

ORGANOCHLORINE CONTAMINANTS IN BIOTA FROM THE HUDSON RIVER, NEW YORK

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FWS PROJECT TITLE: INVESTIGATION OF EXPOSURE OF MIGRATORY BIRDS
TO PCBs, PCDDs, PCDFs AND ORGANOCHLORINE PESTICIDES
ALONG THE HUDSON RIVER

HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

HUDSON RIVER NATURAL RESOURCE TRUSTEES

STATE OF NEW YORK

U.S. DEPARTMENT OF COMMERCE

U.S. DEPARTMENT OF THE INTERIOR

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EXECUTIVE SUMMARY

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

The Hudson River supports a rich array of ecological resources that interact in complex ways, and provides habitat for a wide range of plants and animals. As part of the NRDA, the Trustees are documenting exposure of the natural resources of the Hudson River to PCBs.

One of the species for which the Hudson River provides habitat, and which has been exposed to PCBs, is the bald eagle (*Haliaeetus leucocephalus*). Bald eagles are at risk of accumulating PCBs because they are at the top of the food web. Eagles prey on fish and scavenge carcasses of birds, mink, otter, and other organisms that may contain PCBs. Because much of the eagles' diet may contain PCBs, they are at risk of accumulating concentrations that are associated with adverse health impacts.

In the 1990s, the Trustees began monitoring Hudson River bald eagle nests for reproductive success. As part of those studies the Trustees collected samples from bald eagles for contaminants analysis; samples of the eagles' prey have also been collected for contaminants analysis.

This report addresses bald eagle fat and plasma samples, and bald eagle prey samples collected from the Hudson River in 1998-1999 and analyzed by the U.S. Geological Survey, Biological Resources Division, Columbia Environmental Research Center in Columbia, Missouri.

Specifically this report provides the analytical results for the following twenty-two samples which were analyzed for total PCBs and selected congeners, organochlorine pesticides, non-ortho substituted PCB congeners, and 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins and dibenzofurans:

- 10 whole fish samples;
- 8 migratory bird samples (3 species);
- 2 sparrow egg composite samples;
- 1 bald eagle fat sample; and,
- 1 bald eagle plasma sample.

Within this set of samples, total PCB concentration in the fish ranged from 487 parts per billion (ppb) to 5174 ppb. Total PCB concentration in bird samples ranged from 97 ppb wet weight (ww) in an egg to 190 ppb in an adult bird. Note that the egg units are not fresh wet weight.



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U.S. Geological Survey- Biological Resources Division
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**Organochlorine Contaminants in Biota from the Hudson River,
New York**

by

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FWS Project Title:

Investigation Of Exposure Of Migratory Birds To PCBs, PCDDs, PCDFs And
Organochlorine Pesticides Along The Hudson River

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Project History:

The US Fish and Wildlife Service's New York Field Office is investigating chemical contamination and contaminant dynamics in biota from the Hudson River. Over the last decade, up to 40 bald eagles have been wintering along a 30-mile stretch of the Hudson River in New York between Danskammer Point and Croton Point. Releases of young eagles in the 1980's have resulted in the establishment of two nesting pairs along the Hudson and more are expected. However, the two established breeding pairs have been unsuccessful in producing offspring.

Bald eagle populations and factors influencing their productivity have been studied extensively at other locations but, since the breeding pairs now residing along the Hudson are the first in approximately one hundred years, no previous studies have been undertaken for the Hudson River birds. In this study, contaminants have been quantified in the serum and fat of an adult bald eagle and in a number of eagle prey species from the area. A number of other migratory bird species have also been analyzed for the same contaminants. Organochlorine pesticides, congener-specific polychlorinated biphenyls (PCBs), polychlorinated dibenzo-dioxins (PCDDs), and -furans (PCDFs) were targeted. Also, p,p'-DDE, a breakdown product of DDT which has been demonstrated to significantly impair bald eagle productivity, has been quantified in all samples. Together with information gained from studies on bald eagle productivity conducted in other areas, this information will be used to quantify the exposure of bald eagles living and nesting on the Hudson River to these contaminants within the food chain. The objective of the study is to estimate the effects of these contaminants on the adult birds and their productivity, and to obtain further information on sources and transport of these compounds within the Hudson River system.

Biota sampled by US F&WS were analyzed by the Organic Chemistry Section of the Columbia Environmental Research Center. The following analytes were targeted:

- Total PCBs and selected PCB congeners,
- Organochlorine pesticides
- Non-*ortho* PCB congeners
- 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and -dibenzofurans

A total of 22 samples were investigated:

- 10 whole fish,
- 8 migratory birds (3 species)
- 2 sparrow egg composites
- 1 bald eagle fat and 1 bald eagle plasma

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I. Summary of Analytical Methods for Sample Preparation

The 22 samples in this set consisted of 10 whole fish, 8 migratory birds (adults, nestlings, embryos), 2 sparrow egg composites, one bald eagle fat and one bald eagle plasma. The CERC database numbers assigned to these samples were 17138-17159.

