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Feathers As Bioindicators Of Pcb Exposure In Clapper Rails

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Feathers as bioindicators of PCB exposure in clapper rails

(TITLE)

BY

Jay W. Summers

THESIS

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Feathers as bioindicators of PCB exposure in clapper rails

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Abstract

In this study we used feathers as a biomonitor for exposure to the polychlorinated biphenyl (PCB) Aroclor 1268. Clapper rails have been used as an indicator species of environmental damage for the LCP superfund site located in Brunswick, GA USA. LCP is contaminated with Aroclor 1268, which has been used in limited amounts elsewhere and therefore can be used as a contaminant marker. Aroclor 1268, including congener profiles, were quantified using gas chromatography (GC). Concurrently each sample was quantified for total Aroclor 1268 using an enzyme-linked immunosorbant assay (ELISA) and compared to the GC results to determine if ELISA was a cost effective method for quantifying or qualifying PCBs in feathers. We also conducted experiments to determine if contamination is truly endogenous rather than exogenous by exposing clean feathers to contaminated mud and cleaning them with varying methodologies. GC analysis showed that "clean" feathers exposed to contaminated mud had no measurable residues of PCBs regardless of washing method. Results showed that ELISA consistently quantified PCB loads one order of magnitude less than the GC. Based on sample replication, extraction recovery, and sample spike, it appears that GC is the more reliable method of detection

and that ELISA methods may be better used for qualitative exposure assessment for this particular Aroclor.

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The greatest appreciation is extended to my advisor, Dr. Karen Gaines, for her constant support and guidance throughout my academic career at Eastern Illinois University. The overwhelming patience, insight and perseverance that Dr. Gaines has shown me throughout my time at Eastern Illinois University will stay with me for the rest of my life.

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Introduction and Literature Review

Persistent organochlorine pollutants (POPs) have become a concern as environmental contaminants because of their slow degradation, tendency to bioaccumulate and chronic, detrimental effects on wildlife as well as humans. Polychlorinated biphenyl's (PCBs), a subgroup of POPs, tend to be highly persistent and therefore degrade very slowly once they enter a system. Of the thousands of POP chemicals, there are 209 different PCBs that vary from each other by level of chlorination and substitutent position (USEPA 1990). By nature, POPs are lipophilic and hydrophobic and partition strongly into the organic matter fraction in soils. Specifically, they adhere to lipids in organisms and become stored in adipose tissue. POPs persist in biota because the metabolism of lipids is relatively slow and therefore these chemicals accumulate in food chains (Jones and de Voogt 1999).

Though the manufacturing of PCBs has been discontinued in the United States since the 1970's, PCBs have been globally manufactured for decades. In Russia, approximately 130,000 tons have been produced and in Japan 60,000 tons were created between 1955 and 1972 for use in electrical transformers and capacitors. The waste leaching from this equipment into the environment poses a direct threat to wildlife and humans because of inadequate disposal methods (Kunisuea et al. 2003). Aroclor 1268, the PCB that contaminated marshes around LCP is an uncommon, highly chlorinated PCB mixture that was used to lubricate graphite electrodes used in processing equipment (Kannan et al. 1998). Monsanto Corporation sold PCBs under the trade name Aroclor followed by a 4 digit number. The first two digits usually refer to the number of carbon atoms in the biphenyl skeleton (12 for PCBs); the second two numbers indicate the

percentage of chlorine by mass in the mixture. Thus, Aroclor 1268 has 12 carbon atoms and contains 68% chlorine by mass (USEPA 2002). Adsorption to organic materials, sediments and soils will typically increase with chlorine content of the PCBs. PCB congeners with low chlorine content are commonly more volatile and more soluble (Callahan et al. 1979).

The Linden Chemical Plant is a relatively small industrial facility surrounded by approximately 500 to 600 acres of tidal marshlands (Fig. 1). Excluding the tidal marshlands, the property is occupied by process buildings, offices, treatment and disposal units, and tank storage facilities. The surrounding marshlands of LCP consist of tidal creeks, which form branching networks that flood and drain with tidal fluxes, and tidal pools that are formed at high tide and later exposed at low tide (Gaines et al. 2003). From 1968 to 1994, waste sludge (used in paints, transmission fluids and other coating compounds) containing lead (Pb), mercury (Hg) and PCBs was pumped into surface impoundments constructed along the tidal marsh at the Linden Chemical Plant (LCP) site near Brunswick, GA. The facility was declared a Superfund site when it closed in 1994 and subsequent discovery of elevated levels of metals and organics in several wildlife species followed shortly thereafter (USEPA 1990). LCP purchased Aroclor 1268, a highly chlorinated PCB, from the sole manufacturer, Monsanto Corporation, which produced only a limited amount. Based on this unique feature, Aroclor 1268 can be used as a chemical marker to determine whether biota have resided in LCP (Novak et al. 2006).

In 1989, the U.S. Environmental Protection Agency (EPA) performed a field investigation of seven onsite impoundments. Aroclor 1268 was found in the majority of sources; Hg was detected in all seven sources and Pb in six sources. Hg and Pb were also found in ground water and a surface water body in the vicinity of the site. In 1991, a Georgia Environmental Protection Division / Department of Natural Resources investigation discovered elevated levels of Hg in crab tissue and oyster samples in the surrounding waters, as well as extremely high levels of PCBs in sediment and crab tissue (USEPA 2002). By 1996, 150 tons of mercury and 25,000 tons of contaminated soil had been recovered and shipped to off-site disposal grounds (USEPA 2002). Within the last 5 years, however, little has been done in terms of remediation.

