SERUM/CELL DISTRIBUTION OF PCB CONGENERS IN BALD EAGLE BLOOD SAMPLES

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Introduction

One means of assessing exposure of avian species to organic environmental contaminants such as polychlorinated biphenyls (PCBs) is through collection and analysis of their blood. Levels and patterns of contaminants in blood can be correlated with levels and patterns in water, sediment and eagle prey as a means of determining exposure pathways. This approach has been used for assessing the reproductive health of bald eagles (*Haliaeetus leucocephalus*) along PCB-contaminated reaches of the Hudson River, New York, USA. The bald eagle is a threatened species. Therefore, the best option for assessing organic contaminants is to sample blood from live eagles. This is a difficult and time-intensive task, and the volume of blood that can be collected is limited to a few milliliters. Bald eagle blood was centrifuged into serum and cell compartments. Determination of the differences in PCB congener concentrations and patterns between the blood compartments is necessary for appropriate uses and interpretation of the data. This paper presents data on PCB congener distribution, including the dioxin-like non-*ortho*-PCBs, between serum and cells of several bald eagle blood samples.

Materials and Methods

The bald eagle blood samples used for this study were part of larger investigations led by the New York Department of Environmental Conservation and the US Fish and Wildlife Service. Samples of the serum and cell components, separated by centrifugation, were frozen and provided to CERC-USGS. Sample mass was typically less than 4 grams (<5 mL). The cells and serum samples were dehydrated by addition of anhydrous sodium sulfate and method recovery surrogates were added. Samples were extracted with dichloromethane and a portion (2-5%) of the extracts were used to gravimetrically determine percent lipid. Co-extracted biogenic materials were removed using low-pressure gel permeation chromatography followed by high performance size exclusion chromatography. The extracts were then fractionated on two-layered octadecyl silica/activated silica gel columns into two fractions. The first fractions were chromatographically separated by high performance porous graphitic carbon[1] (PGC) into two fractions: *ortho*-chlorinated PCB congeners (PGC1) and non-*ortho*-chlorinated PCBs (PGC2).

Approximately 140 PCB congeners were measured in the PGC1 fraction with a dual-column GC/ECD system. Analyses were performed using Hewlett-Packard 5890 Series II GCs with cool on-column capillary injection systems and Hewlett-Packard model 7673 autosamplers. The GC analytical columns, both of which had 3-m long retention gaps, were 60-m x 0.25-mm x 0.25-µm DB-5 and DB-17 capillary columns (Agilent Technologies, Palo Alto, CA). Fully resolved PCB congeners were quantified on a single column, while those requiring confirmation were quantified on both columns. A mix of several Aroclors was used for PCB congener calibration. These

standards have been quantified based on pure primary PCB standards (Accustandard, New Haven, CT) and are used as secondary standards[2]. Nine levels of calibration for each congener were used for quantification. In terms of total-PCB concentrations, the calibration curve covered a range from 10 to 8000 ng/mL.

The non-*ortho*-PCBs (PGC2) were determined by GC/HRMS, monitoring two sequential mass windows during the chromatographic separation. GC/HRMS analysis was performed with a Hewlett-Packard 5890A capillary gas chromatograph interfaced to a VG 70 high-resolution mass spectrometer[3]. A Hewlett-Packard 7673 autosampler was used to introduce 2 µL of the extracts onto a 2.5-m x 320-µm deactivated fused silica retention gap via heated (285°C) direct, on-column injection with a Restek spiral uniliner. The analytical column was a 50-m x 200-µm x 0.11-µm Ultra-1 (Agilent Technologies, Palo Alto, CA) capillary column. A calibration curve describing the response of each native congener (0.25 to 2,500 pg/µL) to that of its ^{13}C-labeled surrogate was used. Quantification was inherently corrected by the ^{13}C-labeled surrogates, which accounted for analytical losses during isolation procedures and variations in the instrumental analysis.

Results and Discussion

Levels of PCB congeners, non-*ortho* PCBs, total PCBs, and percent lipid of cells and serum are presented on a wet weight basis in Table 1. Lipid levels were <1%. There is significant uncertainty (± 50%) in the percent lipid determination due to small sample sizes, low lipid levels and method limitations. On a lipid-weight basis, serum-to-cell compartments of these bald eagle bloods had total PCB concentration ratios between 0.8-3.2. The measurement of the very low percent lipids in the eagle blood samples must be more accurate to make the lipid normalization, as they strongly influence the relationship between serum and cell PCB concentrations. On a wet weight basis the separated cells had lower levels of PCBs; typically 2-4 times lower than that found in the serum. Again the different percent lipid values of the cells and serum appear to explain this. However, the ratios of PCBs in serum to PCBs in cells correlated poorly, to the ratios of serum-to-cell lipids (all $r^2 < 0.6$), not surprising given the uncertainty in the lipid measurement.

