Chlorophenol and Alkylphenol Concentrations in Sediment and Mussel Tissues Collected from Selected Locations in Kentucky Lake, USA

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Introduction

Pentachlorophenol (C_6HCl_5O; MW: 266.35) and nonylphenol ($C_{15}H_{24}O$; MW: 220.17) are synthetic organic chemicals that do not occur naturally. Pentachlorophenol (PCP) was one of the most widely used biocides in the United States. Chlorophenols (CPs) are used extensively in cleaning products, paints, herbicides, pesticides, mollucicide, algicide, disinfectant, as an ingredient in antifouling paint, paper production, textile manufacturing and several other consumer products[1,2]. Widespread use of CPs, particularly, PCP has resulted in the detection of its residues in air, rain, snow, groundwater, surface water, waste wood, fish, aquatic invertebrates, as well as human urine, blood and milk[1, 3-5]. PCP is regarded toxicologically relevant as a priority pollutant and its background level in the environment is a matter of ongoing concern. Furthermore, the production of tetrachlorophenol and petachlorophenol results in release of highly toxic and environmentally stable impurities such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)[3]. Alkylphenols (APs) such as nonyl- and octylphenol are products of aklyphenol polyethoxylates (APEO) degradation[6,7]. 4-Nonylphenol (NP) may be produced from trimerized proplylene and phenol giving rise to several isomers. 4-Octylphenol (OP) is synthesized from dimerized isobutylene. APs are used as nonionic surfactants in paints and detergents. Since AP's persistent and hydrophobic characteristics, these chemicals adsorb to particles and accumulate in sediments. These chemicals are redistributed by sediment mixing and bioaccumulate in the aquatic food chain and affect the health of the nearby wildlife and human populations.

Kentucky (KY) Lake (Figure 1) is one the major human-constructed lakes in the US. It serves as an ultimate repository of substances entering this watershed from portions of seven southeastern states, which include a sizeable fraction of the U.S. chemical processing, agricultural chemical products and electronics manufacturing industries[8]. Although a few studies have examined the levels of chlorinated organics in the KY Lake

and the lowermost Tennessee River, there have been no reports on the distribution on the levels of chlorophenols and alkylphenols in sediment and/or biological tissues from this region. In this study, sediment, and freshwater mussels were collected from selected locations in KY Lake and Lake Barkley and analyzed for CPs and APs. Furthermore, wood samples from abandoned docks, navigational towers and wood found in the lake bottom were also analyzed to examine the sources of CPs to the lakes.

Materials and Methods

Open circuit SCUBA diving was used to collect samples from Lake Barkley and Kentucky Lake. The samples were collected during the months of August through December 2003. Surface sediments samples (~0-5 cm) were collected using pre-cleaned I-CHEM jars. All samples were stored on ice on site. Mussels were separated by species and age. Prior to storage, length, width, height, and mass was measured for each mussel. Samples were stored at –20ºC in pre-cleaned I-CHEM jars until further analysis. The chlorophenol analytical method used was based upon Becker *et al.*[4]. Instrument was calibrated using series of known amount of standards. Calibration R^2 >0.97 and response factors were calculated. Dibromooctofluorobiphenyl, and 2,4,6-Tribromophenol (TBP) were used as internal standards. The average recovery of TBP was 108%.

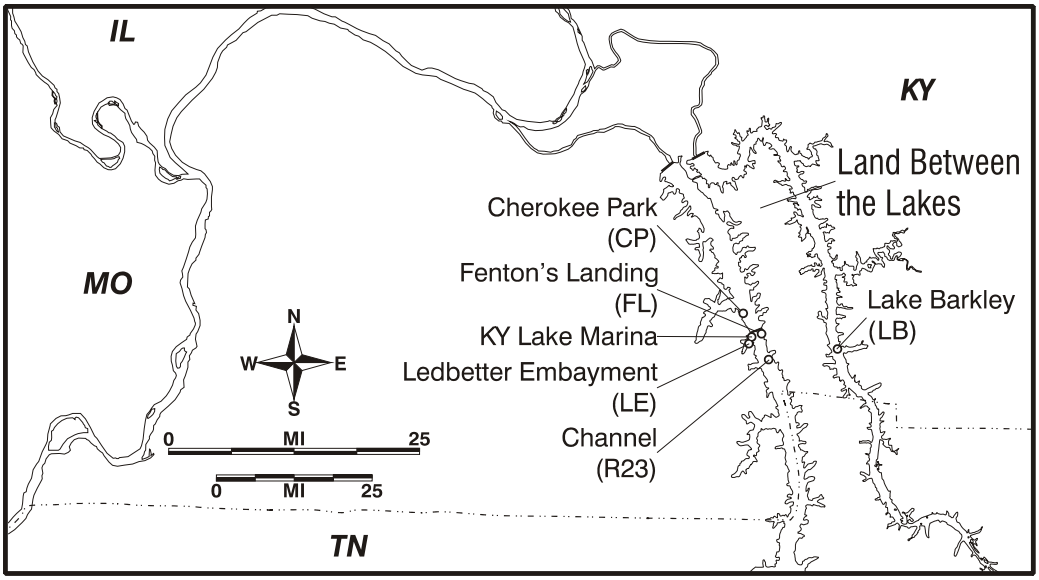

FIGURE 1 Map showing sampling locations in the Kentucky Lake and Lake Barkley, USA.