Clapper rails (*Rallus longirostris*) are opportunistic feeders that typically prey on invertebrates in tidal marshes (Heard 1982). In coastal salt marshes, home ranges of clapper rails are smaller than northern varieties, ranging from 0.04 ha (Roth et al. 1972) to 1.66 ha (Zembal et al. 1989) during the February – August nesting season, and 0.10 to 2.00 ha in winter months (Roth et al. 1972). This makes them an ideal species to study the effects of site specific contamination such as those found at LCP. Previous studies have shown a high degree of double-stranded DNA breakage in adult clapper rails inhabiting LCP (Novak et al. 2006), eggshell integrity problems (Rodriguez-Navarro et al. 2002), and bone mineralization abnormalities from chicks hatched from LCP (Rodriguez-Navarro et al. 2006).

High levels of Aroclor 1268 have been found in clapper rail adults and chicks inhabiting the marshes associated with LCP, thus making this congener mixture a direct fingerprint to the source of the pollution at the LCP site (Cumbee, et al. 2008; Novak et

al. 2006). Clapper rails are a good indicator species for toxicants like PCBs because of their strong site fidelity and predictable diet of benthic organisms. Recently, feathers have been used successfully as a non-lethal tool to detect PCB concentrations in birds (Dauwe et al. 2005; Jaspers et al. 2006,2007). Although these keratinous tissues tend not to accumulate high levels of lipophillic chemicals such as PCBs due to their low fat content, methods have been refined to detect trace amounts (Covaci et al. 2002). Based on this success, evidence suggests that bird feathers can be used as a measure of contaminant exposure (Dauwe et al. 2005; Jaspers et al. 2006,2007). Testing for POPs in keratinous tissue offers a non-lethal approach when compared to traditional matrixes such as internal tissues. Due to the non-invasive nature, monitoring feathers will glean more information regarding contaminant exposure and duration especially for endangered or otherwise compromised species such as the endangered populations of clapper rails inhabiting the west coast of the United States that are consistently exposed to organic pollutants. Moreover, because of predictable molting patterns, feathers offer the opportunity to examine phylogeny and population structure of both migratory and resident species, and can be useful in understanding the consequences of human activities (Smith et al. 2003).

Technological advancements in the field of immunoassay have proceeded rapidly in recent years. Such assays have been shown to be highly sensitive and specific when assaying polycyclic aromatic hydrocarbons (PAHs) (Fritcher et al. 2002), as well as low or moderately chlorinated PCB levels, in matrices such as soil and water using a methanol extraction technique (Fránek et al. 1997). However, limited work has been done investigating the reactivity of higher PCB congeners using hydrophobic solvents

such as hexane. Recently, Zajicek et al. (1996, 2000) have shown that PCBs can be successfully extracted from soil and fish tissue using hexane-based extractions as long as all residues are eliminated from the extract when it is re-eluted in methanol. However, some studies do warn that more highly chlorinated PCBs may not react well with certain enzyme conjugates in ELISA, thus providing erroneously low results. Alternatively, there can be high cross-reactivity with other Aroclors with the lack of specificity producing erroneously high results (Lasrado et al. 2004, Zajicek 2000).

The purpose of this study is to quantify Aroclor 1268 burdens in clapper rail feathers from birds residing in the LCP superfund site in Brunswick GA and compare results using two different analytical methods: gas chromatography (GC) and Enzyme Linked Immunosorbant Assay (ELISA). Further, although it has been established that POPs can be extracted from feathers and other keratinous tissues, there has been limited information available to determine that these burdens are truly endogenous. Therefore, an additional purpose of this study is to ensure that the measured Aroclor 1268 residues in clapper rail feathers were only acquired from their blood during the growth rather than from exogenous sources. Subsequently, the specific objectives of this study were to: (1) expose clean chicken feathers to Aroclor 1268 contaminated mud from the LCP superfund site and wash them using various methods to determine if feather contaminant loads could be from exogenous sources; (2) quantify Aroclor 1268 in clapper rail feathers from birds residing in and near the LCP superfund site using both GC and ELISA techniques; (3) investigate the relationship between GC results from feather samples with those found within liver and muscle tissue from the same birds in a previous study (Cumbee et al. 2008) as well as the congener profile of these tissues, crabs and sediment

collected from within the home ranges of clapper rails analyzed in this study; (4) determine the efficacy of ELISA to quantify Aroclor 1268 contaminant loads in feathers by comparing these measures to GC analysis.

Methods

Clapper rails were collected using a shotgun from the LCP estuary and also nearby control sites Frederica and Troupe Creek (n = 24) in the months of November and December 1999 and January 2000 (Fig. 1; Tissues were used in other toxicological studies (Cumbee et al. 2008; Gaines et al. 2003; Novak et al. 2006; Rodriguez-Navarro et al. 2002). Additional clapper rails were collected in September 2006 (n = 35) and the following Spring in May-June 2007 (n = 21). Birds were stored at -20°C until dissection. Since clapper rails have only a post-nuptial molt in the late fall (fledglings also grow their adult feathers at that time), collection months should have no influence on feather PCB load.

The primary congeners 180, 187, 194, 196, 199, 200, 201, 202, 206, 207, 208 and 209 that compose Aroclor 1268 were quantified in primary flight feathers. Aroclor 1268 standards (Supelco, Inc.) were used as spikes to determine procedural recovery rates, as well as to determine analytical accuracy and precision.

