Analysis of Polychlorinated Biphenyl (PCB) Residues in Whole-body Stoneroller Minnows Collected March 13-14, 2001 from the Bayou Creek System

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INTRODUCTION

Stoneroller minnows (*Campostoma anomalum*) were collected along with sunfish from Big and Little Bayou Creeks on March 13-14, 2001. Reference stations BB1A (upstream of BB1) and Massac Creek (MC) also were sampled. Whole body stoneroller minnows were analyzed for Aroclors 1248, 1254, and 1260 for all stations collected. A total of 30 fish were analyzed from which 22 fish were from Big Bayou Creek and 6 from Little Bayou Creek. No stonerollers were found at station LB3. Therefore, 2 creek chubs (*Semotilus atromaculatus*) were analyzed from that station. For the Massac Creek station, 2 stoneroller minnows were analyzed.

METHODS

Fish collection

Fish were collected by use of back-pack shocker and by seining. Collections were conducted by UK and Division of Waste Management personnel. Fish that did not meet requirements were returned to the stream. Collected fish were wrapped in aluminum foil, tagged, bagged, and placed on ice (4 ^{o}C) for transport to the laboratory. Fish species were identified and stored in the freezer (-15 ^{o}C) until extraction.

Tissue extraction and clean-up

Fish were measured for length and whole body weight, and minnows were prepared as described below.

PCBs in whole body were extracted and analyzed using standard U.S. EPA methods (Watts, 1980; U.S. EPA, 1997; Erickson, 1997). The fish samples were ground with sufficient anhydrous sodium sulfate to produce a dry powder and the powder was then 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 the sample with 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 4μL 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 (0.5μm 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 all samples collected.

RESULTS

Fish analyzed for this study were collected from 10 stations on Big Bayou Creek; 3 stations on Little Bayou Creek; and one reference site on Massac Creek (Tables 1, 2). Since no stoneroller minnows were found at LB3, 2 creek chubs were substituted. Length of stoneroller minnows from Big Bayou Creek ranged

from 73 to 119 mm, with an average length of 95 mm (Table 1). Minnow length ranged from 91 to 104 mm for specimens form Little Bayou Creek, with an average length of 99 mm (Table 2). Whole body weights ranged from 4.26 to 21.71 g (Avg. 11.19 g) and from 10.80 to 15.63 g (Avg. 12.77 g) for Big and Little Bayou Creeks, respectively (Tables 1, 2).

In Big Bayou Creek, Aroclor 1248 was detected in all fish collected (22 out of 22); Aroclor 1254 was detected in 14 out of 22 fish; and Aroclor 1260 was detected in 19 out of 22 fish (Table 1, Fig. 1). The highest total Aroclor concentrations were from station BB4 at 0.382, 0.464, and 0.343 µg/g for 1248, 1254, and 1260, respectively. This station is just downstream of effluent 008. Aroclor 1254 was more prevalent at stations BB4 through BB9 (Fig. 1). For total PCBs, 4 out of 22 fish from Big Bayou Creek were above 1.0 µg/g, that is 1.0 ppm (Table 1, Fig. 2). These results and those for total PCB concentrations for minnows from Big Bayou Creek are further summarized in Figure 3.

All stoneroller minnows from Little Bayou Creek showed Aroclors 1248, 1254, and 1260 (Table 2-3, Fig. 4). The highest total PCBs were detected in the two minnows from station LB2 (*i.e.* at 2.57, 1.71 µg/g). Only Aroclor 1248 was detected in the creek chubs from station LB3 and the values were 0.92 and 0.56 µg/g (ppm).

DISCUSSION AND CONCLUSIONS

Of the 30 fish analyzed from the Bayou Creek system, all were contaminated with PCBs. Concentrations of total PCBs were much higher for this system than for Massac Creek that was used as a reference. Concerning the latter system, only one fish was found to contain detectable PCB and the concentration was 0.01 µg/g. By comparison, mean total PCB concentrations ranged from 0.05 at BB1 to 0.85 at BB4 on Big Bayou Creek and 0.14 (LB4) to 2.14 (LB2) on Little Bayou Creek. Maximum values for total PCBs were 1.18, 1.18, 1.03, 2.57 and 0.92 for stations BB1A, BB4, BB5, LB2 and LB3, respectively (Table 4). These and other results (Birge and Price, 2001a; Birge and Price 2001b) clearly indicate that PCBs are prevalent throughout all reaches of the Bayou Creek system that have been monitored. This includes station BB9 on Big Bayou Creek which is situated about 10.7 Km downstream of station BB1 that is located near the south-western corner of the PGDP facility. BB9 is within 2.8 Km of the Ohio River confluence and total PCB values averaged 0.65 at this downstream station. This value was much higher than that reported for the Massac Creek reference station.