Quality Control:

The following QC samples were analyzed with the samples:

- 4 procedural blanks
- 4 matrix blanks (control bluegill; chicken eggs)
- 4 matrix spikes (control bluegill; chicken eggs)
- 4 positive controls (Saginaw Carp)

Matrix QC samples (blanks and spikes) prepared from clean bluegill tissue and chicken eggs obtained from a local grocery were analyzed with each set of samples. Positive control samples were prepared from CERC's standard positive control matrix (common carp tissue from Saginaw Bay, MI). A total of four of each category of QC sample (procedural blank, matrix blank, matrix spike, and positive control) were analyzed with the samples. Additionally, one of each sample type was prepared, processed, and analyzed in duplicate or triplicate, where sample size was sufficient.

All samples, including QC samples were spiked with surrogate compounds before extraction to monitor recoveries through the cleanup procedures. Since the samples were processed through two separate analytical procedures, two different sets of internal standards were used. Where congener-specific PCBs, PCDDs, and PCDFs were targeted, the following compounds were used:

- PCB 030 (2,4,6-trichlorobiphenyl)
- PCB 204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl)
- ¹³C-labeled non-*ortho* PCB congeners (4)
- ¹³C-labeled 2,3,7,8 substituted dioxin/furans (17)

In the analytical protocol targeting organochlorine pesticides, the following compounds were added:

- PCB 030 (2,4,6-trichlorobiphenyl)
- PCB 204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl)
- Tetrachloro-*m*-xylene
- cis-Permethrin

The following compounds were added to matrix spikes according to the analytical protocol to which they were subjected:

- Organochlorine pesticides (23)
- PCBs (mixed Aroclors 1242, 1248, 1254, 1260)
- native (¹²C) dioxin and furan congeners

Sample Preparation:

Two different analytical protocols were performed portions of each sample. In each protocol, the samples were dehydrated by addition of anhydrous sodium sulfate and method recovery standards were added. Samples were extracted with methylene chloride, and a small portion of the extract (1%) was used to determine percent lipid (1). In the analytical protocol targeting congener-specific PCBs, PCDDs, and PCDFs, extracts were cleaned with acid- and base-treated silica gels and adsorbent chromatography on activated silica gel (2,3). All extracts were further purified by High Performance Gel Permeation Chromatography (HPGPC) (4) before fractionation on high performance Porous Graphitic Carbon (PGC) (5) into the following fractions:

- PGC-1 *ortho*-chlorinated PCB congeners
Analysis by gas chromatography (GC)/electron-capture detection (ECD)
- PGC-2 non-*ortho*-chlorinated PCBs
Analysis by GC/high resolution mass spectrometry (GC/HRMS)
- PGC-3 polychlorinated dibenzo-p-dioxins and -furans (PCDD/PCDFs)
Clean-up by alumina chromatography (6) before GC/HRMS analysis

Organochlorine pesticides extracts were first cleaned on gravity-driven gel permeation chromatography (7) followed by HPGPC (4). The extracts were then fractionated on a two-layered octadecyl silica/activated silica gel column into fractions containing PCBs and four of the targeted OCs (SODS-1), and a second fraction containing the remainder of the OCs (SODS-2) (8). Due to very small sample sizes, six samples (DB# 17138, 17139 and 17156-17159) were processed through a modified procedure in which the entire sample was processed through the organochlorine pesticide method, with SODS-1 undergoing further fractionation on PGC for analysis for PCBs, PCDDs, and PCDFs.

II. Congener-specific PCB Analysis and Results

The sample extracts were adjusted to a final volume of 10 mL, and 500 ng of the internal standard (octachloronaphthalene) was added. Individual PCB congeners were measured in PGC-1 (sample #s 17140-17149, 17152-17155) or SODS-1 (sample #s 17138, 17139, 17150, 17151, 17156-17159) fractions by GC/ECD. Results of the PCB analysis are presented in Table 1.

Instrumentation:

Analyses were performed as described in CERC SOP P.195 (9), using Hewlett-Packard 5890 Series II GCs with cool on-column capillary injection systems and Hewlett-Packard model 7673 autosamplers. For all analyses, 3-m sections of 0.53 mm i.d. uncoated and deactivated (Restek Corp., Inc.) capillary retention gap were attached to the front of each analytical column by a "Press-Tight" (Restek Corp., Inc.) union. The analytical column was a 60-m x 0.25-mm DB-5 (0.25 μ m 5% phenyl-, 95% methylsilicone, J&W Scientific). The H₂-carrier gas was pressure regulated at 25 psi.