Chicken feathers obtained directly from local farms in east-central Illinois were used as feather blanks (i.e. birds had no prior exposure to Aroclor 1268). Three washing methods and two incubation periods were used to determine if duration of outside exposure and washing method affected the amount of Aroclor 1268 left on the outside of the feather. Feathers (n=24) were incubated at room temperature for either 4-6 hours or overnight (~12-16 hours) in mud containing 1672 ng g^{-1} of Aroclor 1268 (Cumbee et al.

2008) taken from close proximity to the LCP superfund site (LCP estuary). After incubation, four feathers (2 from each incubation period) were either washed with DDI only; DDI / 1% liquinox; DDI / 1% liquinox and methanol (GC grade); or DDI / 1% liquinox, methanol, and n-hexanes (GC grade). This procedure was repeated allowing the feathers to dry completely before placing the feather in a drying oven at 23°C.

Feather sampling methods for clapper rails were adapted from Covaci et al. (2002). Based on results from the chicken feather experiment described above which demonstrated that each of the 4 washing procedures removed exogenous contamination (see results below), feathers from the LCP area were washed with a 1% liquinox solution in a singular whirlpak bag, rinsed with deionized water, and washed again in GC grade n-hexanes to remove external contamination. Feathers were dried and then cut with stainless steel scissors into fragments 1-3 mm to increase surface area and optimize PCB extraction. Approximately 250 mg of feathers were placed in a cleaned glass vial with 8ml 4M HCl and 5ml dichloromethane (DCM) then incubated overnight in a 40°C H_2O water bath. The DCM layer was transferred to a 20ml vial with a Teflon- lined cap. The extraction was repeated with an additional 3ml DCM to optimize PCB extraction from the feather medium and the extracts were combined.

To remove traces of HCl in the DCM extracts, samples were filtered through 500 mg of anhydrous $NaSO_4$. The DCM was then concentrated to 1ml under nitrogen evaporation. A hydrocarbon and moisture trap was used to prevent contamination of the analyte from the nitrogen stream.

Columns of acidified silica were used to remove residual organic material from the extract. The acidified silica was prepared by slowly adding 27 ml of sulfuric acid to

50 g of Silica Gel 60 (70-230 mesh; EM Science) while stirring to maintain homogeneity. The mixture was stirred for another 60 minutes following the acid addition. Approximately 780mg of acidified silica was weighed out for each sample and added to a 6ml glass column lined with a Teflon frit. The columns were conditioned with 2ml hexane:DCM and 2 ml hexane, respectively. 1 ml of hexane was added to the extract to create a non-polar eluent of 1:1 DCM/hexane. The 2 ml samples were loaded onto the column, eluted with 4 ml 1:1 DCM/hexane, and collected in a clean glass vial. The eluate was then concentrated to 50 µl under nitrogen and brought up to 1 ml with hexane. Due to the continued presence of residual hydrocarbons and other non-PCB organic material, a second cleanup step was necessary. A second set of columns were prepared as previously described and the samples were eluted with 4ml hexane. The final eluate was blown dry and then raised to the volume of 1ml with 100% methanol.

GC analyses were performed on an Agilent (Atlanta, GA) 6890 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm I.D., 0.25 um film thickness; J&WScientific, Folsom, CA), electronic pressure control (EPC), and an electron capture detector (ECD). Samples were introduced via auto-injection at 250°C, splitless mode. Chromatographic conditions were 54°C, held isothermal for one minute, 54-160°C at 20°C min^{-1}, isothermal for 5 minutes, then 160°-270°C at 3°C min^{-1}, held isothermal for 3 minutes; with constant pressure at 11 psi. Samples were quantified using a six-point calibration curve derived from dilutions of certified congener (Ultra Scientific) and Aroclor 1268 (Sigma-Aldrich) standards. All congeners were identified based on retention time and elution order relative to the standards. Previous analyses performed on GC-MS confirmed GC-ECD identification (Cumbee et al. 2008). Six major

congeners were selected from the Aroclor mixtures. All selected congener peaks were at least 25% of the highest Aroclor component.

Detection of Aroclor 1268 with ELISA was performed using the Abraxis PCB Magnetic Particle Assay Kit specific for Aroclor 1268. Concentrations of 0.00, 0.50, 2.50, 10.0, and 50.0 ng g^{-1} were used to calibrate the photometric analyzer. Calibration curves were accepted if coefficient of determination values were > 0.990 with replicate coefficient of variation values of < 10%. Test kit procedures for water samples were followed. Therefore, feather samples were diluted with DDI water to create a 1:1 mixture with methanol prior to ELISA analysis as based on the kit instructions. 25% of the feather samples were replicated for quality assurance purposes for each ELISA (consisting of 50 potential samples) including chicken feathers spiked with Aroclor 1268 (50 ng g^{-1}) and certified Aroclor 1268 standard (50 ng g^{-1}) in methanol standard (Supelco).

A paired t-test (Microsoft Excel software ®) was used to test the hypothesis of no difference in Aroclor 1268 concentrations between samples run on the GC and the ELISA. All tests were considered significant at $P < 0.05$.

Results

No Aroclor 1268 was detected in any of the 24 chicken feathers based on GC analysis, regardless of washing technique or incubation duration. However, ELISA results from chicken feathers cleaned by the methods listed above ranged from 1.5 to 7.1 ng g^{-1}.

