ORGANOHALOGEN POLLUTANTS IN SEDIMENT AND FISH SAMPLES COLLECTED FROM CLARKS RIVER, KENTUCKY, USA

Vidyasagar Kummarikunta, Nanditha Billa, Bommanna G. Loganathan

Department of Chemistry and Watershed Studies Institute, 1201 Jesse D. Jones Hall, Murray State University, Murray, KY 42071-3300, USA.

Abstract

Polychlorinated biphenyls (PCBs), chlorinated pesticides and polybrominated diphenyl ethers (PBDEs) were analyzed in sediment and fish samples collected from selected locations in Clarks River, Kentucky, USA. Standard procedures were followed to analyze the target compounds using GC-ECD. The results revealed that total PCB concentrations in Clarks river sediment samples found in the range of <0.1 ng/g dry weight to 1.29 ng/g dry weight followed by chlorinated pesticides in the range of <0.1 ng/g dry wt to 3.57 ng/g dry wt and PBDE in the range of <0.1 ng/g dry wt to 3.12 ng/g dry wt. Total PCB concentrations in the fish samples are in the range of 0.4 ng/g dry wt to 6.44 ng/g dry wt followed by chlorinated pesticides are in the range of 0.1 ng/g dry wt to 133 ng/g dry wt and PBDE are in the range of <0.1 ng/g dry wt to 7.34 ng/g dry wt. PCB 66, 101 and 153 were the prominent congeners detected, and in chlorinated pesticides, *trans* nonachlor and DDE were prodominant. In PBDEs, PBDE 47, 30 and 100 are prominent peaks present in all of the samples analyzed. The levels of PCBs and chlorinated pesticides in Clark's River fish were below the Food and Drug Administration (FDA) established limits for human consumption.

Introduction

Organohalogen compounds, such as polychlorinated biphenyls (PCBs), chlorinated pesticides and polychlorinated diphenyl ethers (PBDEs) are well known persistent organic pollutants, bioaccumulate and biomaginfy in food chain and cause harmful effects in wildlife and humans[1,2]. Although PCBs and several chlorinated pesticides were banned production and use in the United States more than three decades ago, residues of these compounds are still found in environmental and biota. PBDEs are currently being used in a variety of household products as flame retardant and increased production from 40,000 lb in 1992 to 147,985,293 pounds in 1999[3]. PBDE are used as additives in polymers, and also in computers, plastics and televisions. The major concern regarding these compounds is that they can persist in the environment, bioaccumulate in organisms and may cause toxic health effects. Several studies have demonstrated that environmental exposure to these compounds produce adverse effects including neurotoxicity, reproductive failure developmental behavioral abnormalities and carcinogenic effects in wildlife and humans[4,5]. Earlier studies on these compounds in western Kentucky region were mainly focused on Kentucky Lake, Barkley Lake and Lower Tennessee river[6]. However, very limited information is available on these persistent organic pollutants in Clarks River sediment and fish. Clarks River receives discharges from adjacent agricultural, some industrial wastewaters from western Kentucky. The objective of this study was to describe contamination profiles of PCB congeners, Chlorinated pesticides and PBDEs in sediment and fish samples collected from Clarks River and also to find out whether the fish samples collected from this river are safe for human consumption.

Materials and Methods

Figure 1 shows the map of the Clarks River in western Kentucky and sampling locations. Three sites were selected for sampling sediment and fish samples. Site 1(36° 36'764'' N and 88° 17' 279'' W) (near I-94 bridge) predominantly receive agricultural drainage. Site 2 (Squire Holland Rd. bridge: 36°39'238'' N &

88°16' 766'' W), upstream of this site receives discharges from three local chemical industries and some agricultural drainage. Site 3 (Almo Road: 36°41'499'' N & 88°16' 393'' W), upstream of this site receives discharges from adjacent farms and local domestic/residential discharges. The coordinates of the sample locations were determined by Garmin GPS. Sediment samples were collected using a garb sampler and fish samples were collected by using backpack electro fishing method. Samples were placed in a cooler with ice and transported to the laboratory. After sampling, the fish samples were identified and measurements (i.e total length and standard length) were made (Table 1). The sediment and dissected fish samples were transferred to a pre-cleaned I-chem. bottles and stored under < -20°C until further analysis. The sediment and fish samples were freeze-dried for 72 hours using the Labconco freeze dry system (model 7753).