The temperature program for the PCB analysis was as follows: initial temperature 60 °C, immediately ramped to 150 °C at 15 °C/min, then ramped to 250 °C at 1 °C/min, and finally ramped to 320 °C at 10 °C/min, and held for 1 min. The temperature of the ECD was held at 330 °C.

General Detection and Quantification Procedure:

Capillary GC/ECD data were collected, archived in digital form, and processed using a PE-Nelson chromatography data system which included the model 970 interface and version 4.1 of Turbochrom™ chromatography software on a Pentium microcomputer. Six levels of PCB standards, a combination of Aroclors 1242, 1248, 1254, 1260 in 1:1:1:1 w/w/w/w ratio (designated A1111), were used for PCB congeners calibration, with total PCB concentrations ranging from 200 to 8000 ng/mL. An instrumental internal standard (IIS) method with octachloronaphthalene (OCN) was used to calculate the concentrations of the targeted compounds. Samples were processed and analyzed in three batches; PCB congener results are presented in Table 1, designated by their CERC database number and are cross-referenced to their field identification number. Concentrations are expressed as nanograms of analyte per gram of sample (wet weight).

Quality Control Procedures and Results:

Recovery data for PCBs 030 and 204 are presented in Table 3. All concentrations are reported in nanograms per gram. Quality control data for procedural and matrix blanks, spikes, replicates, and positive controls are presented in Table 2. The method detection limits (MDLs) for individual PCB congeners and for total PCBs are based on procedural blank (PB) results according to the method outlined by Keith *et al.* (10, 11). Briefly, an average and standard deviation are determined. The MDL (ng) is calculated using the following formula:

$$\text{MDL} = (\text{PB Avg}) + 3(\text{PB SD}).$$

The MDL is then expressed in units of concentration: mass of analyte per mass of sample. If sample masses are within 10% of each other, an average mass is calculated for the entire set. The set of eight samples analyzed separately are noted in Table 1 with an asterisk. Therefore, there are two sets of MDLs calculated for these samples. The lowest MDL for this set of samples was 0.01 ng/g (12) for individual PCB congeners and 2.9 ng/g for total PCB concentrations.

Triplicate analysis of biota sample 17141 (A, B, C) showed precision better than 20% RSD for most of the PCB congeners present at concentrations 10-20 times the MDL. Nearer the limits of detection, variability increases (following measurement theory), and some PCBs in this low concentration range had higher %RSD's. Two other peaks reported as combined PCBs showed 25 and 37% RSD. The precision of the gas chromatographic analysis, peak measurement decisions, and quantification was determined by triplicate injection to be 3.4%.

Accuracy of the method is monitored through quality control. Analytical standards have been verified against certified standards. The method efficiency is monitored by analysis of positive control-Saginaw Bay carp. The following items are used to monitor recoveries:

1. Procedural recovery standards in each sample
2. PCB-spiked control bluegill tissue and chicken egg

The procedural recovery compounds, PCBs 030 and 204, which elute in the PGC-1 fraction, are presented in Table 3. PCB 030, a trichlorobiphenyl, is representative of more volatile early eluting PCBs (Cl₁ - Cl₃). PCB 204, an octachlorobiphenyl, is less volatile and representative of later eluting PCBs (Cl₄ - Cl₁₀). Recoveries averaged 77 ±14% for PCB 030 and 83 ±12% for PCB 204 in the biota analysis (Table 3).

Recoveries were within the QC criteria (50 - 125%), with two exceptions, samples 17141-A and PB 060198 had PCB 030 recoveries at 28% and 47%, respectively; 29% for PCB 204 in the 17141-A sample and 66% in the PB sample. Recoveries of spiked total PCBs were 96% for the tissue spikes. Positive control fish samples (Saginaw Carp) compared to within 96% of the running average of all previous analyses of this matrix by this laboratory for total PCBs.

III. Organochlorine Pesticide Analysis and Results

Organochlorine pesticide fractions (SODS-1 and SODS-2) were adjusted to a final volume of 2, 5 or 10 mL, depending on the mass of sample extracted, and internal standard (octachloronaphthalene) was added at 100, 250 or 500 ng, respectively. Organochlorine pesticides were measured in both fractions by GC/ECD. Results of the OC pesticide analysis are presented in Table 4.