Internal spiking was performed to assess the recovery rates of our PCB extraction procedure. Two spikes were quantified using GC and then compared to values obtained

from ELISA. Each 50 ng g^{-1} spike yielded a recovery of 38.5 and 38.8 ng g^{-1} respectively, or a 78% recovery of Aroclor 1268 as determined by the GC. ELISA quantified these 2 spikes (each run 4 times due to lack of precision) from 19.2 to 84.1 ng g^{-1} (Chicken 1: n=4, $\bar{x}$ = 43.89, SD = 30.12 ng g^{-1}; Chicken 2: n=4, $\bar{x}$ = 41.81, SD = 24.05 ng g^{-1}). The Chicken 1 and Chicken 2 spikes within the same ELISA run were between 0.2% - 20.8% of their replicates. Replicate samples run on the GC ranged from 2% - 3% of each other (n = 7). In contrast, the replicates run on the ELISA ranged from 49% - 365% of the initial sample (n = 27, $\bar{x}$ = 108.62%, SD = 68%). Approximately 25% (compared to 10% for the GC) of the ELISA samples were replicated to gain insight on the distribution of ELISA precision.

Six clapper rails were taken from either control site (Frederica and Troupe Creek), located within 10 km of the LCP site (Figure 1).

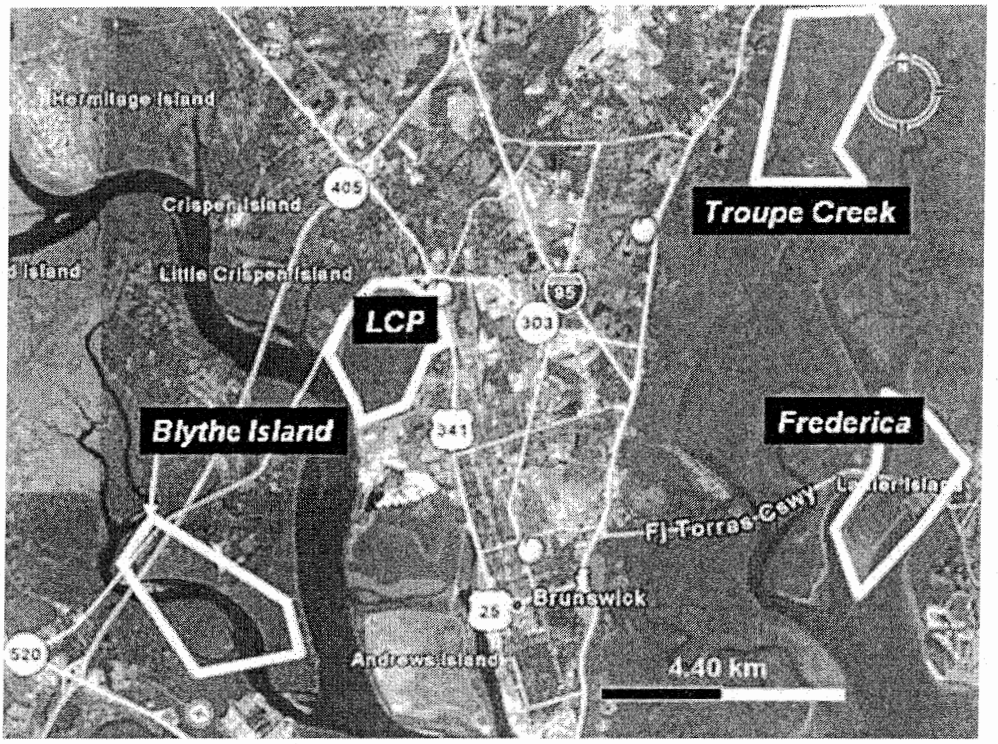

Figure 1: The Linden Chemical Plant (LCP) study site and the three control sites (Troupe Creek, Frederica Island and Blythe Island) that were used to collect clapper rail samples, located near Brunswick, GA, USA. Control samples were harvested from Troupe Creek (n=3) and Frederica (n=3) in the months of January and February, 2002.

Clapper rail feather samples from these control sites all fell below detection limit (BDL) of the GC. However, these same samples quantified for Aroclor 1268 using ELISA produced values between 20 and 75 ng g^{-1} (Table 1).

Table 1: Comparison of Aroclor 1268 concentrations (ng g^{-1}) as determined by gas chromatography (GC) and enzyme-linked immunosorbant assay (ELISA). Mean concentrations as determine by GC ($\bar{x}$ =869.40, sd = 948.72) were significantly higher (P < 0.0001, t =1.66, df=150) than those quantified using the ELISA methods ($\bar{x}$ =83.89, sd = 82.56). Birds taken from control sites Troupe Creek (TC) and Frederica (FR) are indicated at the top of the table. Blank cells indicate that the sample could not be run.

ID	GC	ELISA
TC 01	0.0492	38.1516
TC 02	60.1633	31.7917
TC 08	21.4104	75.2613
FR 01	21.1226	41.4857
FR 06	77.4040	45.1064
FR 07	0.5298	20.7004
LCP 01	206.1800	23.3944
LCP 02	191.0100	77.5529
LCP 03	255.2573	49.1883
LCP 05	534.3658	129.3546
LCP 06	131.6234	20.9283
LCP 11	581.4447	5.6250
LCP 12	560.0652	45.5190
LCP 13	407.6329	44.7115
LCP 14	418.7841	45.1942
LCP 15	408.0995	67.1315
LCP 103	346.9151	31.8381
LCP 104	88.8101	68.5625
LCP 106	246.6134	35.5645
LCP 107	209.2804	33.9747
LCP 108	625.0162	5.1172
LCP 337	2708.3030	92.3000
LCP 358	336.0525	31.1751
LCP 567	2176.8020	46.8070
01 May 2007	539.7370	
02 May 2007	661.9495	244.9138
03 May 2007	2379.3620	280.3817
04 May 2007	170.3779	187.7403
05 May 2007	3508.6130	356.5258
06 May 2007	1035.6460	259.8364
07 May 2007	176.9223	145.7117
08 May 2007	228.6263	112.4889
09 May 2007	766.2422	100.2822
10 May 2007	777.5694	166.0083
11 May 2007	383.2310	141.3603
12 May 2007	363.4067	212.3551
13 May 2007	232.1780	170.6435
14 May 2007	223.4171	259.1024
15 May 2007	8.4838	104.7148
16 May 2007	129.8135	287.0000
17 May 2007	251.1476	194.0546

