

**Analysis of Polychlorinated Biphenyl (PCB) Residues
in Fish Collected March 13-14, 2001
from the Bayou Creek System**

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INTRODUCTION

Fish were collected from Big and Little Bayou Creeks on March 13-14, 2001. The new reference station, upstream of BB1 and designated BB1A, also was collected. In addition, Massac Creek (MC) was sampled and served as a reference station independent of the Bayou Creek system. Fillet samples were analyzed for Aroclors 1248, 1254, and 1260 for all fish collected. A total of 33 fish were analyzed from Big Bayou Creek that consisted of 22 longear sunfish (LS), 4 bluegills (BG), 6 green sunfish (GS) and 1 channel catfish (ChCAT). From Little Bayou Creek, 6 LS and 5 GS were analyzed for a total of 11. For the station at Massac Creek we analyzed 2 LS and 1 GS. In addition to fish, water samples were taken for water quality measurements.

METHODS

Water Quality

Samples for water quality measurements were collected in 1-L Cubitainers and were placed on ice until delivery to the laboratory. Water quality parameters included pH, conductivity, alkalinity and hardness that were measured according to procedures described by APHA (1995). The measurements were performed with a pH meter (Orion Research EA920), a conductivity meter (Amber Science Model 604), the bromocresol green-methyl red titrimetric, and the EDTA titrimetric procedures, respectively.

Fish collection

Fish were collected by use of back-pack shocker and seining. Collections were conducted by UK and Division of Waste Management personnel. Fish that did not meet

our requirements were returned to the stream. Collected fish were wrapped in aluminum foil, tagged, bagged in plastic containers by collecting station, and placed on ice (4 °C) for transport to the laboratory. Fish species were identified and stored in the freezer (-15 °C) until extraction.

Tissue extraction and clean-up

Fish were measured for length and whole body weight, and fillets were taken with solvent-cleaned surgical instruments. The fillets were then weighed and macerated as described below. Otoliths (sagittae) were removed from each specimen for age determinations (Boxrucker, 1986).

PCBs in fish tissues were extracted and analyzed using standard U.S. EPA methods (Watts, 1980; U.S. EPA, 1997; Erickson, 1997). The muscle fillet samples (Mean weight 0.88, 2.15, and 1.91 g for Massac, Big Bayou and Little Bayou Creeks, respectively) were ground with 10g anhydrous sodium sulfate and the powder extracted with petroleum ether in a Soxhlet apparatus for 5-h. The extracts were concentrated to near dryness in a Roto-evaporator (Buchi Model RE121). Reconstituted samples (5.0 mL in iso-octane) were then cleaned of interferences as described below and then analyzed by gas chromatography. A 1.0 mL sub-sample was taken for lipid determinations prior to clean-up. Lipid and pesticide clean-up was performed according to procedures given by Erickson (1997) and U.S. EPA (1997, SW-846 Method 3620B). Elemental sulfur was then removed by shaking 2-propanol (2 mL) and tetrabutylammonium sulfite (2 mL), adding ultra-pure water (8 mL) and reshaking. The organic extract was removed and mixed with 2.0-mL concentrated sulfuric acid (Jensen *et al.*, 1977; U.S. EPA, 1997, SW-846 Method 3660B, sulfur cleanup). A 4 μ L sub-sample was then analyzed by gas chromatography.

Analysis by Gas Chromatography

Samples were analyzed for Aroclors 1248, 1254, and 1260 according to SW-846 Method 8082 (polychlorinated biphenyls by gas chromatography; U.S. EPA, 1997). Analysis was performed using a Hewlett-Packard (HP) Model 5890A gas chromatograph equipped with an electron capture detector and an HP Model 7673A Automatic Sampler. Samples were analyzed using a 60m X 0.53mm ID SPB-5 (0.5 μ m film) fused silica megabore column (Supelco, Inc.) with ultra-high purity helium and nitrogen as carrier and makeup gases, respectively. PCB peak heights were quantified using an HP Model 3396A integrator and multiple-peak linear regression analysis was performed with Lotus-123® software. Aroclor levels were calculated from heights of 6 to 9 peaks for Aroclors 1248 and 1260 and 4-6 peaks for Aroclor 1254. Five external standards were used for calibration curves and for every tenth sample, either a solvent blank or a standard was analyzed. The Lotus program regresses data from PCB standards to the sample being analyzed. Each peak selected for each Aroclor class was statistically analyzed (e.g., standard deviation; standard error; relative deviation).