Instrumentation:

Analyses were performed as described in CERC SOP P.459 (13), using Hewlett-Packard 5890 Series II GCs with cool on-column capillary injection systems and Hewlett-Packard model 7673 autosamplers. For all analyses, a 3-m section of 0.53 mm i.d. uncoated and deactivated (Restek Corp., Inc.) capillary retention gap was attached to the front of the analytical column by a "Press-Tight" (Restek Corp., Inc.) union. The analytical column for the SODS2 fraction was a 30-m x 0.25-mm DB-35ms (J&W Scientific). The H₂-carrier gas was pressure regulated at 11 psi. The temperature program for the analysis was as follows: initial temperature 90 °C, immediately ramped to 165 °C at 15 °C/min, held 3 minutes, then ramped to 260 °C at 2.5 °C/min with a 5 minute hold, and finally ramped to 320 °C at 10 °C/min, and held for 1 min. The temperature of the ECD was held at 330 °C.

General Detection and Quantification Procedure:

Capillary GC/ECD data were collected, archived in digital form, and processed using a PE-Nelson chromatography data system which included the model 970 interface and version 4.1 of Turbochrom™ chromatography software on a Pentium microcomputer. Six levels of OC pesticide standards were used for calibration, with

each pesticide as concentrations ranging from 1 to 80 ng/mL. An instrumental internal standard (IIS) method with octachloronaphthalene (OCN) was used to calculate the concentrations of the targeted compounds. Samples were analyzed and processed in three batches; Organochlorine pesticide results are presented in Table 4, designated by their CERC database number and are cross-referenced to their field identification number. Concentrations are expressed as nanograms of analyte per gram of sample (wet weight).

Quality Control Procedures and Results:

Quality control data for procedural and matrix blanks, spikes, replicates, and positive controls are presented in Table 5. All concentrations are reported in nanograms per gram. The method detection limits (MDLs) for individual compounds are calculated by the method described in the previous section.

The precision of the pesticide analysis was determined to be 36% by triplicate analysis of bullhead eagle nest sample #17141. The concentrations of certain pesticides in one of the triplicates were significantly different than in the other two portions taken. The data indicates that this variability was due to a combination of poor fractionation on SODS and overconcentration of the sample extract. The overconcentration resulted in losses of the more volatile components.

Pesticide-spiked chicken egg and bluegill tissues were used to monitor the recoveries of the 26 pesticides of interest. A generic recovery value for all 26 pesticides is not applicable. Recovery information for each individual pesticide is detailed in Table 5. Recoveries of DDT-related compounds averaged $98 \pm 28\%$. Chlordane components averaged $99 \pm 14\%$. Recoveries of PCA and HCB typically range from 50 – 80% with this analytical method. The measurement of one pesticide, o,p-DDT can be compromised by a PCB congener when PCB levels are high. In a spiked matrix its recovery can appear to be elevated by the presence of PCBs. The matrix spike, where both OCs and PCBs were present, indicated an o,p-DDT recovery of 139%.

Procedural Recovery compounds, tetrachloro-meta-xylene and cis-permethrin were used in this analysis (as test compounds) to monitor recoveries of OC pesticides in each sample. Tetrachloro-meta-xylene, eluting in the first fraction (SODS-1) had variable recoveries, including some in excess of 125%. Therefore, the data was not recovery corrected for this compound. For this data set, the matrix spike recoveries in Table 5 are a better indicator of the behavior of the pesticides in the samples.

The gas chromatographic analysis itself is very reproducible. We monitor this portion of the analysis, from injection through the detailed data interpretation process, by triplicate analysis of a sample extract. The average precision for all 26 pesticides was 4%.

IV. Non-*ortho*-PCB Congener Analysis and Results

The non-*ortho*-PCB fractions (PGC-2) were transferred to conical autosampler vials, evaporated to less than 50 μL with nitrogen, and then spiked with 5 ng of internal standard (50 μL of 100 $\text{pg}/\mu\text{L}$ ^{13}C -labeled 2,2',4,5,5'-PeCB (PCB #101) in nonane). The final volume was adjusted to about 50 μL with nitrogen blow-down. Non-*ortho*-PCBs were determined by gas chromatography/high resolution mass spectrometry (GC/HRMS), monitoring two sequential mass windows of selected ions during the chromatographic separation (14, 15).

Instrumentation:

GC/HRMS analysis was performed with a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high resolution mass spectrometer. An HP 7673 autosampler was used to introduce 2 μL of the extract from a conical vial onto a 5 m x 320 μm deactivated fused silica retention gap via heated (285 $^{\circ}\text{C}$) direct on-column injection with a Restek spiral Uniliner. A 50 m x 200 μm x 0.11 μm Ultra-1 capillary column was used to resolve non-*ortho*-PCBs from most interferences. The GC oven was held at 120 $^{\circ}\text{C}$ for 1 min, programmed to 240 $^{\circ}\text{C}$ at 2.2 $^{\circ}\text{C}/\text{min}$, then ramped to 310 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$, and a final hold of 5 min. Helium carrier gas was maintained at 45 psig with an initial linear velocity of 27 cm/s . The analytical column was put into the MS interface, heated at 310 $^{\circ}\text{C}$. All column-to-column connections were made with fused silica press-tight connectors.