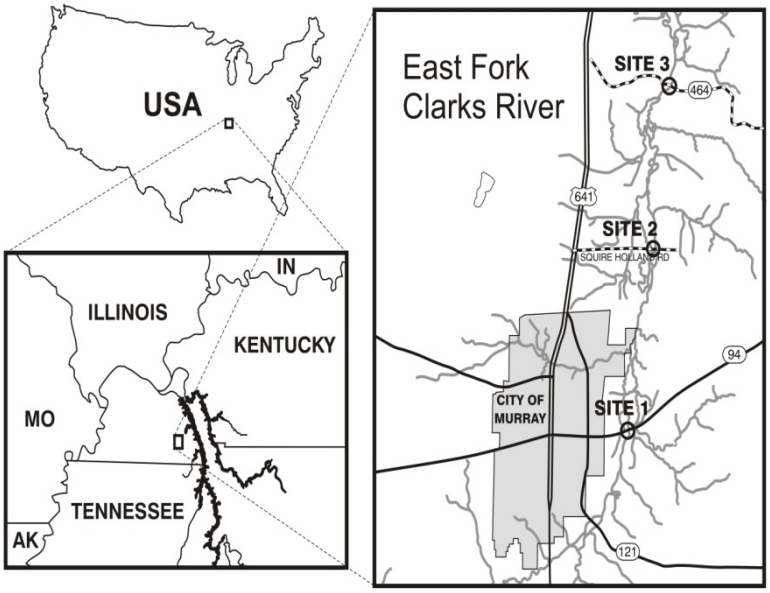

Figure 1. The map showing Clarks River and sampling locations.

PCB congeners, chlorinated pesticides and PBDE were analyzed in sediment and fish samples by using standard procedures[7]. The freeze-dried sediment sample (approximately 25 g) or freeze-dried fish sample (approximately 6 g) were Soxhlet extracted using 3:1 methylene chloride and hexane mixture for 17 h. 4, 4'-dibromooctaflurobiphenyl (DBOFB) in hexane, was used as the surrogate standard and was spiked in each sample before Soxhlet extraction.

Sediment: After extraction, the sample extracts were concentrated to 10-mL using Rapid Vap Labconco Evaporation system and exchanged to hexane. Elemental sulfur present in the sediment samples was removed using freshly activated copper treatment. Then extract was concentrated to 5 mL using gentle stream of nitrogen gas and then subjected to silica gel column chromatography to separate the PCBs from pesticides. In the first fraction (F1), PCBs, HCB, 4, 4'- DDE, Aldrin and trans- Nonachlor were eluted using 110 mL of ultra pure hexane. The second fraction (F2) containing most of the chlorinated pesticides and PBDEs were eluted with 120 mL of 20% methylene chloride in hexane.

Fish: After extraction, the samples was then concentrated to 10 mL using Rapid Vap Labconco Evaporation system. To remove the fat from the fish samples, Florosil dry column chromatography technique was followed. Then extract was concentrated to 5 mL using nitrogen gas and then subjected to silica gel column chromatography to separate the PCBs from pesticides.

The fractions (F1 &F2) extracts were concentrated to Rapid Vap apparatus to 10 mL followed by evaporation to 1 mL by using a gentle stream of nitrogen gas. Then extract was transferred to auto sampler vial and 1µL was injected on to gas chromatograph equipped with electron capture detector (GC-ECD).

Table 1. Details of fish samples collected from Clarks River, Kentucky, USA. (TL: Total length; SL: Standard length).