Quality Assurance

Permanent bench records were kept of all assays and annotated as required under Good Laboratory Practices (*Federal Register*, 40 CFR, Part 160, August 17, 1989). All printouts and graphic recordings were filed and are open for inspection. These bench records will be archived within two years after the close of the project but retrievable upon request. Chain of Custody was maintained for the samples collected.

RESULTS

The results of water quality are presented in Table 1. Overall, pH in Big Bayou Creek increased within and downstream of the effluent receiving zone, with BB8 demonstrating the highest pH value of 7.74. Conductivity ranged from 141 to 448 $\mu\text{MHOs/cm}$ and was highest at stations BB6 and BB7. Alkalinity ranged from 60 to 120 $\text{mg CaCO}_3/\text{L}$ in Big Bayou Creek and 24 to 32 $\text{mg CaCO}_3/\text{L}$ in Little Bayou Creek. Hardness ranged from 32 to 120 $\text{mg CaCO}_3/\text{L}$ and 64 to 88 $\text{mg CaCO}_3/\text{L}$ for Big and Little Bayou Creeks, respectively. Hardness levels were highest for stations BB6 through BB9.

The species of fish used in this study included 30 longear sunfish (*Lepomis megalotis*) (LS); 12 green sunfish (*Lepomis cyanellus*) (GS); 4 bluegill (*Lepomis macrochirus*) (BG); and 1 channel catfish (*Ictalurus punctatus*) (ChCAT). This gave a total of 47 specimens that were analyzed (Tables 2, 3). Fish collected were relatively young. All fish from MC were 1 year or less, only 3 out of 33 fish in Big Bayou were 2 years old, and 1 out of 11 fish from Little Bayou was 2 years old. The mean lengths and whole body weights were 73 mm and 7.24 g for MC; 90 mm and 14.37 g for Big Bayou Creek; 87 mm and 13.22 g for Little Bayou Creek.

In Big Bayou Creek, Aroclor 1248 was detected in 20 of 33 fish collected, Aroclor 1254 was detected in 4 out of 33 fish, and Aroclor 1260 was detected in 3 out of 33 fish (Table 2). A GS at station BB6 had the highest concentration of Aroclor 1248 (0.07 $\mu\text{g/g}$), and a GS at station BB9 had the highest Aroclor 1254 content (0.09 $\mu\text{g/g}$). The two GS at BB6 and BB9 had the highest total PCB concentrations of 0.26 and 0.25 $\mu\text{g/g}$, respectively.

All fish collected from Little Bayou Creek contained Aroclor 1248 (11 of 11); 9 out of 11 contained Aroclor 1254; and 7 of 11 contained Aroclor 1260 (Table 3). The highest total PCB was detected in a GS from LB2 (1.24 µg/g) and the second highest was a GS from LB3 (0.60 µg/g).

In summary, 11 of 19 fish from Big Bayou Creek contained total PCBs at or above 0.05 µg/g which is of possible human health concern under the Great Lakes PCB Protocol used by the State of Kentucky. The highest values were 0.26, 0.13, 0.11 and 0.25 µg/g for fish collected at Big Bayou Creek stations BB6, BB7, BB8, and BB9, respectively. These results suggest probable health risk at these stations and support placing a fish consumption advisory on Big Bayou Creek. PCB pollution appeared greater than observed in July 2000 (Birge and Price, 2001).

Most serious PCB pollution was observed in Little Bayou Creek where 11 of 11 fish contained total PCBs at or above 0.10 µg/g. Seven of the 11 values were above 0.20 µg/g and the highest value was 1.24 at station LB2. Clearly, the present fish consumption advisory for Little Bayou Creek should be kept in force. These findings take on greater concern due to the fact that only “young” fish were recoverable during the collecting effort. These and other results indicate a reduction in older “age classes” of fish with Big Bayou Creek and further study is indicated.

