacements followed established protocols[19].

Chronic toxicity evaluation involved maintaining another set of 20 fish as controls in a similar aquarium containing only water and dichloromethane, with an identical dosing unit.

Every 5th day for 30 days, 2 to 3 fish were sacrificed to harvest gill, kidney, liver, and intestine tissue for bioaccumulation analysis using GC-MS. Tissue samples were fixed in 4% formaldehyde. Subsequent drying, maceration, and extraction using dichloromethane followed established procedures[20, 21].

Cleanup of tissue extracts was carried out using glass filter columns containing Celite 545 filter aid, with anhydrous Sodium Sulphate to absorb moisture. Extracts underwent washing, concentration, and collection for analysis of PCB 169 accumulated. Analysis was carried out using GC-MS calibrated for PCB analysis, where Mass spectra and standard chromatograms of PCB 169 were obtained, as shown in Figures 1, 2 and 3, respectively.



Ramps

	Rate (°C/min)	Temp. (°C)	Hold Time (minutes)
Initial:		60	1.00
Ramp 1:	15.0	180	1.00
Ramp 2:	6.0	280	5.00

☐ Post Run Conditions
Temperature (°C):
Time (min):
Pressure Left (kPa):
Pressure Right (kPa):
Acquisition Time (min)
● Oven Run-Time: 31.67
○ Specific Time:

Oven
☐ Enable Cryo
Max Temp (°C): 350
Prep Run Timeout (min): 10.00
Equilibration Time (min): 0.50

Figure 1: Chromatographic Conditions for PCB Analysis

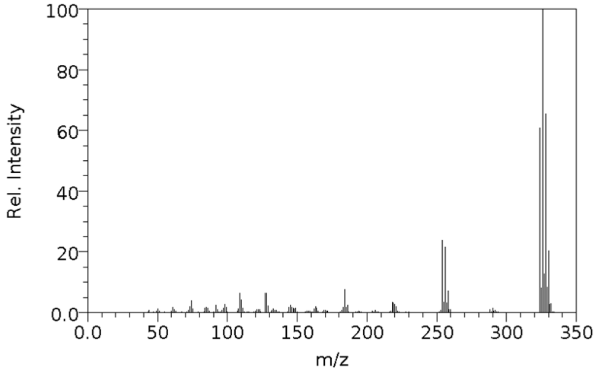


Figure 2: Mass spectra of PCB-169 (3, 3′, 4, 4′, 5, 5′ – Hexachlorobiphenyl)

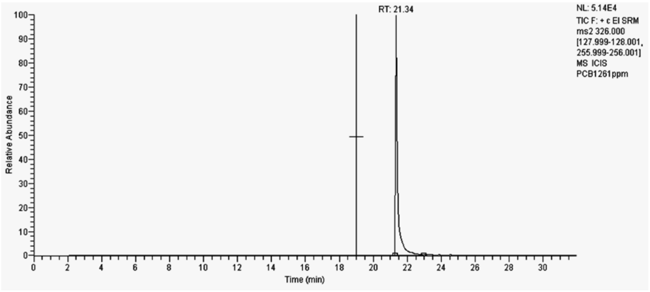


Figure 3: Standard Chromatogram of PCB-169 (3, 3′, 4, 4′, 5, 5′ – Hexachlorobiphenyl)

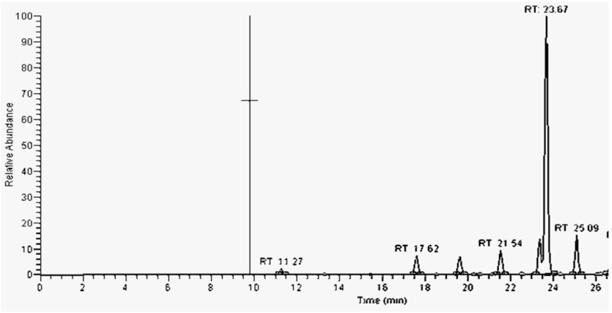


Figure 4: Chromatogram showing PCB 169 in Rasbora Gill (10 Days)

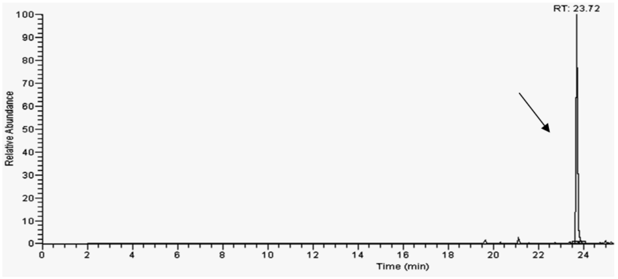


Figure 5: Chromatogram showing PCB 169 in Rasbora Kidney (30 Days)

RESULTS AND DISCUSSION

Tissue samples gathered every fifth day throughout the experiment were subjected to analysis to determine their PCB 169 content. Several chromatograms illustrating the GC-MS analysis are displayed in Figures 4 to 7. These findings are represented in Figure 8.