General Detection Procedure:

The VG GC/HRMS system was tuned to 10,000 R.P. and calibrated using perfluorodecalin, and mass windows were established for two groups of non-*ortho*-PCBs. Group 1 from 23-47:00 min included ions for Cl_4 -biphenyls #77 and 81 and Cl_5 -biphenyl #126; Group 2 from 47:05-64 min included ions for Cl_6 -biphenyl #169. Within each mass window, two most abundant ions were measured for positive identification and quantitation of each analyte. The ion responses were quantified and averaged, unless interferences occurred. Within each mass window, additional ions monitored the responses of higher chlorinated, potential interfering PCB congeners, Cl_{4-8} naphthalenes (PCNs), Cl_{3-5} terphenyls (PCTs), Br_5 - and Cl_6 -diphenyl ethers (residual carryover from PGC-1), and Cl_4 -PCDF (to ensure no breakthrough of PCDFs).

Quantitation of Analytes:

With isotope dilution MS quantitation, the amount of each analyte detected is inherently corrected to account for losses through the whole analysis (isolation of analytes and instrumental analysis) because ^{13}C -isotopically labeled surrogates added at the beginning are recovered or lost in the same percentage as the native target analytes. A calibration curve describing the response of each native congener to that of its ^{13}C -labeled surrogate was used directly in the calculations and its range of values were determined in the calibration procedure. Each calibration curve was specifically matched to the range of analyte responses in the sample set.

Concentrations of the native PCB congeners in standards ranged from 0.25 to 2,500 pg/ μ L.

Chromatographic and Mass Spectral Resolution:

PGC separates non-*ortho*-PCBs from other PCB congeners with nearly 99.9% efficiency. However, even this 0.1% carryover of major PCB congeners can interfere with gas chromatographic/mass spectral analysis: fragment ions are not fully resolved by high resolution MS and thus overwhelm the response of the lower level non-*ortho*-PCBs. Therefore, a 50-m Ultra 1 column is used (instead of the more commonly used DB-5 column) to chromatographically resolve most non-*ortho*-PCBs from major PCBs: non-*ortho*-Cl₄-PCB 81 elutes about 9 sec earlier than Cl₅-PCB 87, non-*ortho*-Cl₄-PCB 77 elutes about 10 sec later than Cl₆-PCB 136 and 10 sec earlier than Cl₅-PCB congener 110, and non-*ortho*-Cl₆-PCB 169 elutes when no other PCBs elute. For continuing QC checks on chromatography, molecular ion responses of these major PCB congeners are measured to ensure that their fragment ion responses do not contribute an interference >10% to the responses of the respective non-*ortho*-PCB. Column performance is verified by analyzing standards of individual congeners, labeled congeners, and congeners from Aroclor spiked mixtures.

Because non-*ortho*-Cl₅-PCB 126 is only minimally resolved from Cl₆-PCB 129, PCB 129's molecular ion response is monitored to assure that its fragment ion response (3.5% abundance) does not contribute an interference of >10% to the response of PCB 126. PCB 129's molecular ion response must not exceed three times that of PCB 126.

Adequate mass resolution is verified while monitoring ions for Cl₄₋₈ PCNs throughout the sample set. The Cl₅₋₇ PCNs ions monitored differ by about 0.1 Da from the ¹³C-Cl₄₋₆ PCB surrogates, assuring a continual check on mass resolution. For each mass window, lock-mass and lock-mass-check ions were used to maintain and verify the accuracy of mass measurement.

Criteria for Confirmation:

For the positive identification and quantitation of each congener, the following criteria were established and met in this study:

1. Peak areas for the selected ion responses must be greater than three times background noise.
2. Native ion peaks must occur at retention times from -1 to +3 sec that for the corresponding ¹³C-labeled ion peaks, that elute about 1 sec earlier.
3. The ion ratio for the two principal ion responses must be within the acceptable range (generally $\pm 15\%$). These ion ratios were determined experimentally for the system during calibrations, compared with the theoretical values, and were tracked.

Method efficiency by calculating percent recovery of ¹³C-surrogates:

To account for variations in GC/HRMS analysis, a known internal standard amount was spiked into the final extract and used to calculate the amounts of the surrogates recovered in the final extract. The efficiency of the extraction and cleanup procedure was measured by comparing the quantity of the surrogates detected in the *final* isolated extract (at GC/HRMS analysis) with the quantity spiked into the sample.