It is also clear that PCB exposure is “current”. This is based on two lines of evidence. First, as recently reported (Birge and Price, 2001b), 23 of 25 fish collected in March 2001 were within the “first-year” age class and all contained PCBs. Most values were above the 0.05 µg/g concentration considered potentially hazardous to human health, depending upon the exposure scenario used for risk analysis. The second concern is based on the ration of Aroclor 1248

to 1254 and/or 1260. The former Aroclor mixture contains greater proportions of less chlorinated PCB congeners which have less environmental persistence. Near equal or higher proportions of 1248 to 1254 and 1260 may be taken as indications of current or recent PCB contamination. Compared to Aroclor 1260, 1248 occurred in greater proportions at most stream stations monitored (Table 5). In addition, in the creek chubs analyzed, only Aroclor 1248 was detected. Also, compared to Aroclor 1260, proportions of 1254 were greater at all but one stream station (Table 5). Only Aroclor 1248 was detected at all stations monitored. Obviously, the Aroclor ratios will depend largely on the proportions of the Aroclor mixtures used and disposed to the environment within any specific time frame. However, all three classes noted here have been detected at PGDP dating back for a decade or more (Birge *et al.*, 1992). Had PCB pollution been curtailed within the past few years, we would expect to find lesser quantities of PCB detected as Aroclor 1248 and a greater shift over this time to Aroclor 1260. Station LB2 on Little Bayou Creek and BB4 on Big Bayou Creek were the most contaminated with PCBs. It is important to establish all PCB sources affecting these and other stations.

Table 1. PCB concentrations in wholebody stoneroller minnows from Massac Creek (MC) and Big Bayou Creek collected March 13-14, 2001.

Station	Date	Sample	Length (mm)	Whole Body Wt. (g)	mg fat /g tissue	Aroclor Conc. (μg/g) 1248	1254	1260	Total
MC	03/14/01	PSR1	103	13.958	30.65	0.015	<0.014	<0.014	0.015
MC	03/14/01	PSR2	77	4.255	28.57	<0.047	<0.047	<0.047	<0.047
BB1A	03/13/01	PSR1	79	6.359	23.75	0.460	0.452	0.268	1.180
BB1A	03/13/01	PSR2	78	6.002	17.09	0.024	<0.033	<0.033	0.024
BB1	03/13/01	PSR2	104	13.293	27.92	0.024	<0.015	0.012*	0.036
BB1	03/13/01	PSR3	96	11.050	23.47	0.030	<0.018	0.016*	0.046
BB1	03/13/01	PSR4	96	9.680	26.60	0.032	<0.021	0.029	0.061
BB2	03/13/01	PSR1	87	8.240	23.89	0.025	<0.024	0.034	0.059
BB2	03/13/01	PSR2	73	4.556	45.25	0.045	<0.044	0.044	0.089
BB3	03/13/01	PSR1	90	6.795	34.05	0.037	<0.029	0.052	0.089
BB3	03/13/01	PSR2	77	4.258	30.24	0.091	<0.047	0.066	0.157
BB4	03/13/01	PSR1	116	17.630	24.65	0.084	0.157	0.082	0.322
BB4	03/13/01	PSR2	119	20.890	44.19	0.221	0.283	0.233	0.737
BB4	03/13/01	PSR3	115	16.513	30.65	0.341	0.464	0.343	1.148
BB4	03/13/01	PSR4	110	15.815	22.94	0.382	0.453	0.343	1.179
BB5	03/13/01	PSR1	96	10.208	32.55	0.342	0.436	0.255	1.034
BB5	03/13/01	PSR2	100	15.980	58.28	0.190	0.251	0.129	0.571

* PCB detected but value was below Minimum Quantitation Limit (MQL).

Table 1, continued. PCB concentrations in wholebody stoneroller minnows from Massac Creek (MC) and Big Bayou Creek collected March 13-14, 2001.