Of still greater concern, the ratios of Aroclor 1248 to Aroclor 1260 were surprisingly high, possibly indicating that recent PCB pollution has occurred and that PCB are in bioavailable form. For example, the ration of 1248 to 1260 (*i.e.* 1248/1260) was 2.11 for all fish collected from Big Bayou Creek where PCBs were detectable. With respect to stream stations BB5 through BB9, the ratio was somewhat higher at 2.54.

The ratio of 1248 to 1260 for fish from all stations on Little Bayou Creek was 2.04. These results contrast with those reported for the collection in July 2000 (Birge and Price, 2001) and may indicate an increase in PCB contamination all the way downstream to the last station monitored.

REFERENCES

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Table 1. Water quality results for stream water samples¹ from the Bayou Creek system collected March 13-14, 2001.

Station	pH	Conductivity (μ MHOs/cm)	Alkalinity (mg CaCO ₃ /L)	Hardness (mg CaCO ₃ /L)
MC	7.04	144	20	52
BB1A	7.14	143	60	64
BB1	7.12	141	20	72
BB2	7.09	146	12	32
BB3	7.22	162	20	52
BB4	7.19	182	20	60
BB5	7.32	181	24	64
BB6	7.48	439	20	112
BB7	7.61	448	20	120
BB8	7.74	404	20	108
BB9	7.39	422	28	120
LB2	7.38	299	28	88
LB3	7.54	250	32	68
LB4	7.40	194	24	64

¹ Water samples taken during fish collection.

Table 2. PCB concentrations in fish from Massac Creek (MC) and Big Bayou Creek collected March 13-14, 2001.

Station	Date	Type	Length (mm)	Age (Years)	Whole Body Wt. (g)	Fillet Wt. (g)	mg fat /g tissue	Aroclor Conc. ($\mu\text{g/g}$)			
								1248	1254	1260	Total
MC	03/14/01	LS1	72	1	7.760	1.058	10.49	0.052	<0.019	<0.019	0.052
MC	03/14/01	LS2	71	1	6.698	1.021	4.31	<0.046	<0.046	<0.046	<0.046
MC	03/14/01	GS1	65	1	4.886	0.569	3.34	<0.060	<0.060	<0.060	<0.060
BB1A	03/13/01	LS1	86	2	11.312	1.598	4.94	<0.040	<0.040	<0.040	<0.040
BB1A	03/13/01	LS2	78	1	9.845	1.512	4.89	0.039*	<0.041	<0.041	0.039*
BB1A	03/13/01	LS3	68	1	6.799	1.107	8.31	<0.024	<0.024	<0.024	<0.024
BB1	03/13/01	BG1	98	1+	15.126	2.703	8.64	0.070	<0.023	<0.023	0.070
BB1	03/13/01	BG2	102	1+	17.573	2.708	3.66	<0.055	<0.055	<0.055	<0.055
BB1	03/13/01	BG3	107	1+	21.478	3.400	3.74	<0.054	<0.054	<0.054	<0.054
BB1	03/13/01	LS1	122	2+	35.587	4.853	3.51	<0.057	<0.057	<0.057	<0.057
BB3	03/13/01	LS1	88	1	14.234	2.175	4.64	0.090	<0.043	<0.043	0.090
BB3	03/13/01	LS2	85	1	11.526	1.828	6.13	0.024*	<0.033	<0.033	0.024*
BB3	03/13/01	LS3	81	1	9.423	1.300	6.38	0.026*	<0.031	<0.031	0.026*
BB3	03/13/01	GS1	75	<1	7.299	1.049	4.24	0.044*	<0.047	<0.047	0.044*
BB4	03/13/01	LS2	89	1	14.296	1.985	5.74	0.032*	<0.035	<0.035	0.032*
BB4	03/13/01	LS3	90	1	13.434	2.110	4.64	0.058	0.032*	<0.043	0.090
BB4	03/13/01	LS4	85	<1	11.319	1.787	4.87	0.061	0.033*	<0.041	0.094

* PCBs detected, however value below Minimum Quantitation Limit (MQL).

Table 2, continued. PCB concentrations in fish from Massac Creek (MC) and Big Bayou Creek collected March 13-14, 2001.