Quality Control Results:

Total mass (pg) of native non-*ortho*-PCBs in the procedural blanks are normalized to sample size (in this case 15 g in Table 6) In the procedural blank of 6/8/98, values are at or below the lowest concentrations in the samples. Non-*ortho*-PCB concentrations are also low in the bluegill (matrix) blank, but are slightly higher in the chicken egg (matrix) blank (Table 6), especially for PCBs 81 and 77.

In the Aroclor-spiked bluegill and chicken egg samples, the most abundant non-*ortho* congener, PCB 77, is within 20% of the historic mean determined for our mixed Aroclor spiking standard. Less abundant non-*ortho* congeners PCBs 81 and 126 in the Aroclor-spiked samples are also within 20% of the respective mean. PCB 169 is too low for meaningful comparisons.

Average non-*ortho* PCB concentrations (Table 6) in the two positive control Saginaw Bay carp samples are also all within 20% of their respective historic mean based on 52 previous QC samples.

Percent recoveries of the ¹³C-labeled surrogates (Table 7) range from 8 to 127%, but only three samples (17141-A, 17156, and 17157) have recoveries below the QC range (25-125%). In replicate A of sample 17141, significant sample extract loss must have occurred at or before HP-PGC, because other PCB congeners were also affected based on 20% recoveries of surrogates for cPCB analyses (see above). For 17156 and 17157, selective loss of the ¹³C-non-*ortho*-Cl₄-PCBs, especially congener 81, indicates that PGC chromatography likely shifted these surrogates into the PGC-1 fraction because of an overload of total PCBs. With isotope dilution quantitation, however, the corresponding native PCB 81 and 77 concentrations are still accurate because their values are self-corrected by the technique.

Ion ratios of the primary ions for all detected analytes in both samples and calibration standards generally varied within the QC range ($\pm 15\%$ of theoretical), except where noted by LQ (< method quantitation limit due to inaccurate ion ratio). Thus most concentrations associated with LQ are less precise and more approximate values just above the detection limit.

V. 2,3,7,8-Cl Substituted Dioxin and Furan Analysis

PCDD/PCDF fractions from PGC (PGC-3) were eluted through basic alumina according to CERC SOP P.193 (6) for removal of potential co-contaminants such as

chlorinated diphenyl ethers and residual PCNs and PCBs. A total of 1 ng of the internal standard, ^{13}C -labeled 1,2,3,4-TCDD, was added to each semiconical autosampler vial prior to transferring the PCDDs/PCDFs. The final extract was concentrated to a volume of ~25 μL under a stream of nitrogen. PCDFs and PCDDs were determined by GC/HRMS by monitoring five sequential mass windows of selected ions during the chromatographic separation according to SOP P.482 (16).

Instrumentation:

GC/HRMS analysis was performed using a HP 5890A capillary gas chromatograph interfaced to a VG 70-AS high resolution mass spectrometer. An HP 7673 autosampler was used to introduce 2 of 25 μL of the extract from a conical vial through a spiral uniliner onto a 5 m x 320 μm deactivated fused silica retention gap via a heated (285 $^{\circ}\text{C}$) direct inlet. The analytes of interest were separated on a 50 m x 200 μm x 0.11 μm Ultra-2 (Hewlett Packard) capillary column with an initial hold of 1 min at 120 $^{\circ}\text{C}$ followed by a ramp to 200 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$, another ramp to 300 $^{\circ}\text{C}$ at 2.3 $^{\circ}\text{C}/\text{min}$, and a final hold of 5 min. The helium carrier gas was maintained at 44 psig with an initial linear velocity of 25 cm/s. All column-to-column connections were made using fused silica press-tight connectors.

General Detection Procedure:

The VG GC/HRMS system was tuned to 10,000 R.P. and calibrated using perfluorokerosene, and mass windows were established for five ion groups to measure Cl_{4-8} PCDFs and PCDDs. These windows were monitored sequentially during the temperature program. Within each mass window, two most abundant ions were measured for positive identification and quantitation of each analyte. The ion responses were quantified and averaged, unless interferences occurred. Within each mass window, additional ions monitored any responses from potentially interfering Cl_{5-9} -PCDEs and Cl_{5-7} -PCTs, and dioxin-like Cl_{6-7} -PCNs, Cl_{3-8} dibenzothiophenes (PCDTs), and Cl_{3-8} phenanthrene and anthracenes.

Quantitation of Analytes using the Method of Isotope Dilution:

A calibration curve describing the response of each native congener to that of a ^{13}C -labeled surrogate congener was used directly in the calculations and its range of values were determined in the calibration procedure. Each calibration curve was matched to the range of analyte responses in the sample set.

