ENVIRONMENTAL PROTECTION AGENCY
REGION IV
SURVEILLANCE AND ANALYSIS DIVISION
ATHENS, GEORGIA 30601

2/1/77

Mr. Sam L. Spencer
Chief, Fisheries Section
Dept. of Conservation and Natural Resources
64 N. Union Street
Montgomery, AL 36130

Dear Mr. Spencer:

At long last I have received from the EPA laboratory in Duluth, MN the results of the PCB analyses on fish collected in your State in the Spring of 1976. I am enclosing these results along with the method used in preparation and analyses.

We do appreciate your cooperation in this study and hope the information can be of use to your staff. If you have any questions, please don't hesitate to call me.

Sincerely,

W. H. Peltier
Ecology Branch

Enclosures

cc: James W. Warr
AL Water Improvement Com.

| Sample Area | Sample Composition | PCB's (ug/gm) 1016/1242 | 1254 |
|---|---|---|---|
| Choccolocco Creek<br>1 mile above Hwy 71<br>bridge in Talladega<br>County near<br>Anniston, AL | 2 Goldfish<br>4 Channel Catfish<br>4 Gizzard Shad<br>1 Redear Sunfish<br>4 Largemouth Bass | 10.5 | 78.8 |
| Dennison Slough<br>Wilson Reservoir, AL | 3 Carp<br>6 Gizzard Shad<br>3 Largemouth Bass<br>3 Redear Sunfish | 0.26 | 7.00 |
| Coosa River weir<br>State Line | 2 Goldfish<br>1 carp<br>4 Largemouth<br>4 Gizzard<br>2 bullhead | 5.0 | 60.0 |

whole fish

## Methods

### Preparation of Samples

The fish were collected by state and federal field crews and shipped frozen to Duluth. The fish in each sample were homogenized as a composite and given laboratory numbers consecutively from 1 to 58. Sub-samples weighing 30 gms and 20 gms were exhaustively extracted with a Soxhlet extractor using a 1:1 mixture of hexane and methylene chloride.

The 20 gm sample extract was concentrated and placed on a 20 gm Florisil column. The PCBs and related chemicals were eluted with 250 ml hexane, and the extract was concentrated to 100 ml for initial EC screening.

The 30 gm sample was concentrated to 100 mg/ml lipids in methylene chloride for cleanup by gel permeation chromatography. The extract was cleaned up by sequential elution of 5 ml aliquots on a bed of SX-2 Bio Reads (Bio Rad Laboratories) using methylene chloride as a solvent. The combined fractions containing the PCBs were passed through a column containing 15 gm Celite 545 impregnated with 9 mls of 1:1 $H_2SO_4$ and fuming $H_2SO_4$ (30% $SO_3$). The extract was concentrated to 1-3 ml for screening with FID gas chromatography and then GC/MS analysis.

### Electron Capture GLC Analysis

The extracts of the 20 gm tissue samples were analyzed for PCBs using a Hewlett Packard 5700A gas chromatograph equipped with a Model 3352B Laboratory Data System, an automatic sampler, and linearized

argon-methane detector. The column was a 6 ft. x 1/8 inch (O.D.) glass column packed with 80/100 mesh Gas Chrom Q coated with 4 percent/ 6 percent SE-30/OV-210. The column temperature was programmed from 160°C to 220°C at 2°C/min. followed by an 8 min. hold at 220°C.

The chromatograms were interpreted by a program prepared by Mr. Frank Puglisi of the Environmental Research Laboratory - Duluth in which 16 reference PCB peaks were monitored. Eight reference peaks corresponded to PCBs found in Aroclor 1016 and Aroclor 1242, and eight peaks corresponded to PCBs found in Aroclor 1254. The program estimated the quantity of either Aroclor 1016 or 1254 based on the individual peak areas and then determined the mean and standard deviation of the respective mixtures based on all reference peaks found. Peaks with areas more than twice the standard deviation were considered contaminated by an interfering chemical and were eliminated from the PCB estimate of the mixture. At least 4 of the 8 reference peaks were required to be present before an estimate of the PCB mixture was reported as an recognizable PCB mixture.

GC/MS Analysis

The extract of the 30 gm tissue samples were first screened using a flame ionization detector (FID) and then by GC/MS using identical GLC conditions. The column was a 6 ft. x 1/8 in. (O.D.) glass column packed with 80/100 mesh Gas Chrom Q coated with 3 percent OV-101. The temperature was programmed from 100°C after a 5 min. hold to 225°C at 4°C/min.

The GC/MS analysis was performed on a Varian Mat CH-5 mass spectrometer interfaced with a Varian 6021 data system. Spectra were acquired repetitively every 7 seconds during the gas chromatograph elution and, after mass conversion on the 6021 computer, were transmitted to a Varian 620L computer for data formating and analysis.

The screening of the GC/MS data was accomplished by selecting ions in the mass spectra of chemicals of interest and searching all of the spectra for the GC peaks where these ions occur most intensely.

The most rapid screening of this data is the mass chromatogram which is simply a plot of the intensity (heigh) of the characteristic ion, or mass, as a function of GC retention time which is stored in the computer as the spectrum number (i.e. one spectrum every seven seconds). This produces a new "gas chromatogram" in which peaks are observed only if a chemical is present that has the selected characteristic ions.