Station	Date	Type	Length (mm)	Age (Years)	Whole Body Wt. (g)	Fillet Wt. (g)	mg fat /g tissue	Aroclor Conc. (µg/g)			
								1248	1254	1260	Total
BB5	03/13/01	LS1	93	1	16.586	2.713	6.58	0.042	0.019*	<0.030	0.061
BB5	03/13/01	LS2	93	1	16.844	2.566	4.68	0.042*	<0.043	<0.043	0.042*
BB5	03/13/01	LS3	94	<1	16.614	2.593	6.56	0.036	<0.031	<0.031	0.036
BB5	03/13/01	LS4	86	1	11.310	1.779	6.83	0.033	0.016*	<0.029	0.049
BB6	03/13/01	LS1	105	1+	22.931	3.357	4.89	0.070	<0.041	0.018*	0.087
BB6	03/13/01	LS2	90	1	13.747	1.717	4.34	0.051	<0.046	<0.046	0.051
BB6	03/13/01	LS3	83	1	11.579	1.702	5.73	0.039	<0.035	<0.035	0.039
BB6	03/13/01	GS1	121	2	28.791	4.086	4.07	0.130	0.078	0.049	0.256
BB7	03/13/01	LS1	100	1	19.329	2.764	5.23	0.071	<0.038	0.022*	0.093
BB7	03/13/01	LS2	77	<1	8.775	1.319	11.98	0.022	0.018	0.008*	0.048
BB7	03/13/01	LS3	80	<1	8.249	1.136	5.90	0.037	<0.034	<0.034	0.037
BB7	03/13/01	GS1	71	1	6.481	0.832	4.57	0.081	0.049	<0.044	0.130
BB8	03/13/01	LS1	101	1	21.558	3.193	4.92	0.086	<0.041	0.024*	0.109
BB8	03/13/01	LS2	87	<1	12.999	2.081	4.71	0.069	<0.042	<0.042	0.069
BB8	03/13/01	BG1	96	1+	15.420	2.502	3.68	<0.054	<0.054	<0.054	<0.054
BB8	03/13/01	GS1	70	<1	6.490	0.902	5.54	0.058	<0.036	<0.036	0.058
BB9	03/14/01	GS1	95	1	14.953	2.313	3.50	0.124	0.087	0.042	0.254
BB9	03/14/01	GS2	100	1	17.835	3.006	3.71	0.059	0.045*	<0.054	0.105
BB9	03/14/01	ChCAT1	84	---	5.374	0.273	3.48	<0.057	<0.057	<0.057	<0.057

* PCBs detected, however value below Minimum Quantitation Limit (MQL).

Table 3. PCB concentrations in fish from Little Bayou Creek collected March 14, 2001.

Station	Date	Type	Length (mm)	Age (Years)	Whole Body Wt. (g)	Fillet Wt. (g)	mg fat /g tissue	Aroclor Conc. ($\mu\text{g/g}$)			
								1248	1254	1260	Total
LB2	03/14/01	LS1	87	1+	13.20	1.960	5.23	0.078	0.088	0.079	0.245
LB2	03/14/01	LS2	78	<1	9.19	1.470	4.66	0.153	0.141	0.135	0.428
LB2	03/14/01	GS1	104	1+	27.20	3.926	4.06	0.467	0.503	0.269	1.240
LB3	03/14/01	LS1	85	<1	12.36	1.933	5.43	0.234	0.122	0.072	0.429
LB3	03/14/01	GS1	70	<1	6.71	0.939	4.85	0.333	0.182	0.085	0.600
LB3	03/14/01	GS2	67	<1	5.16	0.585	11.20	0.053	0.019	<0.018	0.072
LB4	03/14/01	LS1	100	1	21.61	3.175	3.21	0.088	0.059	0.072	0.219
LB4	03/14/01	LS2	98	2	14.32	2.096	4.65	0.075	<0.043	0.027*	0.102
LB4	03/14/01	LS3	86	1	9.90	1.357	3.76	0.078	<0.053	0.024*	0.102
LB4	03/14/01	GS1	91	1	12.48	1.945	3.34	0.267	0.120	0.075	0.461
LB4	03/14/01	GS2	95	1+	13.33	1.657	7.27	0.100	0.030	0.021*	0.151

* PCBs detected, however value below Minimum Quantitation Limit (MQL).