ID	GC	ELISA
18 May 2007	458.1313	163.0512
19 May 2007	600.2654	
20 May 2007	119.3159	128.0653
21 May 2007	179.8194	86.3118
01 August 2006	636.3124	29.1692
2 August 2006	1253.8870	25.7611
3 August 2006	3858.7430	72.8986
4 August 2006	1521.8600	
5 August 2006	1605.2960	29.4697
6 August 2006	1489.1240	
7 August 2006	508.9747	18.3660
8 August 2006	419.3156	19.0040
9 August 2006	1286.5720	19.5170
10 August 2006	854.8788	19.2510
11 August 2006	954.1642	168.1321
12 August 2006	1351.3310	38.1315
13 August 2006	2405.1490	71.5984
14 August 2006	995.9070	24.1839
15 August 2006	1671.7660	24.8099
16 August 2006	1076.9250	46.3191
17 August 2006	1733.2660	18.5946
18 August 2006	4828.0080	239.2115
19 August 2006	676.8408	41.9109
20 August 2006	1408.1833	81.5683
21 August 2006	969.9887	22.2449
22 August 2006	627.4374	64.7719
23 August 2006	3300.3122	254.1982
24A August 2006	185.2835	
24B August 2006	947.9202	25.5303
25 August 2006	2433.1790	60.3408
26 August 2006	1727.6230	34.1667
27 August 2006	401.5539	21.3640
28 August 2006	908.3660	41.6187
29 August 2006	472.8353	12.7520
30 August 2006	406.7255	18.7884
31 August 2006	268.7376	19.9380
32 August 2006	1788.5510	29.2037
33 August 2006	655.5740	11.5985
34 August 2006	479.2011	44.7723
35 August 2006	504.5688	20.0547

The majority of samples taken from LCP contained Aroclor 1268 (Table 1). The mean value for samples run with GC was 869 ng g^{-1}, while the mean obtained with ELISA was 83 ng g^{-1}. Results from the GC varied significantly from results acquired from ELISA ($P < 0.0001$). Specifically, values from ELISA results showed almost a 10 fold reduction from results obtained from the GC (Figure 2).

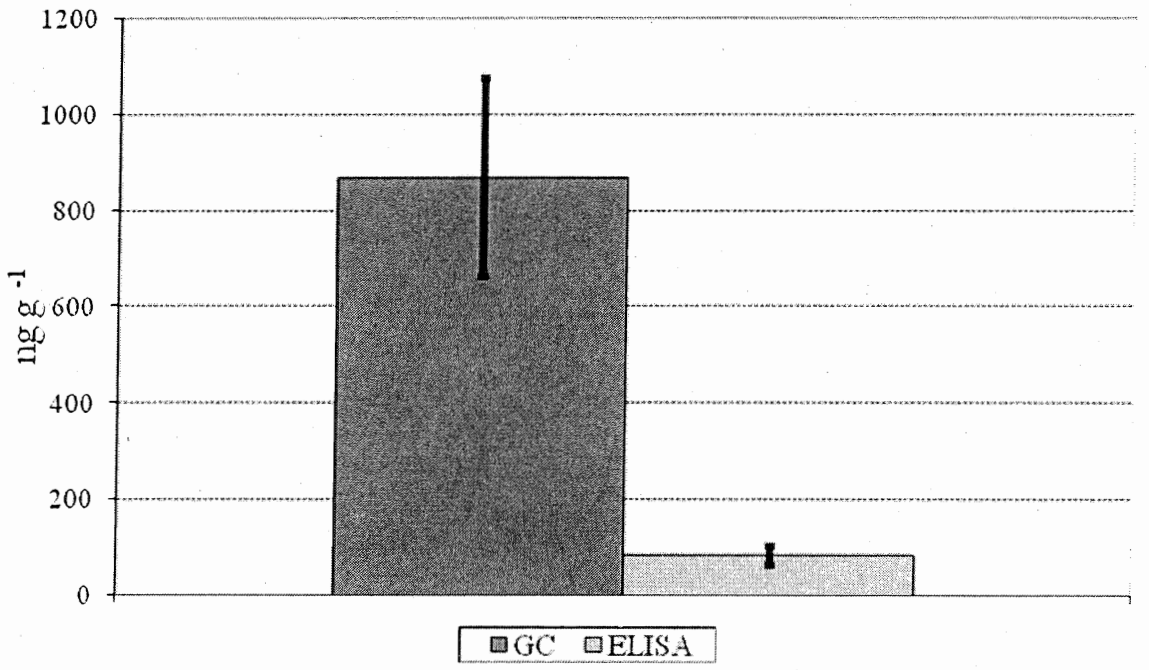

Figure 2: Mean concentrations and 95% confidence limits of Aroclor 1268 detected using gas-chromatography (GC) and enzyme linked immunosorbant assay (ELISA) methods in clapper rail feathers (ng g^{-1}). Feathers were taken from birds harvested at and near the contaminated LCP superfund site in Brunswick, GA, USA.

Eleven congeners of Aroclor 1268 were examined using GC (Table 2). Of these eleven, six congeners (BZ 202, 201, 196, 208, 206 and 209) comprised 85% of the sample.

