



## Barrett Brown

Class: Senior

Major: Chemistry

The opportunity to apply knowledge acquired in the classroom to practical “real world” problems was the primary reason I decided to become involved in research at Murray State. I have always been interested in both biology and chemistry, so I selected a project that encompassed both fields. As I plan to become a physician, the project I chose also provided me with a valuable insight into the potential effects of environmental pollutants on public health.

## ABSTRACT

### A Study of Chlorophenol Concentrations in Kentucky Lake

Widespread use of chlorophenols in industry, agricultural and consumer products has resulted in environmental contamination. Exposure to chlorophenols can cause harmful effects in plants and animals, including humans. In this study, sediment, mussel tissues and wood samples from two local reservoirs (Kentucky Lake and Lake Barkley) were analyzed for dichlorophenol (DCP), trichlorophenol (TCP), tetrachlorophenol (TeCP), and pentachlorophenol (PCP). PCP concentrations ranged from below detection limits (0.6 ng) to 86 ng/g dry weight in sediment samples and from 660 to 2270 ng/g dry weight in freshwater mussel tissue samples. Elevated concentrations in mussel tissues indicate bioaccumulation of chlorophenols in freshwater mussels. Accumulation patterns of chlorophenols in sediment and mussel tissues were similar and in the following order: PCP>DCP>TeCP>TCP. Results provide evidence that (1) detectable levels of chlorophenols are present in Kentucky Lake sediments and mussel tissues, (2) chlorophenols bioaccumulate in freshwater mussels in a specific pattern, and (3) among other sources, wooden structures, including abandoned docks and navigational towers, may contribute to the contamination of chlorophenols in these two lakes.

## FACULTY MENTORS



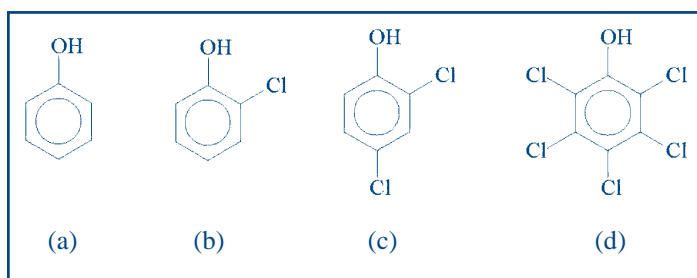
**Bommanna Loganathan** holds a joint appointment in MSU’s Department of Chemistry and Center for Reservoir Research. His research interests focus on environmental pollutants and the effects these pollutants have on wildlife and on human health. He is involved in trace level analysis of these compounds in environmental and biological samples taken from the Kentucky Lake watershed, human exposure studies, and cancer risk assessment studies. Through his involvement in MSU’s Howard Hughes award and through other external grants, Loganathan has involved numerous undergraduate and graduate students in research.



**David Owen**, director of Murray State University’s Chemical Services Laboratory, is a professor in the Department of Chemistry. His research interests range from synthetic inorganic and organic chemistry, through heterocycles, the solid state, and surface chemistry, to applications of microcomputers to detection systems and undergraduate instruction. During his 27 years of service to the university, Dr. Owen has engaged numerous undergraduates and graduate students in research in the chemical sciences.

# A Study of Chlorophenol Concentrations in Kentucky Lake

Pentachlorophenol ( $C_6HCl_5O$ ; MW: 266.35) (PCP) is a colorless or white crystalline solid (pure form) or a dark grey to brown powder (crude product) that was first manufactured commercially in 1936. Since then, large quantities (> 50 million kgs/year) have been produced and used in a variety of applications all over the world (Baynes, Brooks, Mumtaz and Riviere, 2002; Eisler, 1989). Chlorophenols (CPs) are made by stepwise chlorination of phenols, with higher chlorophenols such as tetra and pentachlorophenols being generated by further chlorination of mono-, di-, and trichlorophenols (Figure 1).

