CONGENER SPECIFIC ANALYSIS AND TOXIC EVALUATION OF PCB CONGENERS IN SEDIMENT AND FISH SAMPLES FROM LOWER TENNESSEE RIVER, KENTUCKY, USA

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Abstract

The objective of this study was to determine contamination levels and bioaccumulation of AHH inducing and non-AHH inducing PCB congeners in sediment and fish samples collected from the lower Tennessee River, Kentucky., USA. Fish species analyzed were largemouth bass *(Micropterus salmoides)*; bluegill *(Lepomis macrochirus)*; longear sunfish *(Lepomis megalotis)*; spotted bass *(Micropterus punctulatus)*; gizzard shad *(Dorosoma cepedianum)*; black crappie *(Pomoxis nigromaculatus).* The sediments and fish samples were processed and analyzed using standard methods. The results revealed that, in sediments, total PCBs (excluding AHH inducing PCBs) concentration ranged from 4-5 ng/g and AHH inducing PCBs ranged from 0.29-0.43 ng/g dry wt. In fish samples, total PCBs ranged from 34.77-622 ng/g dry weight and AHH inducing PCBs ranges from 0.35-29.9 ng/g dry wt. Toxic equivalents (TEQs) for dioxin like PCBs in fish ranged from 1.3×10^{-3} ng to 8.3×10^{-2} ng.

Introduction

Polychlorinated Biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins are the members of the group of halogenated aromatic hydrocarbons (HAHs). This group has been identified as priority environmental pollutants posing significant effects on aquatic and terrestrial animals including humans[1-3]. PCBs are highly stable industrial compounds, widely used as dielectric and heat transfer fluids, and flame retardants. Although PCB production and use has been banned over three decades, PCBs are still present in the environment and cause harmful effects. Non-*ortho*-chlorine substituted PCBs are considered highly toxic, as it elicit toxic effects similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin[4,5]. Therefore, congener specific analysis is essential for proper risk assessment due to PCB contamination in environment and biological samples. Very limited information is available on contamination profiles of aryl hydrocarbon hydroxylase (AHH) enzyme inducing dioxin-like (coplanar) PCBs and di-*ortho*-chlorine substituted PCBs. The objective of this study was to determine contamination levels and bioaccumulation of AHH inducing and non-AHH inducing PCB congeners in sediment and fish samples collected from the lower Tennessee River, Kentucky. Fish species analyzed were largemouth bass *(Micropterus salmoides)*; bluegill *(Lepomis macrochirus)*; longear sunfish *(Lepomis megalotis)*; spotted bass *(Micropterus punctulatus)*; gizzard shad *(Dorosoma cepedianum)*; black crappie *(Pomoxis nigromaculatus).* Very little information is available on the AHH inducing PCBs in sediment and fish from the lower Tennessee River, Kentucky. In this study we analyzed AHH inducing PCBs as well as other PCBs (mono- through decachlorobiphenyls) in the sediments and fish collected from the selected locations lower Tennessee River (Figure 1). Lower Tennessee River is the downstream of Kentucky Lake. A number of industries in the Calvert City Industrial Complex discharge effluents into this river. Lower Tennessee River joins Ohio River in the north (Figure 1).

Methods and Materials

Figure 1 shows sediment and fish sampling location in downstream of Kentucky Lake .