Table 2: Aroclor 1268 congener profile (ng g^{-1}) and congener summation as determined by gas chromatography (GC). Congeners are presented in the Ballschmiter-Zell (BZ) numbering system. Samples whose individual congener quantification fell below detection limits were omitted for the purposes of congener summation. Sample IDs with asterisks (*) were from control sites.

Sample ID	BZ 187	BZ 202	BZ 200	BZ 180	BZ 201	BZ 196	BZ 208	BZ 207	BZ 194	BZ 206	BZ 209	∑PCB
*TC 01									0.34	0.15		0.49
*TC 02	1.33	4.21	3.81	9.38	5.30	5.67	5.51	5.21	8.21	8.66	2.87	60.16
*TC 08				6.32	1.18	2.21	0.11		5.76	5.82		21.41
*FR 01				6.48	1.21	2.27	0.11		5.91	5.15		21.12
*FR 06	1.63	5.16	4.67	11.50	6.49	6.94	6.75	6.39	10.06	14.28	3.52	77.40
*FR 07									0.37	0.16		0.53
LCP 01	11.08	12.60	3.87	9.52	31.68	25.00	21.77	8.76	14.10	56.28	11.53	206.18
LCP 02	12.49	7.62			38.50	28.67	16.77	2.94	8.34	60.21	15.48	191.01
LCP 03	12.20	15.97	4.59	11.31	42.22	35.04	26.61	10.82	18.43	61.09	16.97	255.26
LCP 05	28.86	18.92	11.15	10.77	109.02	83.23	47.62	17.25	34.83	151.13	21.59	534.37
LCP 06	6.94	1.89			29.30	22.47	13.70	1.68	5.22	42.42	8.01	131.62
LCP 11	29.90	8.15		7.09	117.90	92.42	50.09	13.24	33.35	194.98	34.33	581.44
LCP 12	46.48	18.05		7.65	120.41	107.79	47.59	15.94	32.93	146.88	16.34	560.07
LCP 13	24.73	22.89	4.34	10.68	72.11	58.32	42.15	14.83	24.94	111.52	21.12	407.63
LCP 14	27.69	28.38	3.95	9.72	71.33	57.16	42.57	14.66	21.36	113.97	27.99	418.78
LCP 15	29.34	9.98		5.55	88.83	66.12	38.30	11.07	22.61	116.53	19.77	408.10
LCP 103	18.44	8.39		7.34	63.25	48.41	31.63	7.34	21.17	125.28	15.67	346.92
LCP 104					15.64	10.92	11.75		7.23	33.36	9.90	88.81
LCP 106	13.10	14.72	4.43	10.90	44.78	34.88	26.51	10.53	15.67	57.47	13.62	246.61
LCP 107	12.49	5.12	4.63	11.40	35.47	29.22	23.23	6.33	15.18	53.34	12.86	209.28
LCP 108	25.68	28.74		6.25	123.20	96.06	58.96	18.45	40.58	200.83	26.27	625.02
LCP 337	209.73	74.89	16.26	34.17	576.88	503.85	250.17	84.29	143.58	661.06	153.41	2708.30
LCP 358	13.10	4.45			72.01	58.27	28.48	6.86	18.18	113.89	20.82	336.05
LCP 567	164.56	39.49		37.72	462.80	440.17	143.04	48.79	128.76	621.62	89.87	2176.80
May 2007-01	37.00			7.56	101.35	79.04	65.99	23.31	26.96	160.35	38.18	539.74
May 2007-02	49.22	37.23	4.73	17.89	147.74	107.20	69.65	21.55	34.45	147.06	25.23	661.95
May 2007-03	196.01	63.69	13.99	31.22	556.98	492.07	204.38	67.65	132.30	527.30	93.79	2379.36
May 2007-04	6.03	1.36			40.76	27.94	18.55	3.61	7.17	57.55	7.40	170.38