Station	Date	Sample	Length (mm)	Whole Body Wt. (g)	mg fat /g tissue	Aroclor Conc. (μg/g)			
						1248	1254	1260	Total
BB6	03/13/01	PSR1	115	21.710	44.26	0.167	0.218	0.246	0.630
BB6	03/13/01	PSR2	102	14.744	32.26	0.094	0.157	0.109	0.361
BB7	03/13/01	PSR1	92	11.023	31.45	0.087	0.141	0.115	0.343
BB7	03/13/01	PSR2	92	10.182	42.78	0.172	0.293	0.257	0.721
BB8	03/13/01	PSR1	85	8.832	42.50	0.176	0.349	0.184	0.709
BB8	03/13/01	PSR2	85	7.976	37.77	0.155	0.368	0.126	0.649
BB9	03/13/01	PSR1	90	4.466	35.29	0.107	0.182	0.079	0.367

* PCB detected but value was below Minimum Quantitation Limit (MQL).

Table 2. PCB concentrations in wholebody stoneroller minnows from Little Bayou Creek collected March 14, 2001.

Station	Date	Sample	Length (mm)	Whole Body Wt. (g)	mg fat /g tissue	Aroclor Conc. (μg/g)			
						1248	1254	1260	Total
LB2	03/14/01	PSR1	99	10.804	32.96	0.779	0.732	1.062	2.573
LB2	03/14/01	PSR2	104	15.625	56.24	0.617	0.614	0.482	1.712
LB3	03/14/01	PCC1[1]	71	3.297	45.10	0.925	<0.607	<0.607	0.925
LB3	03/14/01	PCC2	76	5.011	27.72	0.560	<0.399	<0.399	0.560
LB4	03/14/01	PSR1	100	13.350	67.06	0.100	0.071	0.041	0.213
LB4	03/14/01	PSR2	91	11.317	39.89	0.051	0.011*	0.014*	0.075

[1] Since no stoneroller minnows were available at station LB3, creek chubs were analyzed.
* PCB detected but value was below Minimum Quantitation Limit (MQL).

Table 3. Mean PCB concentrations in wholebody stoneroller minnows from Massac Creek (MC) and the Bayou Creek system.

Station	Avg. Length (mm)	Avg. Whole Body Wt. (g)	Avg. mg fat /g tissue	Avg. Aroclor Conc. (μg/g)			
				1248	1254	1260	Total
MC	90	9.11	29.61	0.015	N.D.	N.D.	0.015
BB1A	79	6.18	20.42	0.242	0.452	0.268	0.602
BB1	99	11.34	26.00	0.028	N.D.	0.019	0.047
BB2	80	6.40	34.57	0.035	N.D.	0.039	0.074
BB3	84	5.53	32.14	0.064	N.D.	0.059	0.123
BB4	115	17.71	30.61	0.257	0.339	0.250	0.846
BB5	98	13.09	45.41	0.266	0.344	0.192	0.802
BB6	109	18.23	38.26	0.130	0.188	0.177	0.495
BB7	92	10.60	37.11	0.130	0.217	0.186	0.532
BB8	85	8.40	40.13	0.165	0.359	0.155	0.679
BB9	85	7.98	37.77	0.155	0.368	0.126	0.649
LB2	102	13.22	44.60	0.698	0.673	0.772	2.142
LB3[1]	74	4.15	36.41	0.742	N.D.	N.D.	0.742
LB4	96	12.33	53.48	0.076	0.041	0.012	0.144

[1] Since no stoneroller minnows were available at station LB3, creek chubs were analyzed.

Table 4. Maximum PCB concentrations in wholebody stoneroller minnows from Massac Creek (MC) and the Bayou Creek system.

		Maximum Aroclor Conc. (μg/g)			
Station	n	1248	1254	1260	Total
MC	2	0.015	N.D.	N.D.	0.015
BB1A	2	0.460	0.452	0.268	1.180
BB1	3	0.032	N.D.	0.029	0.061
BB2	2	0.045	N.D.	0.044	0.089
BB3	2	0.091	N.D.	0.066	0.157
BB4	4	0.382	0.464	0.343	1.179
BB5	2	0.342	0.436	0.255	1.034
BB6	2	0.167	0.218	0.246	0.630
BB7	2	0.172	0.293	0.257	0.721
BB8	2	0.176	0.368	0.184	0.709
BB9	1	0.107	0.182	0.079	0.367
LB2	2	0.779	0.732	1.062	2.573
LB3[1]	2	0.925	N.D.	N.D.	0.925
LB4	2	0.100	0.071	0.041	0.213

[1] Since no stoneroller minnows were available at station LB3, creek chubs were analyzed.

