Assessment of Polychlorinated Biphenyl (PCB) and Mercury Exposure to Raccoon (*Procyon lotor*) Populations in the Bayou Creek Drainage: An FFOU Field Study

[†] A RESEARCH PROSPECTUS

by

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[†]This outline is a prospectus on proposed research. An official proposal, if solicited, will be forwarded in about 2 weeks through the University of Kentucky Research Foundation, Office of Sponsored Projects.

INTRODUCTION

With the implementation of the Biological Monitoring Plan in the fall of 1987, the Bayou Creek system was found to be contaminated with polychlorinated biphenyls (PCBs). This was traceable for the most part to commercial PCB mixtures that included Aroclor 1248, 1254, and some 1260. Greatest contamination occurred in Little Bayou Creek and this was associated, at least in part, with such effluent outfalls as 011. Due to a high incidence of action level PCB residues (≥2mg/Kg) in edible fish tissues, Little Bayou Creek was placed under a fish consumption advisory by the Commonwealth of Kentucky. The magnitude of PCB contamination in fish from Big Bayou Creek was considerably less and PCB detection in the water column was generally infrequent. These early trends in PCB contamination have been reviewed by Birge *et al.* (1989; 1992).

Later studies by personnel from the Oak Ridge National Laboratory (ORNL) concluded that there has been a steady reduction in PCB contamination in fish from 1992 through 1995 (ORNL, 1994; 1996). More recently, however, studies by Clemson University (Murry and Smith, 1997) reported widespread occurrence of relevant PCB congeners (*i.e.*, 138, 153, 170, 180) in the white-footed mouse (*Peromyscus leucopus*) and the marsh rice rat (O*ryzomys palustris*). Total concentrations ranged from 32.7 to 507 μg/kg (ppb) in liver tissues. Congeners 138 and 153 were found in lower concentrations in animals from the reference site. The latter was located 12 miles south of the Paducah Gaseous Diffusion Plant (PGDP) in Ballard County. These studies revealed no gross abnormalities, but noted liver weights tended to be somewhat higher in PGDP animals and there was a higher frequency of parasitic infection as compared with the reference specimens. Nevertheless, no clear evidence was put forth to indicate reduced population density or reproductive failure in these two species. As Aroclor mixtures (*e.g.*, 1248, 1254) were not analyzed, PCB exposure could not be fully characterized. In addition, patterns of PCB contamination could not be compared with fish or other data.

Although various metals were detected in mice and rats, mercury (Hg) was not analyzed. However, Hg contamination in fish has been reported by Birge *et al.* (1992) and ORNL (1994; 1996). Of the various environmental contaminants detected in the PGDP area, PCBs and Hg hold the greatest propensity to bioaccumulate in biota and biomagnify up the food web (U.S. EPA, 1980a; 1980b; 1985; Kosuda *et al.*, 1993). In addition, they are the contaminants with 1) the most stringent environmental regulatory standards, 2) FDA action levels, and 3) sufficient benchmark data to permit assessments of biological and ecological impact.

OBJECTIVES

This 18-month investigation centers on using the raccoon (*Procyon lotor*) as a sentinel monitor or indicator to further characterize PCB contamination within the Bayou Creek system. Raccoons have been used successfully in numerous studies of PCBs and other contaminants (Bigler *et al.*, 1975; Nalley *et al.*, 1975; Layer *et al.*, 1987; Valentine *et al.*, 1988; Clark *et al.*, 1989; Ford and Hill, 1990; Herbert and Peterle, 1990). Specific objectives include the following:

1) Determine and compare Aroclor residues (*i.e.* 1248, 1254, 1260) in raccoons from Bayou Creek sites adjacent to PGDP and a reference site with comparable habitat. Blood, liver, and adipose tissues will be sampled without sacrificing raccoons (see Work Plan below).

2) Identify specific PCB congeners in raccoon tissues important in characterizing PCB exposure and effects.

3) Analyze Hg in blood, liver, and hair to characterize extent of Hg contamination in raccoon populations.

4) Establish PCB and Hg ratios in blood compared with other tissues. This could facilitate future population studies if necessary. PCB exposure could be assessed using blood sampling, without further trauma to raccoons.

5) Analyze PCB and Hg data to evaluate possible deleterious effects on raccoons; to further characterize magnitude and sources of contamination; and to determine need for further study.