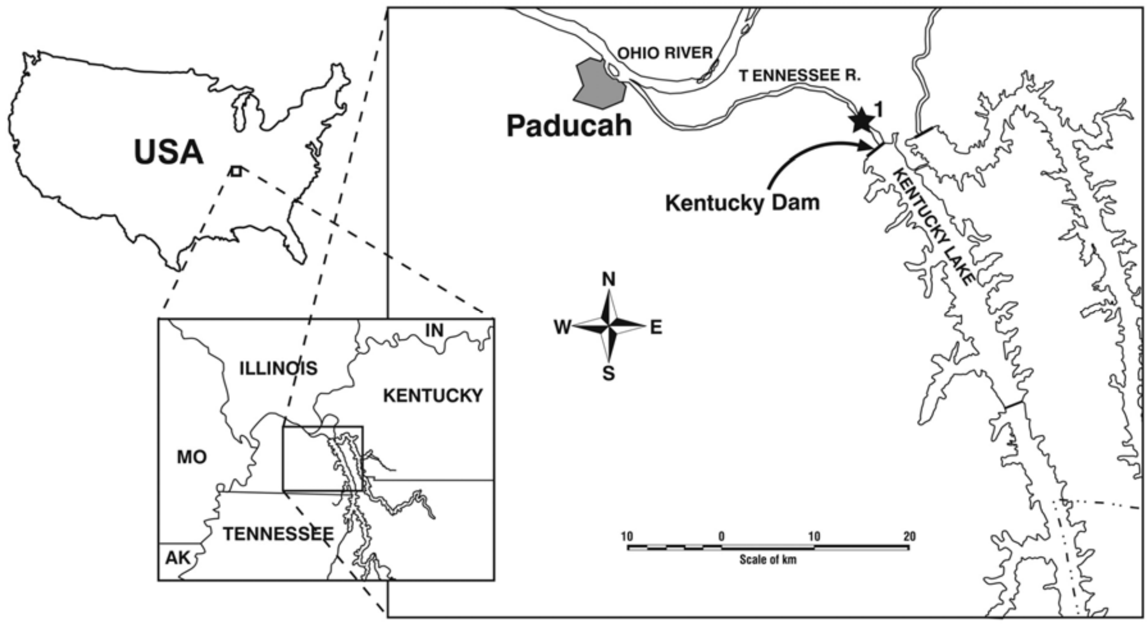

Figure 1. Map showing sediment and fish sampling location (*)

Sediment and fish samples were collected and stored under < -20°C until analysis. Fish samples were identified and measured. Table 1 shows morphometric (total length, standard length, total weight) characteristics of fish samples analyzed. Pooled samples were prepared by dissecting and mixing aliquot of edible portion of fillet. (Table 1) .

Table 1: Details of fish samples obtained from lower Tennessee River, Kentucky, USA

Fish (common name)	Number of fish pooled	Total length (mm)	Std length (mm)	Total weight (g)	Amount used for extraction (g. dry wt)
Black crappie	n=1	240	204	286	6.0
Longear sunfish	n=4	137-153	106-130	47-88	6.0
Spotted bass	n=4	162-180	138-149	58-72	6.0
Gizzard shad	n=4	169-191	46-59	46-59	6.0
Bluegill	n=4	137-143	46-57	46-57	6.0
Largemouth bass	n=1	351	315	768	6.0

The pooled samples were homogenized and freeze dried prior to extraction. Analytical procedures followed were as follows: Approximately 25g and 6g of dry weight of sediment and fish samples respectively were extracted using Soxhlet extraction with 3:1 hexane and acetone mixture. The extract was then concentrated to 10 ml using Rotary evaporator. An aliquot of the fish extract was used for fat content analysis. The extract was concentrated to 5-ml under a gentle stream of nitrogen gas and fish samples extracts were subjected to Florisil column chromatography to remove lipids. Sediment sample extracts were subjected to copper treatment to remove elemental sulfur and sulfuric acid treatment to remove interfering substances. Then the sample extracts were subjected to silica gel column chromatography to remove interfering organic and polar species and to separate PCBs from pesticides. First fraction F1 was eluted using 110ml of hexane in which PCBs, 4,4'-DDE, HCB and trans-Nonachlor were eluted. The second fraction (F2) eluted most of the pesticides and PBDEs with 120 ml of 20% methylene chloride in hexane. F1 fraction was concentrated using Rapidvap to 1ml and 1µl was injected into the gas chromatography equipped with electron capture detector (GC-ECD). GC conditions were: initial temperature: 90ºC; hold time: 1 minute, ramp 1: 90°C to 200°C @ 10°C /min.; hold time: 0 minute, ramp 2: 200°C to 280°C @ 1°C /min.; hold time: 20 min., Injection port temperature 280°C, Detector temperature: 330°C, Carrier gas velocity: Helium @ 1.1 ml/min. the standard reference material SRM 2262 obtained from National Institute of Standards and Technology was used for the quantification of PCB congeners. AHH inducing PCBs were purchased from Ultra Scientific, Kingston, RI, USA. Appropriate quality assurance quality control analysis were performed including reagent blank, NIST QA fish sample, calibration curve with r^2 value of 0.99, surrogate standard and matrix spike recoveries (100 ±30%).