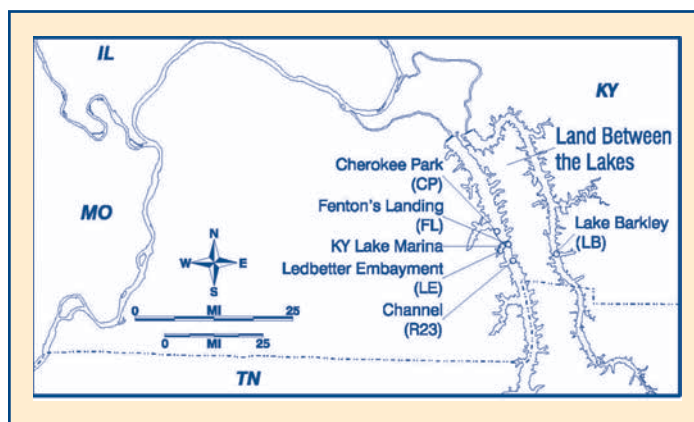


**Figure 1.** Structures of phenol (a) and chlorophenols: (b) 2-chlorophenol, (c) 2,4-dichlorophenol, and (d) 2,3,4,5,6-pentachlorophenol.

Among the CPs, PCP is highly toxic, and it was one of the most widely-used biocides in the United States. CPs are used extensively in cleaning products, paints, herbicides, pesticides, molluscicide, algicide and disinfectant, and as an ingredient in antifouling paint, paper production, textile manufacturing, cable coverings, leather, rope, ink, rubber and antiseptics (Cirelli, 1978; Eisler, 1989). Because of its harmful environmental effects (cancer suspect agent) on wildlife and humans, effective July 1984 the purchase and use of PCP was restricted to certified applicators (Environmental Protection Agency [EPA], 1984). PCP is still used industrially as a wood preservative to control damage by mold, termites, powder post beetles and wood boring insects (Agency for Toxic Substances and Disease Registry [ATSDR], 2001; Baynes et al., 2002; Becker, Buge and Win, 2002). CPs, particularly PCP residues, have been detected in air, rain, snow, groundwater, surface water, fish and aquatic invertebrates, as well as human urine, blood and milk

(Eisler, 1989; Muir and Eduljee, 1999; ATSDR, 2001; Becker et al., 2002). According to TRI 99 (Toxic Release Inventory) - 2001 maximum amount of PCP use in one of the Kentucky facilities was approximately 45,400 kg (99,900 lbs) (ATSDR, 2001). CPs are regarded as a cancer suspect agent and as a priority pollutant, and their levels in the environment are a matter of ongoing concern (Gupta, Ali, and Saini, 2004). Furthermore, tetrachlorophenol (TeCP) and PCP production results in release of highly toxic and environmentally stable impurities such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (ATSDR, 2001). To our knowledge, no studies have been conducted on the levels of CPs in the Kentucky Lake ecosystem. The objective of this study was to determine the levels of CPs in sediment, mussel tissues, and wood samples collected from selected locations in Kentucky Lake and Lake Barkley.

Kentucky Lake and Lake Barkley are two of the major human-constructed lakes in the southeastern United States (Figure 2). Together they serve as ultimate repositories of substances entering their watersheds from portions of seven southeastern states, which include a sizeable fraction of the United States' chemical processing, agricultural chemical products and electronics manufacturing industries.



**Figure 2.** Map showing sampling locations in Kentucky Lake and Lake Barkley.

During the past two decades, mass mortalities of mussels have been encountered in these regional waters, the causes of which have not been elucidated. In some cases, the quality and quantity of mussels harvested for the pearl industry have been substantially reduced (Loganathan, Kawano, Sajwan and Owen, 2001). These events have raised concern about the water quality of Kentucky Lake and the lowermost Tennessee River. Although a few studies have examined the levels of chlorinated organics in Kentucky Lake and the lowermost Tennessee River, there have been no reports on the levels of CPs in sediment and/or biological tissues from this region. In this study, sediment and freshwater mussels were collected from selected locations in Kentucky Lake and Lake Barkley and analyzed for CPs. Since PCP is predominantly used as a wood preservative, wood samples from abandoned docks and navigational towers also were analyzed to find out whether these structures contribute to the contamination of CPs to the lake water. Determining toxicant levels in environmental and biological samples is an essential step in understanding the complex freshwater lake system, viability of its biota, and protection of living resources. This paper presents some preliminary results of CP concentrations in sediment, freshwater mussel tissues, and wood samples. Observations on accumulation patterns of various CPs are described.