A progressive rise in bioaccumulation over the 30-day exposure period was noted across all tissues examined, with the highest accumulation being observed in the gills and the lowest in the liver at the end of the period. Gills, essential for extraction of respiratory oxygen, exhibited the highest PCB 169 accumulation. Initially, bioaccumulation was recorded at 1.88 µg/g after 5 days of exposure, and this increased to 7.12 µg/g by the end of the 30-day period, indicating nearly a four-fold increase. This suggests that a minimal amount of metabolic processing of PCB 169 took place within gill tissue as compared to other tissues, leading to their accumulation in them. This observation also highlights the fact that much

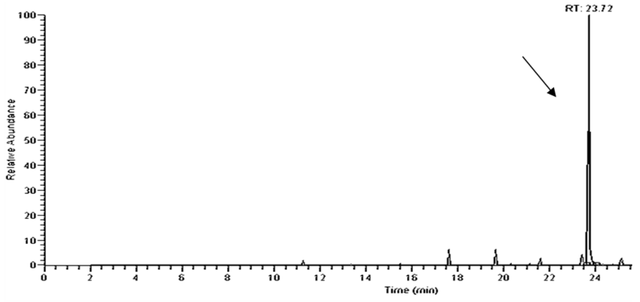


Figure 6: Chromatogram showing PCB 169 in Rasbora Liver (30 Days)

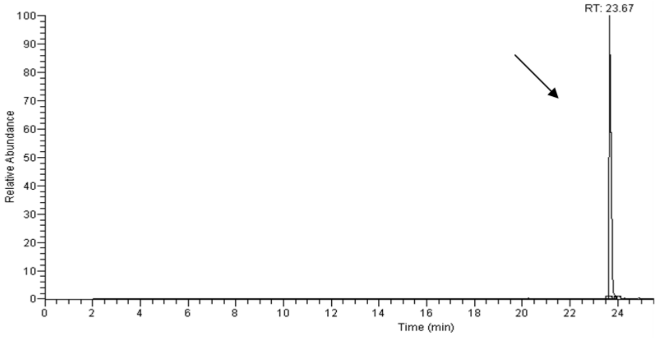


Figure 7: Chromatogram showing PCB 169 in Rasbora Intestine (30 Days)

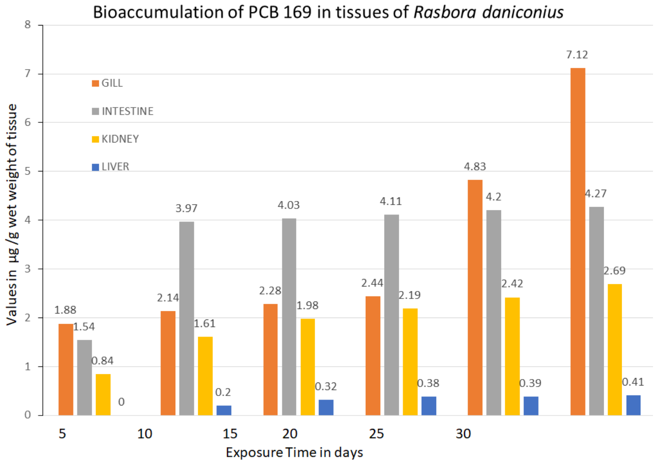


Figure 8: Graphical representation of Bioaccumulation of PCB 169 over 30 days' exposure period

of the PCB 169 stayed back within the gills on exposure to surrounding water, showing inefficient disposal through the efferent branchial vessels of the gills.

Intestinal tissue displayed a slightly lower degree of bioaccumulation, with levels remaining relatively constant throughout the study period (ranging from 3.97–4.27 µg/g). This suggests a tendency for PCB 169 accumulation in the intestine tissue. Further, it may be inferred here that the PCB 169, though absorbed from water in the intestine, did not get transported to the liver for biotransformation. This inference can be supported by the observation that liver tissue exhibited comparatively insignificant and relatively constant bioaccumulation over the 30-day exposure period, with only a slight increase from 0.0 to 0.41 µg/g.

Kidney tissue showed a gradual yet threefold increase in bioaccumulation over the 30-day exposure period, from 0.84 to 2.69 µg/g. This indicates that some PCB could have been said to enter circulation, mainly through gills, was transported to the site of excretion, i.e., the kidneys, and yet could not be efficiently disposed of by the kidneys, as is evident from the above concentration of PCB 169 in the kidney tissue.

In conclusion, PCB 169 demonstrates substantial bioaccumulation in freshwater fish, likely leading to buildup and biomagnification within aquatic ecosystems' food chains, which include other aquatic organisms. The present study found the propensity of PCB 169 to bioaccumulate to vary with the type of tissue. This variability may be used as an indicator of tissue-specific pathology in an environment polluted with PCB 169. It may also serve to trace the routes followed by the PCB in the affected organism. Furthermore, bioaccumulation and biomagnifications of PCB 169 may lead to significant deleterious effects of metabolic as well as histological nature due to its toxic nature across all organisms in food chains in polluted environments, including humans who typically occupy the apex of these food chains.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.