Location	Common Name	*Scientific Name*	TL (cm)	SL (cm)	Weight (gm)
Site 1	Creek Chub Suckers (CCS)	*Erimyzon oblongus*	11.6-12.4 (n=3)	10.4-10.5	21.0-22.2
	Creek Chubs (CC)	*Semotilus atromaculatus*	10.4-10.9 (n=2)	8.7-8.9	12.2-13.3
	Longear Sunfish (LS)	*Lepomis megalotis*	6.5-9.9 (n=6)	5.1-7.9	4.64-15.6
	Spotted Sucker (SS)	*Minytrema melanops*	15.2-18.3 (n=3)	12.5-15.6	33.8-67.2
	Large Scale Stone Roller (LSR)	*Campostoma oligolepis*	10.6-11.7 (n=4)	8.9-9.9	11.4-15.2
	Large Mouth Bass (LMB)	*Micropterus salmoides*	9.1-11.5 (n=3)	7.6-9.6	8.45-17.0
	Golden Red Horse (GRH)	*Movostoma punctulatus*	17.0-19.0 (n=3)	14.2-15.7	57.7-73.1
Site 2	Spotted Bass (SB)	*Micropterus punctulatus*	7.8-17.0 (n=2)	6.5-14.1	5.05-56.9
	Longear Sunfish (LS)	*Lepomis megalotis*	9.0-11.5 (n=4)	7.2-9.5	13.0-31.0
	Stone Roller (SR)	*Campostoma oligolepis*	10.0-12.2 (n=4)	8.5-10.3	12.8-18.0
	Steel Color Minnows (SCM)	*Cypfinella whipplei*	4.5-7.5 (n=10)	3.7-6.2	0.63-3.06
	Bigeye Shinner Minnows (BSM)	*Notropis boops*	5.5-6.4 (n=10)	4.4-5.3	1.05-2.05
Site 3	Bluegill Sunfish (BS)	*Lepomis macrochirus*	8.4-11.5 (n=3)	6.7-9.4	9.10-27.7
	Longear Sunfish (LS)	*Lepomis megalotis*	9.3-10.8 (n=2)	7.4-8.7	13.0-21.5
	Spotted Sucker (SS)	*Minytrema melanops*	11.2-12.1 (n=5)	8.7-9.9	11.3-15.7

PCB congeners, Chlorinated pesticides and PBDE congeners were analyzed using a Varian model CP-3380 gas chromatograph (GC) with Varian model CP-8410 auto injector. The GC system was equipped with a DB-5 capillary column (60m length; 0.25mm i.d.; 0.25µm film thickness, J&W Scientific, USA) and a ^{63}Ni electron capture detector (ECD). The initial column temperature is 90 ˚C with a 1 min hold time and increased at the rate of 5°C to 150°C and ramped at the rate of 2°C to 280°C with a hold time of 20 min. The injector and detector temperatures were 270°C and 330°C respectively. Helium (1.5 mL/ min) and nitrogen (28.5mL/min) were used as a carrier and make up gases respectively. The standard reference material SRM 1941b of organics of marine sediment and SRM QA02FSH5/ Fish homogenate V obtained from National Institute of Standards and Technology were used as quality assurance and quality control samples. Twenty eight PCBs congeners, 15 different chlorinated pesticides and 11 PBDEs congeners were analyzed. PCB congeners, chlorinated pesticides and PBDEs congeners were identified in the sample extract by comparing the retention times from the standard mixtures and quantified from the ECD response and response factors. Appropriate quality assurance and quality control analysis was performed including: reagent blank, calibration curve with r^2 value of 0.99, surrogate recovery (4,4'-dibromooctafluorobiphenyl), NIST SRMs and matrix spike recoveries were 100± 30%.

Results and Discussion

Table 2 shows total PCBs (sum of all detected congeners), 15 different chlorinated pesticides and total PBDEs in sediment samples collected from Clarks River.

Table 2. Concentrations of organohalogen compounds in sediment samples collected from Clarks River.

LOCATION	ΣPCB CONGENERS (NG/G DRY WT.)	ΣPBDE CONGENERS (NG/G DRY WT.)	OCP (NG/G DRY WT.)
Site 1	4.36	3.92	3.85
Site 2	3.37	0.43	1.57
Site 3	5.51	2.05	2.99

Detectable amount of the PCBs, chlorinated pesticides and PBDEs are found in all sediment samples. Relatively high concentrations of the PCBs were found in the Site 3 followed by site 1 and the site 2. Due to limited number of samples analyzed, non-availability of age data of fish, temporal trend cannot be explained. Considering the congener composition, PCB 66/95 is in highest concentration followed by PCB 101, 153. In general, the penta, hexa chlorinated PCB congeners contain relatively higher concentrations than lower chlorinated PCB congeners. The high concentrations of the PBDEs are observed in samples collected from site 1 compared with Site 3and Site 2. The PBDE 47 is in higher concentration followed with PBDE 30, 100 and 99. Among the chlorinated pesticides, trans- Nonachlor, DDE were most frequently detected.