## Method

Open circuit SCUBA was used to collect samples from Lake Barkley and Kentucky Lake. The sample collection sites and dates were as follows: Ledbetter Embayment (LE), Kentucky Lake Marina, and channel (R23) sites were sampled on 7 September 2002; Cherokee Park (CP) and Fenton's Landing (FL) were sampled on 17 December 2002; Lake Barkley (LB) was sampled on 2 November 2002; and LE and Kentucky Lake Marina were sampled on 4 August 2003. Surface sediment samples (0-5 cm) were collected using pre-cleaned I-CHEM jars. All samples were put on ice on-site. Mussels were separated by species and age. Prior to storage, length, width, height and mass were measured for each mussel. Samples were stored at  $-20\text{ }^{\circ}\text{C}$  in pre-cleaned I-CHEM jars until further analysis. For source identification, wood samples from abandoned docks, a navigational tower, and wood found in lake bottom were collected for CPs analyses. Chemical analyses were performed in Murray State University's Chemical Services Laboratory (CSL). The CP analytical method used was based upon Becker et al. (2002). Gas chromatograph equipped with electron capture detector was calibrated using a series of

known amount CP standards. Calibration  $R^2 > 0.97$  and response factors were calculated. 2,4,6-Tribromophenol (TBP) was used as the internal standard. The average recovery of TBP was 108 percent.

Samples were freeze-dried and then homogenized using mortar and pestle prior to Soxhlet extraction. Sediment and mussels were Soxhlet extracted for 17 hours with 3:1 ratio of methylene chloride and hexane. Following extraction, the extracts were rotary evaporated. Sediment extracts were subjected to activated copper treatment to remove sulfur while the lipids were removed using Florisil dry column method. The extracts were fractionated and purified by eluting through 10 g of activated Florisil packed in a glass column (10mm i.d.). The first fraction (F1) containing PCBs and some pesticides were eluted using 75 ml of hexane. PAHs and other pesticides were eluted using 100 ml of 20 percent methylene chloride in hexane. Nonylphenol, octylphenol, and CPs were eluted using 150 ml of 50 percent methylene chloride in methanol. A portion of fraction 3 (F3) was transferred to methanol, acetylated with  $\text{K}_2\text{CO}_3$  solution/acetic anhydride, liquid/liquid extraction with 20 ml of ultrapure hexane, and analyzed using gas chromatography coupled with electron-capture detector (GC-ECD). The gas chromatograph was equipped with a DB-5 capillary column coupled with a  $^{63}\text{Ni}$  electron capture detector. The initial temperature was  $90\text{ }^{\circ}\text{C}$ . This was ramped at  $5\text{ }^{\circ}\text{C}/\text{min}$  up to  $200\text{ }^{\circ}\text{C}$ , followed by a second ramp of  $10\text{ }^{\circ}\text{C}/\text{min}$  up to  $280\text{ }^{\circ}\text{C}$ , with a final hold time of 35 min. The carrier and makeup gases used were helium (2 ml/min) and nitrogen (28 ml/min), respectively.

Wood samples were cut into small slivers then sonicated at  $40\text{ }^{\circ}\text{C}$  in enough pesticide grade methanol to wet all surfaces for 1 hour each time. The extract was then pipetted off, concentrated under a gentle stream of nitrogen gas, acetylated, and analyzed as described above. To check any cross contamination of samples during the instrumental analysis, ultra-pure hexane was injected between samples.

## Results

Among the various analytes measured, dichlorophenol (DCP) was detected in all samples analyzed (Table 1). Trichlorophenol (TCP) was not detectable in most of the sediment samples. Among the CPs measured, PCP concentrations were the highest and ranged from below detection limit (BDL) to  $86\text{ ng/g}$  dry weight of sediment.

**Table 1**

Chlorophenol Concentrations (ng/g dry wt.) in sediments from Kentucky Lake and Lake Barkley.				
Location	DCP	TCP	TeCP	PCP
LE	9.46	BDL	BDL	59.00
KY Lake Marina	15.00	BDL	7.32	BDL
R23	15.10	BDL	BDL	BDL
CP	12.20	BDL	5.70	45.10
FL	15.60	BDL	5.40	BDL
LB1	13.80	5.40	5.90	86.00
LB2a	10.90	BDL	BDL	BDL
LB3	15.50	BDL	5.90	45.90
LE	15.30	BDL	5.40	41.30
KY Lake Marina	14.40	BDL	1.10	14.70

Note. BDL = below the detection limit (0.6 nanogram/g sample); CP = chlorophenol; DCP = dichlorophenol; TCP = trichlorophenol; TeCP = tetrachlorophenol; PCP = pentachlorophenol; LE = Ledbetter Embayment; R23 = KY Lake channel site; CP = Cherokee Park; FL = Fenton’s Landing; LB = Lake Barkley.

