Analysis of Polychlorinated Biphenyls (PCB) in Red-tailed Hawk Blood, Mink and Deer Liver and Kidney

David J. Price, Wesley J. Birge

Over the past several months, we have received requests to analyze a small sample of avian and mammalian tissues. These were "collections of opportunity" by FFOU personnel intended to be used for preliminary PCB screening and for prioritizing further studies.

METHODS

Tissue extraction and clean-up

PCBs in red-tailed hawk whole blood samples were extracted using methods described by Gill *et al.* (1996) and analyzed using standard U.S. EPA methods (U.S. EPA, 1996; Erickson, 1997). A 1.0 mL sub-sample of whole blood was removed from the original sample, 1.0 mL acetic acid was added and vortexed for 1 min., followed with 3.0 mL hexane/dichloromethane (9:1 v/v), and vortexed again. The sample was centrifuged for 2 min. at 1800 rpm and the top layer was decanted to a new tube. These steps were repeated two more times. To the new tube, 1.0 mL concentrated sulfuric acid was added and the sample vortexed again for 1 min. followed by centrifugation for 2 min at 1800 rpm. The organic layer was decanted, 1.5-mL hexane/dichloromethane was added and centrifuged once more. This last step was repeated once. The organic fraction was then concentrated to 0.5 mL. Cleanup of the organic samples were performed, as described by Gill *et al.* (1996), using custom made solid phase extraction (SPE) columns obtained from Supelco, Inc. The extract was eluted with hexane and taken to a final volume of 0.5 mL. A 4 μL sub-sample was then analyzed by gas chromatography. Mink and deer tissue samples (*i.e*. liver and kidney) were extracted and prepared for analysis using methods previously described for fish (Birge *et al.*, 1998).

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, 1996). 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. The temperature program was 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 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

Copies of all chain of custody forms and permanent records are maintained in active files and are available for review by FFOU or the Cabinet for Natural Resources. Original chain of custody forms are maintained by FFOU. Quality assurance for PCB assays included solvent blanks, procedure controls and spiked tissue recoveries (U.S. EPA, 1987; Birge and Price, 1997; Birge *et al.*, 1998).

RESULTS

The PCB assays for mink and deer are given in table 1. There was no detection of Aroclors 1248, 1254, or 1260 in deer liver, observing a detection limit 0.021 and 0.027 mg/Kg. This included two samples from deer 5 and one from deer 2. However, mink liver and kidney contained 1.10 and 0.53 mg/Kg, respectively.

The analysis of Hawk blood involved standardizing new methods for our laboratory, as described above. The initial assays were completed with chicken blood used as a reference tissue. Results are given in Table 2 and PCB recovery ranged from 83.5 to 88.2 percent. Also, no PCB contamination was detected in solvent controls intended to detect any PCB contamination in glassware used in these experiments.

The results obtained for blood taken from four red-tailed hawks are given in Table 3. Two blood samples were analyzed for hawk number 1. There was no detection of Aroclors 1248 and 1254. However, Aroclor 1260 was observed at 0.67 and 0.76 µg/mL (ppm). There was no detection of PCBs in hawk number 2, but Aroclor 1260 was found in blood from hawks number 3 and 4 at 0.09 and 0.06 µg/mL (ppm), respectively. Confirmatory results obtained in the second set of assays of chicken blood are given in Table 4. Recoveries ranged from 79 to 90 percent.

Analyses of PCB in blood from bald eagles were reported by Frenzel and Anthony (1989) and by Anthony *et al.* (1993). Eagles from Oregon and Washington, considered threatened or endangered, contained PCB blood levels in subadults and adults that ranged from 0.014 to 0.53 ppm and 0.018 to 2.4 ppm, respectively. The frequency of detection of PCB was 50 % for adults examined by Frenzel and Anthony (1989). Egg concentrations of PCBs averaged 12.7 ppm and ranged from 4.8 to 26.7 ppm in the study by Anthony *et al.* (1993). Based on results reported for the bald eagle, the red-tailed hawk populations from the PGDP area possibly may be impacted. This opinion is

supported by the 75% PCB detection frequency for these red-tailed hawks and by results of a recent study by McMurry and Smith (1997) in which all of the white-footed mice collected form around the PGDP site contained one or more PCB congeners. Therefore, a more extensive study is recommended.