Chromatographic and Mass Spectral Resolution:

Window switching times were established using a window-defining PCDF/PCDD standard mixture; relative retention times were then established for PCDTs. Chromatographic columns were selected and temperature programmed on the basis that they must resolve 2,3,7,8-TCDD from 1,2,3,7/1,2,3,8-TCDD (and from 1,2,3,4-TCDD) by a resolution factor of at least 0.5. Column performance was verified by analyzing standards of individual components, and observing the chromatographic resolution of the TCDDs, HxCDDs, and HxCDFs. Similarly, relative retention times for all other congeners of interest were evaluated with respect to labeled analogs.

Adequate mass resolution was verified while monitoring ions for Cl₆₋₇ PCNs vs. ion responses of ¹³C-TCDDs and of native TCDD vs. ¹³C-TCDF throughout the sample set. The latter two ions, both at nominal m/z 320, differ by 0.04 Da, requiring a Resolving Power of at least 8000 for complete resolution. Monitoring these ion ratios thereby assures a continual check on mass resolution. For each mass window, lock-mass and lock-mass-check ions were used to maintain and verify the accuracy of mass measurement.

Criteria for Confirmation:

For the positive identification and quantitation of a particular congener, the following additional criteria had to be met:

1. The peak areas for the selected ion responses must be greater than three times the background noise (S/N > 3)
2. For congeners with isotopically-labeled analogs, the ion peaks for the native must occur at retention times from -1 to +3 sec that for the corresponding ¹³C-labeled ion peaks, which elute about 1 sec earlier than the native ion peaks;
3. For OCDF (without an isotopically-labeled analog), ion responses in sample analyses must occur at RRTs from -0.2 to 0.5% of ¹³C-labeled OCDD, analogous to the window above;
4. For the two principal ion responses, the ion ratio must be within the acceptable range (generally ±15%). These ion ratios were determined experimentally for the system during calibrations, compared with the theoretical values, and were tracked for quality assurance.

Calculation of method efficiency (recovery of ¹³C-surrogates): A known amount of internal standard was spiked into the final extract and used to calculate the amounts of the surrogate recovered in the final extract. The efficiency of the extraction and cleanup was measured by comparing the quantity of the surrogates detected in the final isolated extract (at GC/HRMS analysis) with the quantity spiked into the sample at the beginning of the extraction step.

Quality Control Results: In the quality control blanks, amounts of PCDFs and PCDDs are expressed as total mass (pg) divided by 15g to normalize to sample concentrations (Table 8). In these blanks, values are at or below the lowest concentrations in the samples except for OCDF in the carryover blank. OCDD and OCDF are the only analytes in the bluegill or chicken egg blank that exceed 0.4 pg/g. Concentrations of native PCDFs and PCDDs in the spiked bluegill or chicken egg samples are within 25% of that expected except for OCDF and OCDD. Analyte concentrations in the positive control Saginaw carp samples closely compare (within 10-15%) with the average values from over 20 previous QC analyses for those congeners greater than the MQL (Table 8).

Recoveries of most of the ^{13}C -labeled surrogates (Table 9) are within the expected QC range of 25-125 % except for samples HUD BB3, HUD BB4, and HUD WD1 where 2,3,7,8-TCDF and TCDD recoveries were <25%. The native TCDF and TCDD concentrations were still measured - HUD WD 1 had 16.7 pg/g TCDF while all others were < 1 pg/g. The low recoveries were due to volatilization losses during the final solvent reduction. Greater than normal losses of the instrumental standard (^{13}C -1,2,3,4-TCDD) also occurred in these same samples.

Ion ratios of the primary ions for all detected analytes in both samples and calibration standards varied within the QC range ($\pm 15\%$ of theoretical) except where noted by LQ. Values designated as LQ are less than the method quantification limit.

VI. Summary

This report is part of a larger study that is investigating exposure of migratory birds to contaminants along the Hudson River. Fish, birds, eggs, bald eagle fat and plasma were analyzed for organochlorine pesticides, PCB congeners, non-*ortho* PCBs, and polychlorinated dioxins/furans. Quality assurance procedures and results for the different classes of analyses are described in detail in their various sections. In general, QC performance fell well within prescribed limits; exceptions are discussed in the text and noted in the data tables.

Within this set of samples, total PCB concentrations in the fish ranged from 487 ng/g to 5174 ng/g. Total PCB concentrations in bird samples ranged from 97 ng/g in the Savannah sparrow egg and the HUDBB4 bluebird nestling to 190 $\mu\text{g/g}$ in one adult tree swallow sample. The adult tree swallows (HUDTS-1 and 2), one bluebird sample (HUDBB2), and the eagle fat sample (HUD FT1) had the highest concentrations of total PCBs: 83, 190, 78, and 86 $\mu\text{g/g}$, respectively.