METHODS

Animal Collection

Four study areas will be selected as given below in the Work Plan. Raccoons will be taken humanely in Havaheart or similar live traps (Bigler *et al*., 1975; Herbert and Peterle, 1990). Animals will not be sacrificed. Trapping will occur in each study area for a specific period of time (*e.g*., 3 days) taking as many animals as possible. Six to eight animals will be selected from each study area for tissue sampling. If necessary, anesthesia or sedation (Bigler and Hoff, 1974; Bigler *et al*., 1975; Taulman and Williamson, 1992) will be used to simplify handling and transport. Hair samples will be taken in the field. Animals will then be transported to a local veterinary clinic where blood, liver and adipose tissue will be biopsied. Prior to release, animals will be given ear tags and there will be a minimal period of observation to determine state of health. Raccoons will be returned and released at original field locations. Care will be taken to insure humane treatment and to preclude disruption of raccoon populations to the extent possible. Blood will be collected and stored in Vacutainer heparinized tubes. Blood and tissue samples will be kept on ice during transport to the University of Kentucky laboratory, whereupon they will be frozen and stored at 4 °C.

PCB Analysis

Tissue extraction and clean-up

PCBs in raccoon tissues will be extracted and analyzed using standard U.S. EPA methods (Watts, 1980; Erickson, 1997). The wet tissue sample will be ground with 10 g anhydrous sodium sulfate and the powder will be extracted with petroleum ether in a Soxhlet apparatus for 5-h. The extract will be concentrated to near dryness in a Roto-evaporator (Buchi Model RE121). The reconstituted samples (5.0 mL in iso-octane) will be cleaned of interferences as described below and then analyzed by gas chromatography. Lipid and pesticide clean-up will be performed by eluting a 2.0 mL sample through a micro-column of 2.0 g activated 100-200 mesh Florisil® (100 ^{o}C/24 h) with 10.0 mL hexane and evaporated to 2.0 mL (Erickson, 1997; U.S. EPA, 1996, SW-846 Method 3620B, Florisil cleanup). Elemental sulfur will be removed by shaking 2-propanol (2 mL) and tetrabutylammonium sulfite (2 mL), adding ultra-pure water (8 mL) and reshaking. The organic extract will be removed and mixed with 2.0 mL concentrated sulfuric acid (Jensen *et al.*, 1977; U.S.

EPA, 1996, SW-846 Method 3660B, sulfur cleanup). A 4 μL sub-sample will then be analyzed by gas chromatography. PCBs in blood samples will be prepared using the methods described in Gill *et al.* (1996).

Analysis by Gas Chromatography

Samples will be analyzed for Aroclors 1248, 1254, and 1260 according to SW-846 Method 8082, polychlorinated biphenyls by gas chromatography (U.S. EPA, 1996). Analysis will be performed using a Hewlett-Packard (HP) Model 5890A gas chromatograph equipped with an electron capture detector and an HP Model 7673A Automatic Sampler. Samples will be 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. Special columns will be selected as necessary to optimize analysis of individual congeners (Albro *et al*., 1981; Safe *et al*., 1985; Schneider *et al*., 1985; Clarke *et al*., 1989; Anderson, 1991; Erickson, 1997). The temperature program will be set at 160 °C (6 min)-10 °C/min-235 °C (0 min)-0.9 °C/min-260 °C (10 min); Injector temperature, 280 °C; Detector temperature, 300 °C. PCB peak heights will be quantified using an HP Model 3396A integrator and multiple-peak linear regression analysis will be performed with Lotus-123® software. Aroclor levels will be calculated from heights of 6 to 9 peaks for Aroclors 1248 and 1260 and 4-6 peaks for Aroclor 1254. Five external standards will be used for calibration curves and for every tenth sample either a solvent blank or a standard will be analyzed. The Lotus program regresses data from PCB standards to the sample being analyzed. Each peak selected for each Aroclor class will be statistically analyzed (*e.g.*, standard deviation; standard error; relative deviation). Unless the specified number of acceptable peaks per Aroclor are obtained, the sample will be reanalyzed. This will include further clean-up (*e.g.*, pesticides), as well as a new standard curve. All chain of custody and records will be maintained in active files and will be available for review by FFOU, the Kentucky Cabinet for Natural Resources, or PGDP.

Hg Analysis

Hg samples will be prepared using U.S. EPA method 245.6 (Lobring and Potter, 1991). Tissue samples (0.2 – 0.3 g each) will be digested in a 4:1 mixture of concentrated sulfuric and nitric acids at 58 °C, followed by overnight oxidation with potassium permanganate and potassium persulfate at room temperature. Mercury in the digested sample will be reduced with stannous chloride to elemental mercury and quantified by the conventional Hatch and Ott cold vapor atomic absorption technique using a Hg analyzer system (Bachrach Coleman Model MAS-50B).