Results and Discussion

Generally PCBs are insoluble in water. Due to their low water solubility and high organic matter partition coefficient, PCBs attach to the surface of the clay particles and organic matters that are suspended in water or have already settled to the bottom. In the sediment samples, comparable concentrations were found in all the sites of the location and AHH inducing PCBs were minimal when compared to the total PCBs. Total PCBs ranged from 4-5 ng/g of dry wt and concentration of AHH inducing PCBs ranged from 0.29-0.43 ng/g of dry wt as as shown in Figure 2. The range of total PCB concentration was relatively high[6] (ranged from 44-1000 ng/g of dry wt) as reported earlier but when compared to the present results there is a drastic decrease in the concentrations during the last ten years.

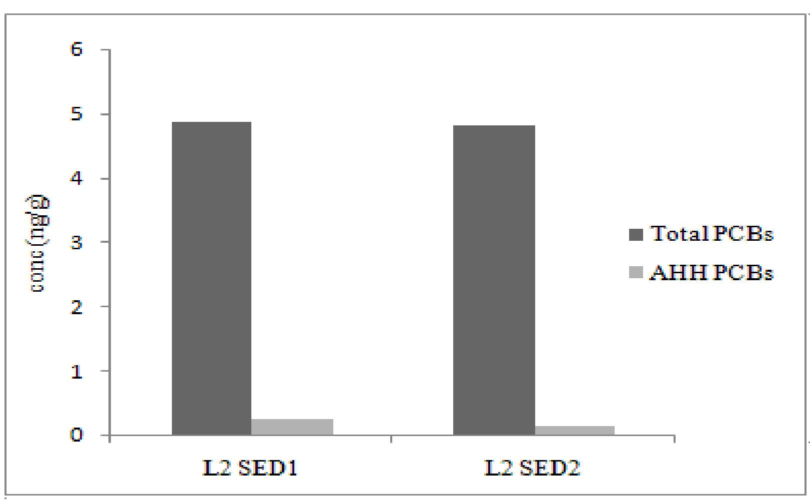

Figure 2: PCB concentrations in sediment samples from Kentucky Lake, USA.

PCBs are lipophillic and are rapidly accumulated by aquatic organisms and easily bioaccumulate in the food chain. From the earlier studies it was clear that Black crappie (a predator fish) has higher concentrations of PCBs than a phytoplankton feeding fish[7]. Detectable amounts of AHH inducing PCBs were found in all the samples analyzed. The concentration of total PCBs and AHH inducing PCBs is higher in fish than in the sediment samples. This difference in concentration in fish may be due to the bioaccumulation in the fat tissues. Age, fat content and feeding habits of the fish are the major factors that influence the bioaccumulation of PCBs in fish. It is clear from the results (Figure 3) that the concentration of the AHH inducing PCBs were much lower than the concentration of the non-AHH inducing PCBs in all the species of fish. Comparatively higher concentration was found in the gizzard shad and the lowest concentration was found in spotted bass (Figure 3).