Sample ID	BZ 187	BZ 202	BZ 200	BZ 180	BZ 201	BZ 196	BZ 208	BZ 207	BZ 194	BZ 206	BZ 209	∑PCB
May 2007-05	258.98	69.14	19.63	48.20	825.64	687.74	302.31	309.52	105.94	734.10	147.43	3508.61
May 2007-06	67.04	37.96	4.22	14.85	242.58	194.66	91.95	26.26	51.47	262.55	42.11	1035.65
May 2007-07	9.33	1.95			43.17	31.54	18.76	2.20	8.79	54.83	6.35	176.92
May 2007-08	12.71	3.25			51.09	40.43	21.28	4.32	11.59	72.19	11.77	228.63
May 2007-09	49.64	12.25	0.00	1.71	180.91	142.39	62.22	15.16	39.13	228.78	34.05	766.24
May 2007-10	59.42	34.87	11.02	17.08	159.76	132.30	74.12	24.14	36.53	194.77	33.57	777.57
May 2007-11	20.06	12.42			88.71	67.98	38.38	8.51	19.46	109.63	18.09	383.23
May 2007-12	19.69	12.65			74.43	55.39	42.09	6.64	14.16	114.39	23.97	363.41
May 2007-13	16.91	13.92			54.26	39.68	22.63	2.68	6.99	67.48	7.61	232.18
May 2007-14	14.31	13.06	4.32	10.64	43.23	34.73	23.04	5.91	17.14	53.77	3.26	223.42
May 2007-15		-5.79							0.36	8.12		2.69
May 2007-16					33.90	24.17	15.03	2.97	6.37	41.87	5.50	129.81
May 2007-17	13.96	18.87	6.00	14.77	40.09	31.67	24.80	8.20	18.18	64.05	10.56	251.15
May 2007-18	26.88	17.12		8.44	107.69	82.63	51.00	14.31	19.99	122.63	7.44	458.13
May 2007-21	8.84	2.63		6.90	34.25	26.37	20.80	4.07	12.17	54.61	9.19	179.82
August 2006-01	42.47	22.17		6.52	142.10	112.12	56.34	15.02	26.45	183.68	29.44	636.31
August 2006-02	81.04	50.60	15.38	26.29	268.48	225.99	121.60	41.37	59.41	302.81	60.91	1253.89
August 2006-03	263.33	184.68	45.96	64.47	847.03	742.84	365.91	118.41	186.94	859.63	179.55	3858.74
August 2006-04	108.90	23.23	0.00	13.66	363.63	321.27	95.20	35.65	85.88	406.04	68.41	1521.86
August 2006-05	114.13	49.32	0.00	12.83	381.51	315.11	147.73	43.04	80.06	383.63	77.93	1605.30
August 2006-06	110.09	26.73		18.79	315.85	284.95	109.89	41.71	74.52	431.38	75.23	1489.12
August 2006-07	35.08	13.06		7.72	109.18	86.90	48.25	13.20	27.80	142.48	25.31	508.97
August 2006-08	24.08	22.33			87.86	71.21	45.58	9.56	16.26	118.73	23.70	419.32
August 2006-09	81.35	59.15	13.40	21.15	265.30	209.77	132.26	40.89	61.48	334.34	67.48	1286.57
August 2006-10	40.01	35.61	3.54	9.14	164.26	135.98	89.44	26.52	35.60	261.53	53.27	854.88
August 2006-11	82.33	50.84	0.00		219.71	161.45	109.65	27.54	34.47	235.49	41.75	954.16
August 2006-12	77.20	45.96	0.62	13.91	295.18	246.86	130.05	38.12	74.69	360.42	68.33	1351.33
August 2006-13	173.53	76.83	1.20	22.49	553.62	460.86	230.50	71.91	113.54	581.74	118.94	2405.15
August 2006-14	4.50	34.17	7.33	17.94	198.38	179.80	96.65	31.98	51.50	315.38	58.27	995.91
August 2006-15	89.04	45.14	7.24	16.18	295.35	264.56	142.89	43.94	85.12	579.32	102.99	1671.77
August 2006-16	62.58	39.98	4.98	18.84	206.06	165.93	115.39	36.39	59.10	305.63	62.05	1076.93
August 2006-17	99.92	70.08	13.95	18.38	334.11	270.51	180.30	56.09	78.69	509.39	101.86	1733.27

Sample ID	BZ 187	BZ 202	BZ 200	BZ 180	BZ 201	BZ 196	BZ 208	BZ 207	BZ 194	BZ 206	BZ 209	∑PCB
August 2006-18	428.38	191.94	51.26	81.09	1029.25	907.67	386.62	125.42	237.31	1210.92	178.14	4828.01
August 2006-19	33.49	13.31		5.87	126.09	115.84	57.48	20.60	32.27	224.40	47.49	676.84
August 2006-20	112.29	70.06	12.46	4.99	305.61	240.92	158.77	45.53	53.03	336.96	67.56	1408.18
August 2006-21	67.91	34.31	11.75	18.33	180.91	159.55	92.55	34.35	44.25	269.73	56.37	969.99
August 2006-22	44.12	2.35	19.66	128.56	108.80	78.56	15.12	19.95	0.00	175.00	35.31	627.44
August 2006-23	328.27	172.83	41.08	42.94	644.95	559.17	343.93	97.09	140.30	783.85	145.90	3300.31
August 2006-24A	9.48	9.36		7.79	33.60	29.65	20.96	5.34	7.10	56.55	5.45	185.28
August 2006-24B	55.66	35.72	7.29	6.87	180.11	140.77	105.62	33.08	48.00	278.30	56.50	947.92
August 2006-25	113.92	41.28	12.56	36.72	467.58	428.76	179.48	75.24	145.49	793.41	138.74	2433.18
August 2006-26	164.97	88.28	9.04	14.75	397.56	318.37	175.90	46.38	72.43	365.21	74.75	1727.62
August 2006-27	30.13	19.22			92.95	69.63	42.28	8.29	16.99	104.27	17.79	401.55
August 2006-28	45.82	49.10	4.27	175.70	143.97	107.41	90.24	33.50	38.92	178.59	40.86	908.37
August 2006-29	26.25	19.43		7.26	104.01	75.00	48.76	15.41	28.70	127.59	20.42	472.84
August 2006-30	18.78	21.13	4.55	11.21	79.82	62.13	42.91	15.05	25.48	100.08	25.58	406.73
August 2006-31	7.61	6.20			52.89	39.61	33.48	6.35	9.12	94.42	19.07	268.74
August 2006-32	116.96	58.29	14.76	22.87	368.55	311.33	162.84	54.76	78.61	507.32	92.26	1788.55
August 2006-33	30.78	23.32			144.02	106.23	76.01	18.27	25.68	192.98	38.30	655.57
August 2006-34	31.43	20.62		8.98	107.52	77.57	58.50	14.91	23.20	121.40	15.08	479.20
August 2006-35	28.37	25.74	3.95	9.72	89.14	69.77	58.27	17.98	28.62	144.09	28.92	504.57

The percent profile of these 6 congeners were similar to clapper rail muscle and liver tissue, whole clapper rail chicks, fiddler crabs, and sediment taken from the LCP site as well as the congener profile of the Aroclor 1268 standard (Figure 3). Specifically, clapper rail feathers contained the same percentage of congeners 202, 208 and 209 as the standard. PCB 206 (2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl) had the highest mean concentration, followed by congener 201 (2,2',3,3',4,5',6,6'-octachlorobiphenyl), and congener 196 (2,2',3,3',4,4',5,6'-Octachlorobiphenyl; Figure 3).