Quality Assurance

Quality assurance for PCB assays will include solvent blanks, procedure controls and spiked tissue recoveries (U.S. EPA, 1987). Certified standards will be used for quality assurance in all PCB and Hg analytical procedures as part of the quality assurance plan. Chain of custody forms, field notes, and laboratory records will be kept according to good laboratory practices (U.S. EPA 1996; Chapter 1) and maintained on file for inspection.

WORK PLAN

Study Area

Four sampling areas will be located and described. They will include a reference site away from the PGDP area, an area on Little Bayou Creek (LBS), an area on Big Bayou Creek (BBS), and the final study area will be in the Western Kentucky Wildlife Management Area (WKWMA) north of the plant. Locations are detailed below but may be modified as dictated by initial field studies.

1) *Reference area.* This site will be chosen from three possible locations based on the results of field study. Areas under consideration include: upper Massac Creek; upper Little Bayou Creek (no PCBs detected there); and the Ballard County area (12 miles south of PGDP) used by Murry and Smith (1997).

2) *The Little Bayou Creek Area (LBA).* These sites will be located between stream kilometers (Km) 9.8 and 7.8. This corresponds approximately to the area between effluent 012 and stream station LB3 (Birge *et al*., 1992).

3) *The Big Bayou Creek Area (BBA).* These sites will be located between stream Km 10.8 and 8.8. This corresponds to the area between stream station BB4 and a point between stations BB7 and BB8 (Birge *et al.*, 1992).

4) *The final study area.* This site will be in or near the WKWMA, either on Big Bayou Creek at Km 7.0 to 5.0 or on Little Bayou Creek at Km 5.0 to 3.0. An alternative location may be the area between the two creeks near their confluence. The final location will be chosen after initial field study.

NOTE: Field locations will be subject to approval by PGDP/DOE and will be adjusted as necessary to comply with any requests.

Collection and Field Study

Study areas will be chosen to include comparable habitat, including access to surface water systems. It is necessary to define any differences in population density or structure attributable to other conditions. Raccoons at each site will be live-trapped for a specified time interval (*e.g*., 3 days). The time will be adjusted as necessary to secure good population samples (*e.g.*, 12 or more raccoons/reference site). Upon collection, each animal will be examined, weighed, and tagged. Short-term sedation will be used if necessary. Collections will be made in the late fall/winter to avoid disturbing the reproductive cycle and to obtain samples of young of the year. Populations will be characterized as follows:

- Trapping success (*i.e*., animal vs. time)
- Ratio of young to adult
- Weight
- Observable condition/health
- Occurrence of abnormalities

Depending on availability, six to eight adult raccoons will be selected at each site for hair, tissue, and blood samples. Others will be released after inspection and tagging. Animals biopsied generally will be returned to their original location within 24-h. Hair, tissues, and blood will be analyzed as noted above.

Selection of PCBs

The analysis of Aroclor mixtures (*e.g.*, 1248, 1254, 1260) is important to characterize fully the extent and magnitude of possible PCB contamination and to allow comparisons with the existing data on fish, sediment, stream water, and effluents as reported by ORNL (1996) Birge *et al*. (1992), PGDP, and others. The selection of specific PCB congeners is somewhat more complex. The study by Murry and Smith (1997) included 11 congeners selected because they were easier to analyze (*i.e.,* less co-elution) and were considered to be persistent in animals. However, they excluded some congeners that 1) could be used as "markers" for specific Aroclor mixtures (*e.g.*, 1248) and 2) are important in evaluating biological effects and performing risk assessments. In particular, these omissions involved certain non-ortho substituted coplaner and mono-substituted coplaner biphenyls that are considered more prone to produce biological effects (Erickson, 1997; U.S. EPA, 1997). Their "absence" or "presence" in animal tissues correlates with lesser or greater risk, respectively. Accordingly, we propose to use the following selection of 8 congeners given in consecutive order: 77, 105, 118, 126, 138, 153, 170, and 180. Congeners 138, 153, 170, and 180 were those most often detected in animal tissues by Murry and Smith (1997), including 138 and 153 that were observed in " reference" animals. We will also analyze for four co-planer congeners that are ranked higher as to potency. These will include congeners 77 and 126 which are non-ortho substituted biphenyls (U.S. EPA, 1997). They are present in commercial Aroclors at lower concentrations than other selected congeners. For example, 77 and 126 have been quantified at 3000 and 88 μg/g (ppm) in Aroclors 1248 and 1254, respectively (Erickson, 1997). Congener 77 can be used as a marker for Aroclor 1248 and the latter frequently has been detected in fish from the Bayou Creek system. Congeners 105 and 118 are mono-substituted biphenyls that occur in Aroclors 1248 and 1254 at 14,000 to 20,000 and 32,000 to 76,000 μg/g, respectively (Erickson, 1997, p246). This selection should provide an adequate basis for examining food web uptake of specific congeners, assessing possible biological effects and tracking components of the commercial Aroclors most important in impact assessment (Alfro *et al.*, 1981; Safe *et al*., 1985; Anderson, 1991).