Table 5. Aroclor ratios for wholebody concentrations of fish from the Bayou Creek system[1].

Station	1248/1254	1248/1260	1254/1260
BB1A	0.53	0.90	1.69
BB1	---	1.50	---
BB2	---	0.89	---
BB3	---	1.09	---
BB4	0.76	1.03	1.36
BB5	0.77	1.39	1.79
BB6	0.70	0.74	1.06
BB7	0.60	0.70	1.17
BB8	0.46	1.07	2.31
BB9	0.42	1.22	2.91
LB2	1.04	0.90	0.87
LB3	---	**---**	**---**
LB4	1.84	6.30	3.42

[1] Aroclor 1248 was detected at all 13 stations, 1254 at 9 stations, and 1260 at 12 stations. Fish from LB3 contained only Aroclor 1248.

Figure 1. Aroclor concentrations in stoneroller minnows collected from Big Bayou Creek on March 13-14, 2001.

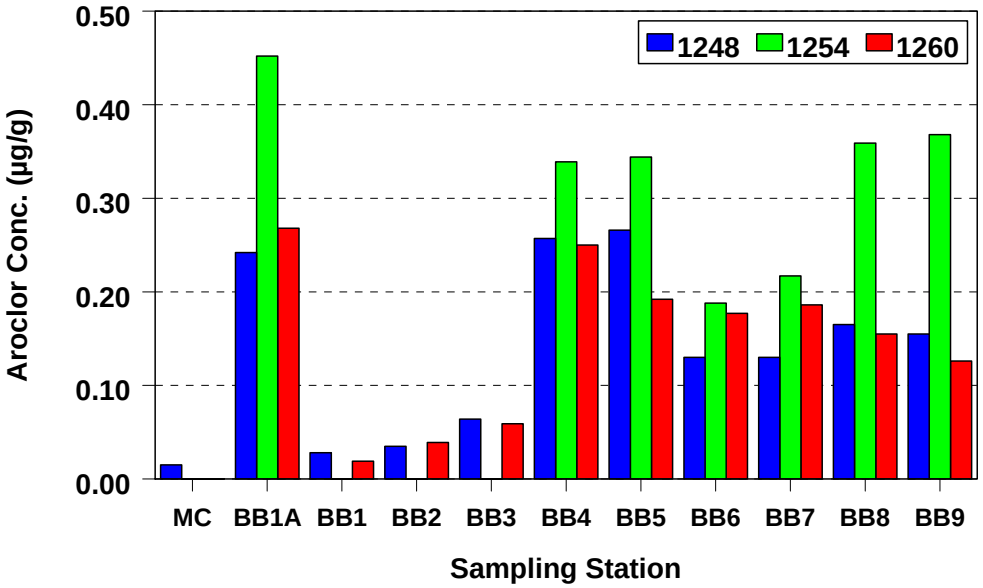


Figure 2. Total Aroclor concentrations in stoneroller minnows collected from Big Bayou Creek on March 13-14, 2001.

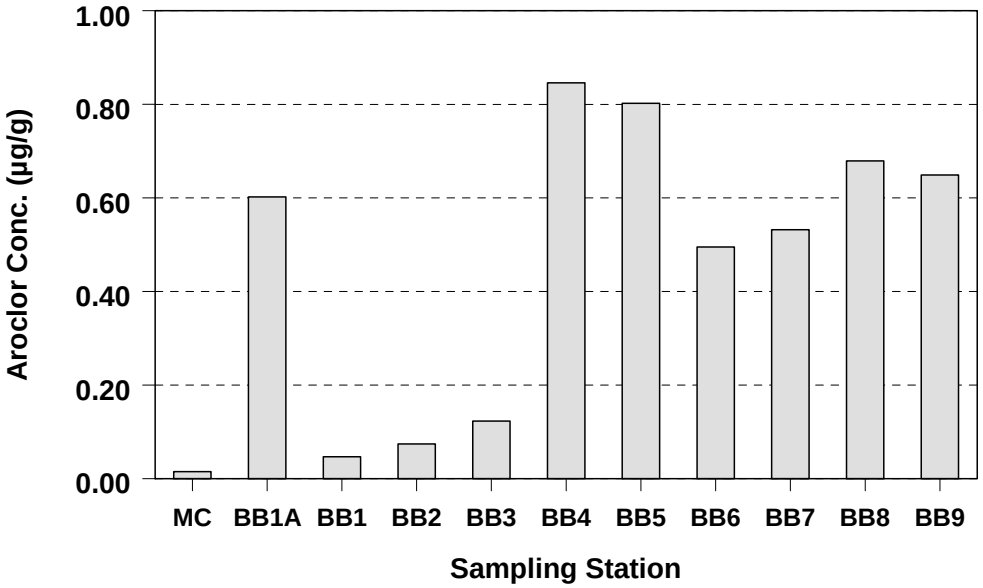


Figure 3. Summary of results for stoneroller minnows collected from Big Bayou Creek on March 13-14, 2001.