Table 1. PCB Concentrations in Deer and Mink from PGDP
Collected August 5 and 14, 1997.

	Aroclor Conc. (mg/Kg)		
Sample Number	1248	1254	1260
DLV5080597PLV1A	<0.021	<0.021	<0.021
DLV5080597PLV1B	<0.027	<0.027	<0.027
DLV2(7a)080597PLV1	<0.021	<0.021	<0.021
MNK1081497PLV1	<0.187	<0.187	1.1
MNK1081497PKID1	<0.228	<0.228	0.53

DFA8080597PFAT1 sample coagulated during final volume determination and could not be injected.

Table 2. PCB results for control and spiked chicken blood samples extracted along hawk blood samples collected May 14, 1997.

Sample Number	Aroclor	µg Spike	Concentration (µg/mL)			Percent Recovery
			1248	1254	1260	
CBO062497PNUM1A[a]	No Spike	---	<0.020	<0.020	<0.020	---
CBO062497PNUM2A	1248	0.500	0.422	<0.020	<0.020	84.4
CBO062497PNUM3A	1254	0.500	<0.020	0.418	<0.020	83.5
CBO062497PNUM4A	1260	0.500	<0.020	<0.020	0.441	88.2

[a] CBO062497PNUM1A was extracted as a control to determine background PCB levels.

Table 3. PCB results for red tailed hawk blood samples collected from PGDP by Matt Vick during 1997.

	Aroclor Concentration (µg/mL)		
Sample Name	1248	1254	1260
HBO051497P#1A[a]	<0.020	<0.020	0.67
HBO051497P#1B	<0.020	<0.020	0.76
HBO051497P#2A[b]	<0.020	<0.020	<0.020
HBO051497P#3A	<0.020	<0.020	0.09
HBO051497P#4A[b]	<0.020	<0.020	0.06

[a] Sample #1 was analyzed on July 7, 1997 and redone on October 10, 1997.

[b] Samples #2 and #4 were clotted upon arrival, a lysing buffer was used to reconstitute the samples. Analyses of the buffer indicated no PCB contamination.

Table 4. PCB confirmatory results for control and spiked chicken blood samples extracted along hawk blood samples collected May 14, 1997.

Sample Number	Aroclor	µg Spike	Concentration (µg/mL) 1248	1254	1260	Percent Recovery
CBO062497PNUM1A[a]	No Spike	---	<0.020	<0.020	<0.020	---
CBO062497PNUM2A	1248	0.500	0.395	<0.020	<0.020	79.1
CBO062497PNUM3A	1254	0.500	<0.020	0.419	<0.020	83.8
CBO062497PNUM4A	1260	0.500	<0.020	<0.020	0.451	90.2

[a] CBO062497PNUM1A was extracted as a control to determine background PCB levels.

REFERENCES

Birge, W.J. and D.J. Price. 1997. Analysis of Metals and PCBs in Environmental Samples from the Bayou Creek Systems. Report submitted to FFOU December 8, 1997. 40 pp.

Birge, W.J., D.J. Price, and M.D. Kercher. 1998. Report to FFOU on Polychlorinated Biphenyl (PCB) Residues in Fish from the Bayou Creek System. Report submitted to FFOU January 30, 1998. 13 pp.

Erickson, M.D. 1997. Analytical Chemistry of PCBs, 2nd edition. CRC Press, Boca Raton, FL. pp.667.

Gill, U.S., H.M. Schwartz, and B. Wheatley. 1996. Development of a method for the analysis of PCB congeners and organochlorine pesticides in blood/serum. *Chemosphere* 32(6):1055-1061.

McMurry, S. and P. Smith. 1997. Polychlorinated biphenyl and metal exposure of small mammals at the Paducah Gaseous Diffusion Plant, McCracken County, Kentucky. Final report presented to Lockheed Martin, Vicki Jones. 28 pp.

U.S. Environmental Protection Agency. 1996. Test methods for evaluating solid wastes, SW-846, 2nd ed. Office of Solid Waste and Emergency Response, Washington, DC.

U.S. Environmental Protection Agency. 1987. Chapter One-Quality Control, update 1992. *In:* Test Methods for Evaluating Solid Waste-Volume IA:Laboratory Manual Physical/Chemical Methods. EPA/SW-86. Washington, DC.