**Table 2**

Age (years), sampling location, and chlorophenol concentrations (ng/g dry wt.) for various species of the mussels collected from Ledbetter Embayment and channel site in Kentucky Lake.						
Species	Location	Age	DCP	TCP	TeCP	PCP
<i>Fusconaia flava</i>	LE	8	170	BDL	70	660
<i>Fusconaia flava</i>	LE	11	140	BDL	30	890
<i>Quadrula quadrula</i>	LE	11	250	54	50	1500
<i>Quadrula quadrula</i>	LE	11	190	53	BDL	1430
<i>Plectomerus dombeyanus</i>	LE	12	190	BDL	BDL	1670
<i>Plectomerus dombeyanus</i>	LE	11	230	70	BDL	1500
<i>Megaloniaias nervosa</i>	R23	20	140	49	49	980
<i>Amblema plicata</i>	R23	15	205	47	58	1290
<i>Amblema plicata</i>	R23	14	210	40	50	2270

Note: BDL = below the detection limit (0.6 ng/g sample); DCP = dichlorophenol; TCP = trichlorophenol; TeCP = tetrachlorophenol; PCP = pentachlorophenol; LE = Ledbetter Embayment; R23 = KY Lake channel site.

In general, CP concentrations were one to two orders of magnitude higher in freshwater mussel tissues than in the corresponding sediments from Kentucky Lake (Table 2). As observed for sediments, DCP was detected in all freshwater mussel tissues analyzed. Also, TCP and TeCP levels were lower than DCP and PCP. Similar to sediments, PCP concentrations in freshwater mussel tissues were the greatest among all the CPs analyzed.

DCP levels in mussels were approximately 200 ng/g and appeared to be unrelated to mussel age, species, or location. PCP levels ranged from 660 to 2270 ng/g dry weight, with a mean concentration of 1220 ng/g dry wt. A 14-year old *Amblema plicata* mussel showed the highest PCP level (2270 ng/g dry wt.). TCP levels in mussels tended to be around BDL and 50 ng/g dry wt. The mean TeCP was found to be 31 ng/g dry wt. (Table 2).

In order to identify the source of CPs in Kentucky Lake sediment and mussels, wood samples (PCP has been largely used in wood preservatives) from abandoned docks, a navigational tower, and wood found in lake bottom were analyzed for CPs (Table 3).

**Table 3**

Chlorophenol concentrations (ng/g dry wt of wood) in wood samples collected from Kentucky Lake.				
Wood Source	DCP	TCP	TeCP	PCP
Navigational Tower	120	BDL	BDL	904
Dock	56.4	102	2190	BDL
Lake Bottom	BDL	BDL	BDL	BDL

Note: BDL = below the detection limit (0.6 ng/g sample); DCP = dichlorophenol; TCP = trichlorophenol; TeCP = tetrachlorophenol; PCP = pentachlorophenol.

DCP and PCP were detected in wood samples from the navigational tower, whereas, abandoned dock wood samples contained DCP, TCP, and TeCP (Table 3). Unlike sediment or mussel tissues, dock wood contained a 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.



## Discussion

CPs are well-known environmental pollutants. PCP, in particular, adversely affects growth, survival and reproduction in sensitive aquatic species at media concentrations of 8-80 µg/L for algae and higher plants, at 3-100 µg/L for invertebrates, and at < 1-68 µg/L for fish. In birds, PCP was fatal at > 285 µg/g contaminated nestling materials (i.e., wood shavings) (Eisler, 1989). Although, the present study did not analyze water samples for CPs, it is evident from sediment concentrations that the levels of PCP in Kentucky Lake may be well below the adverse effect levels reported by earlier researchers. Escher and Schwarzenbach (1996) reported that  $pK_a$ 's of selected CPs (mono- to pentachlorophenols) ranged from 5.0 to 9.4. The  $pK_a$ 's provide strong evidence that most CP compounds will be in an ionized state, therefore leading to greater retention in sediment and biological medium. Because of the chemical property, measurable concentrations of CPs accumulated in Kentucky Lake sediments and mussels (Table 1). Based upon octanol-water partition coefficients, CPs have the ability to bioaccumulate in organisms (Escher and Schwarzenbach, 1996). Chlorinated phenols (e.g., tetra and pentachlorophenols) have increased ability to reside in organic matter when compared to mono- and dichlorophenols due to higher lipophilicities (Masunaga, Susarla, Gundersen, and Yonezawa, 1996). In this study, PCP concentrations were the highest in both sediment and in mussel tissues, corroborating the above property.