Time Table and Reporting

The following tentative schedule for activities can be altered to fit start-up date, budgetary matters, or the activities of PGDP. Synoptic reports will be submitted after each phase of the study.

1. Field sites will be selected and described in July/September, 1998.

2. Animals will be collected, inspected, and biopsied during October/December, 1998.

3. Hg analysis will be performed in January/March, 1999.

4. PCB mixtures and Congener will be analyzed in April/June, 1999.

5. Data analysis and literature reviews will be conducted in July/September, 1999.

6. Draft final report will be submitted in October, 1999.

7. Final report, revised as necessary, will be submitted in December, 1999.

SIGNIFICANCE OF RESEARCH

This investigation should provide useful information on sources and magnitude of "bioavailable" PCBs important to resource management and assessment of effects on biota. Specifically, it will be possible to determine if food web transport of "bioavailable" PCBs is sufficient to affect raccoons and other terrestrial organisms that feed on fish, crayfish, *etc*. Dietary PCB concentrations of 0.3 to 0.64 μg/g (ppm) adversely affected mink reproduction (Platonow and Korstad, 1973). Liver PCB residues in mink were 0.39 to 1.23 μg/g (ppm) for these dietary values. U.S. EPA (1980a) took 0.64 μg/g as the lowest maximum permissible tissue concentration for the protection of wildlife and based the freshwater aquatic-life chronic criteria of 0.14 μg/L on these results (*i.e.*, 0.64). Although raccoons likely are more tolerant to PCBs than mink, liver residues of 1 μg/g or greater may prove problematic. Assessment of the effects of PCBs on raccoon populations will be based on tissue residue levels and field observations noted above. Should liver residues exceed 1 μg/g and/or initial field studies reveal reduced populations in the affected areas, a further investigation may be recommended. The raccoon is an opportune "sentinel" species with which to monitor effects of PCBs on terrestrial wildlife (Bigler *et al*., 1975, Layer *et al*., 1987; Valentine *et al*., 1988) and U.S. EPA (1989) has indicated tissue residues as important in assessing ecological effects. It has been well established that the more sensitive effects of PCBs include reproductive suppression and endocrine disruption (U.S. EPA, 1980a; Waller *et al*., 1995; Palmer and Selcer, 1996).

Mercury also is well known to suppress reproduction, produce terata, and impair the autoimmune system (U.S. EPA, 1980b; Colburn *et al*., 1993; Kosuda *et al*., 1993; Wolfe and Norman, 1998; Wolfe *et al*., 1998). The maximum permissible tissue concentrations set by the U.S. EPA (1980b) were 1.0 μg/g for man, ≤ 1.1 μg/g for mink, and 5-7 μg/g for fish. Birge *et al*. (1979) found Hg tissue residues of 0.04 to 0.06 μg/g to correlate with about 50% mortality or teratogenesis in life stages of fish and amphibians.

Criteria for recommending further study will be based primarily on 1) evidence of reductions in population density or abnormalities observed in field studies and/or 2) tissue (e.g., liver) residue levels of PCB and/or Hg known to produce endocrine disruption, reproductive impairment, or other debilitating effects. That is, tissue residues of PCB or Hg determined in this study will be compared with those reported in other studies where corresponding deleterious effects have been described (Platonow and Korstad, 1973; U.S. EPA, 1980a; 1980b; Herbert and Peterle, 1990; Kasuda *et al*., 1993; Colburn *et al*., 1993; Palmer and Selcer, 1996; Erickson, 1997; Wolfe *et al*., 1998)

BUDGET[‡]

Estimated Direct Costs	
Labor	30,000
Travel	3,500
Reagents and Material	6,000
Instrument operation/ maintenance	3,000
TOTAL	**42,500**

[‡]Must be officially submitted via the University of Kentucky, Office of Sponsored Projects

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