Concentrations of PCBs, PBDEs and Chlorinated pesticides in fish samples collected from Clarks River are shown in Table 3. The total PCBs concentrations in Clarks River fish samples ranged from 10.8 ng/g dry wt to 157.1 ng/g dry wt. The total PBDEs concentrations ranged from 4.4 ng/g dry wt to 97.2 ng/g dry wt. The total chlorinated pesticides concentrations ranged from 20.9 ng/g dry wt to 427 ng/g dry wt. Among the fish samples, shiner minnows (SM) showed the greatest total PCB congeners concentration (157.1 ng/g dry wt) and the lowest concentrations was showed in creek chub sucker (10.8 ng/g dry wt). In the PCB homolog composition revealed that penta and hexachlorobiphenyl contributed greater percentage than mono- and decachlorobiphenyls. Among the total chlorinated pesticides, the creek chubs showed in greatest concentration (427 ng/g dry wt) and creek chub suckers showed in lowest concentration (20.9 ng/g dry wt). Among the chlorinated pesticides, the 4,4' DDE was found to be highest concentrations in all the fish samples followed by the trans- nonachlor, cis chlordane and Dirldrin. The steel color minnows showed the greatest concentration (97.2 ng/g dry wt.) of PBDEs congeners and the lowest concentrations (4.4 ng/g dry wt.) showed in the creek chub suckers. The PBDE 47 is one of the most stable congeners of PBDE, which shows in higher concentrations (56 ng/g dry wt.) compared with other PBDE congeners.

Table 3. Concentrations (ng/g dry wt) of fish samples collected from Clarks River.

Location	Species	ΣPCBs	ΣPBDEs	OCP
SITE 1	Creek Chubs Suckers (CCS)	10.8	4.4	20.9
	Creek Chubs (CC)	97.9	8.6	427
	Longear Sunfish (LS)	55.2	32.3	76.2
	Spotted Sucker (SS)	66.3	35.8	90.4
	Large Scale Stone Roller (LSR)	29.9	13.6	260
	Large Mouth Bass (LMB)	114.0	14.3	130
	Golden Red Horse (GRH)	101.0	6.8	126
SITE 2	Spotted Bass (SB)	57.8	83	210
	Longear Sunfish (LS)	50	76	85.0
	Steel Color Minnows (SCM)	157.1	97.2	212
	Bigeye Shinner Minnows (BSM)	173	68.3	182
	Stone Roller (SR)	23.4	2.81	85.7
SITE 3	Bluegill Sunfish (BS)	15.8	12.5	29.6
	Longear Sunfish (LS)	38.1	78.5	84.5
	Spotted Sucker (SS)	28.9	5.77	109

Table 4 show the food Drug and administration (FDA) action levels for PCBs and selected Pesticides in fish for human consumption.

Table 4. FDA action levels for PCBs and selected pesticides in fish for human consumption

Substance	Action Level
PCBs	2ppm
DDTs	5ppm
HCB	0.2ppm
Cis-chlordane	0.3ppm

The fish samples concentrations were compared with the FDA limits. Total PCBs congeners concentrations of Clark's River fish samples were below the action levels of the FDA. DDTs,HCB and cis-Chlordane concentrations were also below the FDA action levels established for human consumption. The results provide evidence that PCBs, chlorinated pesticides and PBDEs are prevaent in the sediment and fish samples collected from Clarks River. Further analysis is needed in to confirm the contamination levels as well as temporal trends of PCBs, chlorinated pesticides and PBDEs. The results showed that concentrations of the fish samples are much below the action levels of the Food Drug Administration (FDA), therefore, fish from Clarks River are safe for the human consumption. As PBDEs are currently used in variety applications, concentrations of PBDE in environmental (sediment) and biological samples (fish) may increase in the future.

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