Sediment and mussel samples were freeze-dried using FreeZone Freeze Drr System Model 77535 and homogenized using mortar and pestle. The samples were Soxhlet extracted for

17 hours with 3:1 ratio of methylene chloride and hexane. The extracts were rotary evaporated to smaller volume. Sediment extracts were subjected to activated copper treatment to remove sulfur. In the mussel samples, lipid removal was performed using Florisil dry column method. The extracts were fractionated and purified by eluting through 10g of Florisil packed in a glass column (10 mm i.d.). The first fraction (F1) containing PCBs and some pesticides were eluted using 75mL of hexane. In Fraction 2, PAHs and other pesticides (PCB's and pesticide data are not presented in this paper) were eluted using 100mL of 20% methylene chloride in hexane. CPs, NP and OP were eluted using 150mL of 50% methylene chloride in methanol.

An aliquot of Fraction 3 was transferred to methanol, acetylated with K_2CO_3 solution/acetic anhydride, liquid/liquid extraction with 20 mL of ultrapure hexane, and analyzed for CPs using gas chromatography coupled with electron-capture detector (GC-ECD)[4]. The gas chromatograph was equipped with a DB-5 capillary column (60 m long x 0.25 mm i.d. x 0.25 micron film thickness) interphased with a ^{63}Ni electron capture detector. The initial temperature was 90°C. This was ramped at 5 °C/min. up to 200°C. The second ramp was 10°C/min. up to 280°C with a hold time of 35 minutes. The carrier and makeup gases used were helium (2 mL/min) and nitrogen (28 mL/min) respectively. Wood samples were cut into small slivers and extracted using methanol by sonication at 40 ˚C for 1 h. The extract was concentrated under a gentle stream of nitrogen gas, acetylated, and analyzed as described above.

Another aliquot of Fraction 3 was concentrated and solvent exchanged to acetonitrile. The extract was analyzed for NP and OP using reverse-phase high-performance liquid chromatography equipped with a C18 column coupled with a fluorescence detector (Shimadzu RF-10AXL). The HPLC was programmed to inject a 10 µL. Mobile phase comprised of HPLC grade acetonitrile and HPLC grade deionized water (50%-98% gradient of acetonitrile over 20 minutes). The fluorescence detector excitation wavelength was set at 229 nm and the emission wavelength at 310 nm.

Results and Discussion

Table 1 shows the concentrations of various CPs and APs detected in sediments from Kentucky Lake and Lake Barkley. Escher and Schwarzenbach[9] reported that pK_a 's of selected CPs (mono- to pentachlorophenols) ranging from 5.0 to 9.4. The pK_a 's provide strong evidence that most chlorophenol compounds will be in an ionized state, therefore, leading to greater retention in sediment and biological medium. Due to this chemical property, measurable concentrations of CPs accumulated in sediments from Kentucky Lake sediments (Table 1).

TABLE 1. Concentrations (ng/g dry wt.) of chlorophenols (DCP=dichlorophenol, TCP=trichlorophenol, TeCP=trachlorophenol, PCP= pentachlorophenol) and alkylphenols (APs: OP= octylphenol, NP= nonylphenol) in sediments from Kentucky (KY) Lake and Lake Barkley, USA.

Location	DCP	TCP	TeCP	PCP	OP	NP
LE	9.5	BDL	BDL	59.0	BDL	113
KY Lake Marina	15.0	BDL	7.32	BDL	BDL	6.85
R23	15.1	BDL	BDL	BDL	BDL	12.4
CP	12.2	BDL	5.70	45.1	24.46	74.1
Fenton	15.6	BDL	5.40	BDL	24.72	62.9
LB1	13.8	5.4	5.90	86.0	6.15	950
LB2a	10.9	BDL	BDL	BDL	BDL	7.83
LB3	15.5	BDL	5.90	45.9	BDL	1100
LE High Pool	15.3	BDL	5.40	41.3	2.54	16.1
KY Marina High Pool	14.4	BDL	1.10	14.7	3.57	3.23

BDL: Below the detection limit (0.6 nanogram), LE: Ledbetter Embayment, LB: Lake Barkley, R23= Channel, CP;Cherokee Park.

Among the various analytes measured, dichlorophenol was detected all samples analyzed, while nonylphenol and dichlorophenol were the only analytes detected in all sediment samples. Trichlorophenol (TCP) was not detectable in most of the sediment samples. Among the CPs measured, PCP concentrations were the greatest and it ranged from below detection limit (BDL) to 86 ng/g dry weight of sediment. Percent detects for NP was found higher than OP deteects. Sediments in Ledbetter Embayment, Cherokee Park, and Fenton's Landing showed greater levels of NP than R23, probably due to the increased sedimentation of embayment in comparison to channel locations (Table 1).