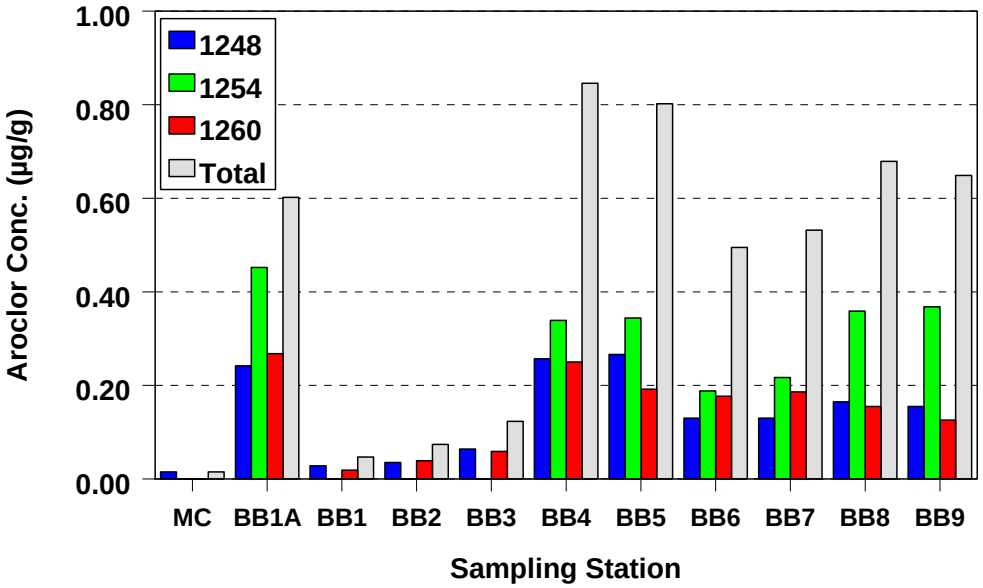
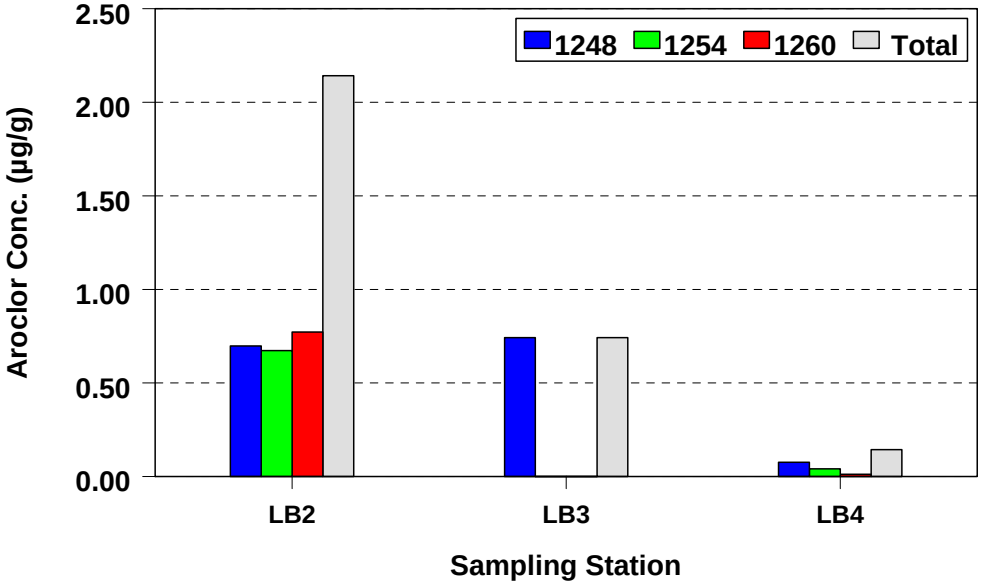


Figure 4. Arolcor concentrations in stoneroller minnows collected from Little Bayou Creek on March 14, 2001.



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