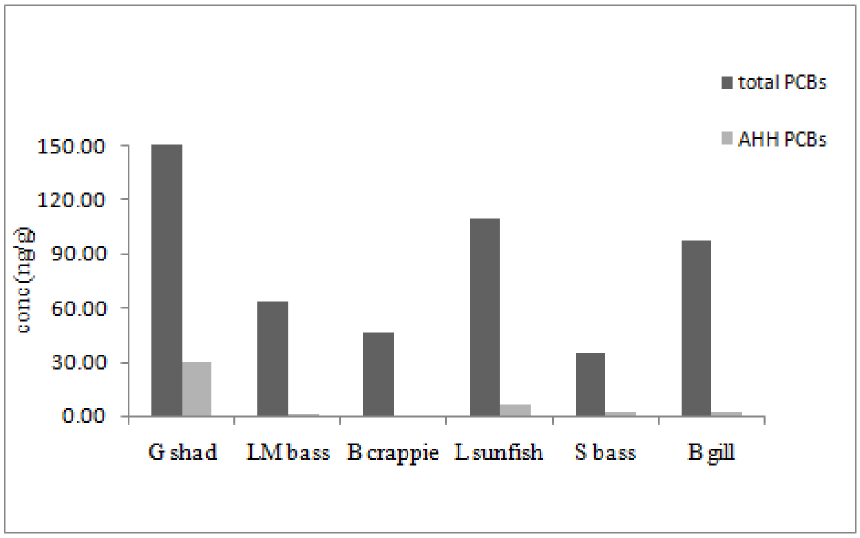

Figure 3: PCB concentrations in fish samples from Kentucky Lake, USA.

These results indicates that the PCB congeners bioaccumulate along the food chain. Toxic potential of PCBs depends on number and position of chlorine atom attached to biphenyl rings. The most toxic PCB congeners are those that have chlorine substitution in the non-*ortho* positions. Specific dioxin-like PCBs are more toxic than the other PCB congeners and they are assigned with the toxic equivalent factors based on the relative toxicity with dioxin[1,5,6]. The most toxic dioxin is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is assigned a value of Toxicity Equivalence Factor of 1.0 . They are 3,4,4',5-tetrachlorobiphenyl (PCB-81), 3,3',4,4'-tetrachlorobiphenyl (PCB-77), 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126), 3,3'4,4',5,5'-hexachlorobiphenyl(PCB-169) with TEF values of 0.0005,0.0001, 0.005, 0.00005 respectively. The TEQ values for the PCB congeners were calculated by multiplying the concentration of the PCB congener with its TEF value. Among all fish samples analyzed, gizzard shad had the highest TEQ of 83.23pg (Figure 4).

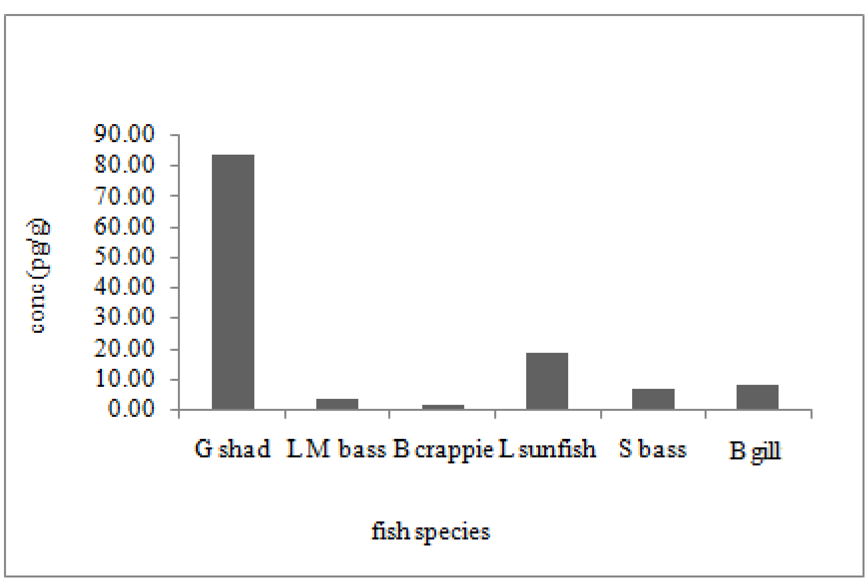

Figure 4: TEQ values of fish samples from Kentucky Lake, USA.

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