Elevated levels of DCP, TCP, TeCP, and PCPs in mussel tissues revealed that all forms of CPs bioaccumulate in freshwater mussels (Table 1 and 2; Figure 3). The accumulation pattern of chlorophenols in sediment and mussel tissues were similar and exhibited the following order: PCP>DCP>TeCP>TCP. Measurable concentrations of CPs in an abandoned dock and a navigational tower made from wood may serve as one of the sources for CP contamination in Kentucky Lake and Lake Barkley. Further study is needed to elucidate baseline contaminant levels, bioaccumulation, biomagnification patterns, and effects on aquatic organisms in Kentucky Lake and Lake Barkley.

## References

- Agency for Toxic Substances and Disease Registry. (2001). *Toxicological Report for pentachlorophenol* [update] (ATSDR Publication No. PB/2001/109106/AS). Atlanta: U.S. Department of Health and Human Services, Public Health Services.
- Baynes, R.E., Brooks, J.D., Mumtaz, M., and Riviere, J.E. (2002). Effect of chemical interactions in pentachlorophenol mixtures on skin and membrane transport. *Toxicological Sciences*, 69, 295-305.
- Becker, R., Buge, H-G., and Win, T. (2002). Determination of pentachlorophenol (PCP) in waste wood - method comparison by a collaborative trial. *Chemosphere*, 47, 1001-1006.
- Cirelli, D.P. Patterns of pentachlorophenol usage in the United States of America - an overview. In K.R. Rao (Ed.), *Pentachlorophenol, chemistry, pharmacology, and environmental toxicology* (pp. 13-18). New York, NY: Plenum Press.
- Eisler, R. (1989). Pentachlorophenol hazards to fish, wildlife and invertebrates: a synoptic review. *U.S. Fish and Wildlife Service Biological Reports*, 85, 1-72.
- Escher, B., and Schwarzenbach, R. (1996). Partitioning of substituted phenols in liposome-water, biomembrane-water, and octanol-water systems. *Environmental Science and Technology*, 30, 260-270.
- Environmental Protection Agency. (1984, July 13). Notice of intent to cancel registration of pesticide products containing creosote, pentachlorophenol (including its salts), and the inorganic arsenical (49, CAR 28666).
- Gupta, V.K., Ali, I., and Saini, V.K. (2004). Removal of chlorophenols from wastewater using red mud: an aluminum industry waste. *Environmental Science and Technology*, 38, 4012-4018.

Loganathan, B.G., Kawano, M., Sajwan, K.S., and Owen, D.A.

(2001). Extractable organohalogen (EOX) in sediment and mussel tissues from the Kentucky Lake and Kentucky Dam Tailwater, USA. *Toxicological and Environmental Chemistry*, 79, 233-242.

Muir, J., and Eduljee, G. (1999). PCP in the freshwater and marine environment of the European Union. *Science of the Total Environment*, 236, 41-56.

Masunaga, S., Susarla, S., Gundersen, J., and Yonezawa, Y. (1996). Pathway and Rate of Chlorophenol Transformation in Anaerobic Estuarine Sediment. *Environmental Science and Technology*, 30, 1253-1260.

## **Acknowledgements**

This research was supported in part by the National Science Foundation's Collaborative Research for Undergraduate Institutions (NSF-CRUI) at Murray State University. The author is grateful to Dr. James Sickel, professor of biology, Murray State University, for his help in field sampling, identification and aging of mussels. The author also appreciates Dr. Howard Whiteman, Dr. David White, and other CRUI investigators for their constructive criticism during presentations of this study at CRUI meetings and at the 2004 Scholars Weeks CRUI symposium. Center for Reservoir Research Contribution number is 102.

Barrett Brown is now at the University of Kentucky Medical School, Lexington. Correspondence concerning this article should be sent to Mr. Barrett Brown. Email: barrett\_brown@msn.com.