Table 2 shows the CP and AP concentrations in freshwater mussel tissues from Kentucky Lake. In general, the concentrations of the analytes determined in mussels were one to two orders of magnitude higher than sediment samples. As observed in sediments, DCP was detected in all freshwater mussel tissues analyzed. Trichlorophenol was found in mussel tissues from Kentucky Lake, but not detected in sediments from Kentucky Lake. Also, TCP and TeCP levels were lower than DCP and PCP.

TABLE 2. Age (yrs.), sampling location (SL), and chlorophenol concentrations (ng/g dry wt.) for various species of the mussels collected from Ledbetter Embayment and channel site in Kentucky Lake.

Species	SL	Age	DCP	TCP	TeCP	PCP	OP	NP
Fusconaia flava	LE	8	170	BDL	70	660	BDL	580
Fusconaia flava	LE	11	140	BDL	30	890	BDL	171
Quadrula quadrula	LE	11	250	54	50	1500	BDL	BDL
Quadrula quadrula	LE	11	190	53	BDL	1430	BDL	BDL

Plectomerus dombeyanus	LE	12	190	BDL	BDL	1670	BDL	BDL
Plectomerus dombeyanus	LE	11	230	70	BDL	1500	BDL	BDL
Megalonaias nervosa	R23	20	140	49	49	980	278	2950
Amblema plicata	R23	15	205	47	58	1290	245	3440
Amblema plicata	R23	14	210	40	50	2270	243	1680

BDL: Below the detection limit (0.6 nanogram).

Similar to sediments, PCP concentrations in freshwater mussel tissues were the greatest among all the CPs analyzed. More chlorinated phenols (example. Tetra and pentachlorophenols) have increased ability to reside in organic matter than mono- and dichlorophenols due to higher lipophilicities[10]. In this study, PCP concentrations were the highest in both sediment and in mussel tissues, corroborating the above property. NP was present in more mussel samples than OP and was found in larger concentrations. Octylphenol levels were lower and less prominent than nonylphenol due to a lower industrial use. Nonylphenol polyethoxylates make up approximately 80 percent of the APEO produced, while octylphenol is the main constituent of the remaining 20 percent[11-13]. Concentrations of the analytes recorded in KY Lake were relatively lower than those in sediments from Tokyo metropolitan area (0.03-13 μg/g)[12] and waste water, septage, ground water from Cape Cod, Massachusetts (0.003-0.113 μg/g)[14]. However, these latter studies were conducted at sites close to major industrialized cities or sewage treatment facilities. Additionally, this study concurred with others in that NP levels were higher than OP levels[11-15] . Nonylphenol may be prone to accumulate in sediments due to a higher affinity, based upon the log K_{ow}, than octylphenol[11, 12]. The determined levels of nonylphenol in this study are close to the range reported in water, sediment and carp from Cayuga River, Ohio[15]. Isobe *et al.* [12] found a seasonal trend (temperature dependent) in that levels of NP and OP tend to be higher in warm than cool water because of increased degradation of APEO's. However, no such pattern was seen for NP. Octylphenol on the other hand showed a temperature dependent trend. In KY lake, during low pool, winter season, OP was not detected in LE or the marina while it was found at these sites at high pool, summer months (Table 1).

As an attempt to identify the sources of CPs to Kentucky Lake sediment and mussels, wood samples (PCP has been largely used in wood preservatives) from abandoned docks, navigational tower, and wood found in lake bottom were analyzed for CPs. Table 3 shows the concentrations of various chlorophenols in wood samples. DCP and PCP were detected in wood samples from navigational tower. Whereas, abandoned dock wood samples contained DCP, TCP and TeCP. Unlike sediment or mussel tissues, dock wood sample contained relatively higher concentration of TeCP (2190 ng/g dry wt.) than other chlorophenols. Wood collected from the lake bottom had no detectable levels of CPs.

TABLE 3. Chlorophenol (DCP= dichlorophenol, TCP= trichlorophenol, TeCP= tetrachlorophenol, PCP= pentachlorophenol) concentrations (ng/g dry wt of wood) in wood samples collected from Kentucky Lake.

Sampling Location	DCP	TCP	TeCP	PCP
Navigational. Tower	120	BDL	BDL	904
Dock	56.4	102	2190	BDL
Lake Bottom	BDL	BDL	BDL	BDL

BDL**:** Below the detection limit (0.6 nanogram).

The results of this study provide evidence that (1) detectable levels of chlorophenols and alkyphenols are present in Kentucky Lake sediments and mussels tissues and (2) these compounds bioaccumulate in freshwater mussels in a specific pattern. The accumulation pattern of chlorophenols in sediment and mussel tissues were similar and exhibited the following order: PCP>DCP>TeCP>TCP.

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