Mono-*ortho* PCB congener concentrations in these samples followed the same trends as for the total PCBs. Concentrations of the prominent mono-*ortho* PCB congeners #118 and #105 were 10 to 1500 times higher in the four samples discussed above than in the lowest two samples (HUDSP and HUDBB4). Mono-*ortho* PCBs in these biota samples ranged in concentration from 10 ng/g to 16600 ng/g.

Organochlorine pesticides were found in these biota samples from the Hudson River, however, many of the pesticides were at low concentrations or were below method detection limits. In each sample, the most prevalent OC pesticide was p,p-DDE, ranging from 32 to 3564 ng/g. Other DDTs related pesticides were much lower in concentration. The p,p-DDD concentration in fish and the eagle samples ranged from 5 - 414 ng/g, while the p,p'-DDD in all bird samples was <1 ng/g. Hexachlorobenzene, alpha-BHC, *trans*-nonachlor, oxychlorodane, dieldrin, heptachlor epoxide were found in all samples. Within the samples reported in this study, the eagle fat had the highest concentrations of pesticides. Methoxychlor was not found in any of the samples.

Concentrations of individual non-*ortho*-PCBs ranged from three to four orders of magnitude from the lowest to the highest samples. Concentrations appear to correlate to the sample's rank in the food chain. Adult tree swallows, two bluebird samples, and the bald eagle fat sample had the highest concentrations, much higher than the fish and eel samples. Generally, PCB 77 is the most abundant non-*ortho*-PCB. However, in two of the four eel samples, PCB 126 was the most abundant. PCBs 77 and 81 are relatively high in only one of the four eels. A possible explanation is that the American eel metabolizes the Cl₄-PCBs 77 and 81 more rapidly than PCB 126.

Concentrations of PCDFs and PCDDs overall ranged from as low as 0.1 pg/g to as high as 364 pg/g. TCDD concentrations were from 0.1 pg/g to 17 pg/g in the bald eagle fat sample (HUD FT1). The PCDFs and PCDDs concentrations were highest in adult tree swallow samples (HUD TS1 and TS2); the level of OCDF in the Henslow sparrow egg (HUD HP) was very high. The dioxin and furan concentrations were lower in wood duck embryos and were even lower in the bluebird nestlings.

Concentrations of PCDFs and PCDDs were generally 10 times higher in the eagle fat than in the plasma. TCDD was up to 6 times higher in the fat than in plasma. The concentration ratio for 1,2,3,6,7,8-HxCDD was 22; the ratio for HpCDD, OCDD, and 2,3,4,7,8-PeCDF was about 13. Eagle prey showed similar PCDF and PCDD profiles to biota from the river. The American eel from an eagle nest (HUD NST 1) showed a close correlation to one from the river (HUD RIV 1). In a similar comparison, the profiles from a brown bullhead from an eagle nest (HUD NST 2) also compared closely with bullhead from the Hudson River (HUD RIV 4).

Samples were screened for polychlorinated naphthalenes (PCNs), polybrominated diphenyl ethers (PBDEs, Br₅ primarily) and for dibenzothiophenes (PCDTs). PCNs, primarily as Cl₄- and Cl₅-PCNs, ranged from 5 pg/g to near 1000 pg/g. There was no constant ratio of PCNs to PCBs observed in the samples. PCNs were very low in the eels and low in both adult tree swallow and wood duck embryo samples. PCNs were found to be much higher in the bullhead and perch and highest in the HUD BB 2 bluebird nestling. The highest PCN levels in these samples were about the same levels as in our positive control carp sample from Saginaw Bay, MI. PCNs are of potential toxicological concern because they are dioxin-like compounds and may contribute to the overall dioxin toxic equivalents; complete studies of their toxic equivalence however have not been performed.

PBDEs, which are flame retardant compounds, can be screened by GC/HRMS by analyzing the PGC-2 fraction. The bulk of the PBDEs are in the PGC-1 fraction, with about 1% occurring in PGC-2. Using PGC-2, samples that contained high levels of PBDEs were pinpointed: The brown bullhead HUD NST 2 samples had the highest PBDEs, perhaps as much as 100 ng/g based on the PGC-2 estimate. The adult tree swallow samples were slightly lower, followed by the fish and eel samples and then

the rest of the samples including the bald eagle fat. Also based on the small amounts in the PGC-2 fraction, polychlorinated terphenyls and Cl₆-diphenyl ethers were measured. These compounds were found to be very low in all samples. PCDTs, which are sulfur-analogs to the PCDFs, were detected in PGC-3 fraction in only some of the samples and at very low concentrations (< 5 pg/g). The PCDT most often detected was tetrachlorinated.

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