Table 1: PCB Concentrations in Bald Eagle Blood Compartments

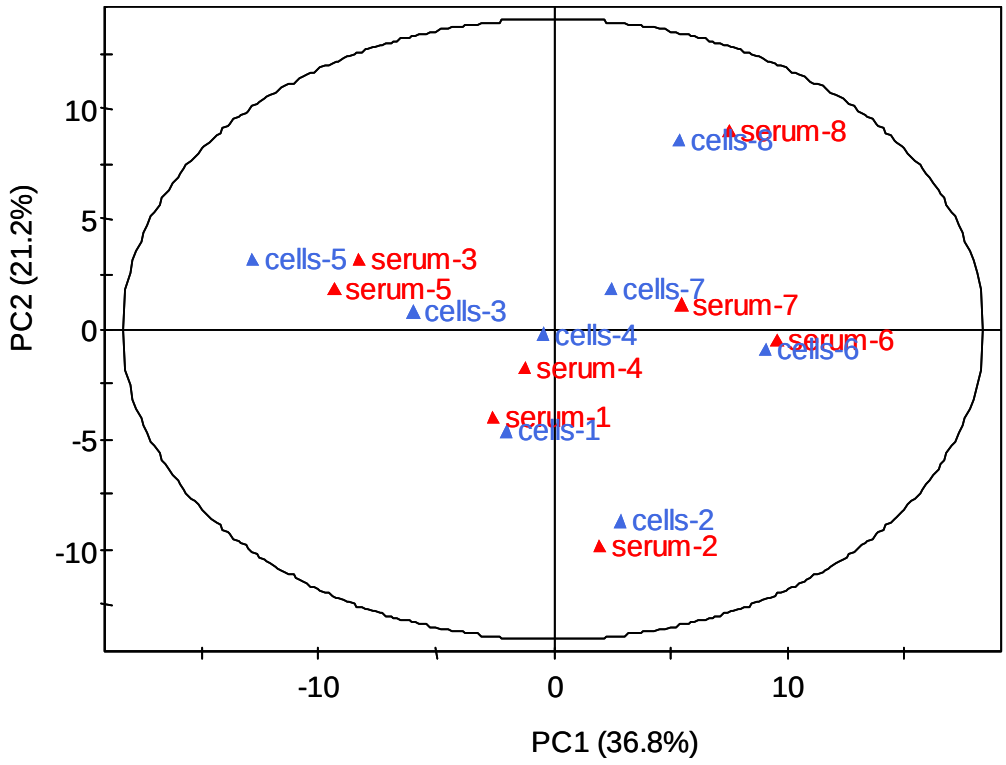
Eagle Blood Samples	Percent Lipid	81 (pg/g)	77 (pg/g)	126 (pg/g)	169 (pg/g)	Total PCBs (ng/g)
Sample 1						
serum	0.4	25	110	87	15	1,000
cells	0.2	22	90	63	8.3	390
Ratio	2.0	1.1	1.2	1.4	1.8	2.6
Sample 2						
serum	0.5	27	140	120	20	1,300
cells	0.2	12	72	45	9	310
Ratio	2.5	2.2	1.9	2.7	2.1	4.2
Sample 3						
serum	0.3	66	400	250	38	2,100
cells	0.2	35	210	110	13	440

Eagle Blood Samples	Percent Lipid	81 (pg/g)	77 (pg/g)	126 (pg/g)	169 (pg/g)	Total PCBs (ng/g)
Ratio	1.5	1.9	1.9	2.3	2.9	4.8
Sample 4						
serum	0.9	39	160	110	20	1,000
cells	0.2	19	88	43	9	240
Ratio	4.5	2.0	1.8	2.6	2.3	4.2
Sample 5						
serum	1.0	530	2,400	1,100	89	14,300
cells	0.2	170	850	310	22	3,600
Ratio	5.0	3.1	2.8	3.5	4.1	4.0
Sample 6						
serum	0.3	13	200	120	26	620
cells	0.2	7	92	53	13	270
Ratio	1.5	1.9	2.2	2.3	2.0	2.3
Sample 7						
serum	0.6	20	110	120	28	560
cells	0.3	12	67	67	13	260
Ratio	2.0	1.7	1.6	1.8	2.2	2.2
Sample 8						
serum	0.5	4	29	17	5	190
cells	0.3	2	22	10	3	110
Ratio	1.7	1.7	1.3	1.7	1.8	1.7

Total avian toxic equivalents (TEQs) were calculated from the serum and cells congener-specific data using the World Health Organization (WHO) toxic equivalency factors (TEFs) values for birds[4]. The calculated total TEQs ranged from 3 pg/g to 100 pg/g in cells and 4 pg/g to 340 pg/g in serum. The TEQs of the cells are 31-75% of the TEQs of the serums. For the TEQ calculation, it is critical that the congener-specific analyses be accurate and that the non-*ortho*-PCBs be fully resolved from interferences.

To determine whether the congener compositions of the serum and cell blood compartments were significantly different, we conducted a principal components analysis (PCA) on the PCB congener data, including non-*ortho* PCBs. The score plot of the first two principal components is presented in Figure 1. This score plot explains 58% of the total variance in the data set with the first two principal components—PC1 36.8%, PC2 21.2%. All samples fell within the 95% confidence ellipse of the calculated PCA model, indicating no significant difference in the patterns of PCBs of the serum and cell components of the eagle blood samples.

Figure 1. Principal Components Plot of Eagle Blood Components



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