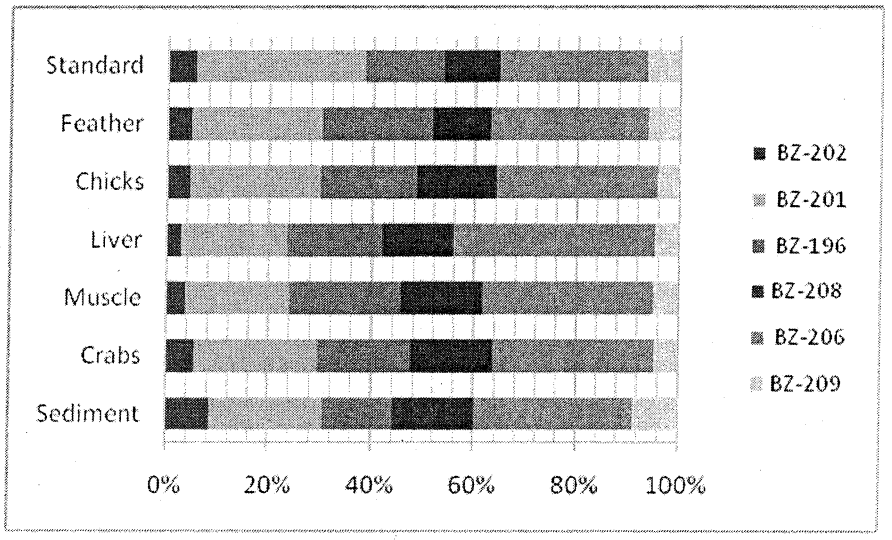

Figure 3: Percent composition of Aroclor 1268 (6 major congeners – Ballschmiter-Zell (BZ) numbering system) as determined via gas chromatography (GC) in biota and sediments collected from the LCP superfund site in Brunswick GA during various time periods in 2000, 2002, 2006, and 2007. The percent compositional breakdown for Aroclor 1268 (standard) is shown as a reference.

The summation of Aroclor 1268 in clapper rail feathers was compared to the liver and muscle tissue values of those same clapper rails (n=8) (Cumbee, et al. 2008) revealing that rail feathers contained approximately 2-3 orders of magnitude less Aroclor 1268compared to muscle and liver tissues from the same bird (Table 3). However, the

trend of minimum versus maximum feather load did not correspond to the PCB loads in the matching tissues.

Table 3: Aroclor 1268 concentrations in muscle and liver tissues from clapper rails collected from the LCP study site (Cumbee et al. 2008) that were included in the current feather study ($\mu g\ g^{-1}$). All samples were analyzed by gas chromatography (GC).

ID	FEATHER	MUSCLE	LIVER.
LCP 103	0.346	9.4	NA
LCP 104	0.088	1.7	8.3
LCP 106	0.246	5.0	20.0
LCP 107	0.209	18.0	56.0
LCP 108	0.625	49.0	499.0
LCP 358	0.336	19.0	157.0
LCP 567	2.176	18.0	126.0
337 LCP	2.708	71.0	568.0

Discussion

The discrepancies between the amount of Aroclor 1268 quantified based on the ELISA versus the GC methods may in part be due to cross-reactivity. The quantitative magnetic ELISA that was used combines an antibody specific for Aroclor 1268 with an enzyme-labeled PCB. Aroclor 1268 has been shown to exhibit a 16.5% cross-reactivity with other Aroclors (Lawruk, et al. 1996), which may cause an over estimation if other Aroclor compounds are present. However, based on the congener profiles detected by the GC, only Aroclor 1268 was present in the sample. Also, most samples were under estimated by the ELISA compared to the GC making this phenomena unlikely. Moreover, Aroclor 1268 is present in very high quantities in the LCP marsh and any other Aroclor mixture would be from global distribution. Not only were most of the feather samples consistently under estimated for the amount of Aroclor 1268 in the sample compared to the GC, these estimates were consistently an order of magnitude

less, with the mean and standard deviation being almost exactly an order of magnitude reduced. Although ELISA run samples were consistently less than the same extracts run on the GC, the replicates run on the ELISA ranged from 49% - 365% of each other showing a lack of precision. Weathering of material can cause change in congener distributions which may influence or interfere with anti-PCB antibodies binding to the analyte (Zaijcek et al. 2000). This is not a likely explanation for the ELISA under-detection because the congener profiles from our feathers were not statistically different than those of the standard.

Other studies examining the efficacy of ELISA to detect POPs including PCBs in samples have been successful. However, most use a methanol-based extraction method that is conducive to the ELISA and photometer absorption. Moreover, these studies have also demonstrated Aroclor 1268 has a strong binding reaction with other solvents such as isooctane and hexane that remained in the extract even in trace amounts (Zajicek et al. 1996, 2000). These same studies have shown that the evaporative removal of hydrophobic solvents with subsequent dissolution in 100% methanol gives near quantitative recovery of model PCB congeners. Samples from this study were blown down dry with a gentle nitrogen stream and re-eluted with methanol. A plausible explanation for the consistent underestimation of PCBs in the feather samples could be the presence of such residues although none were visible to the naked eye. Zajicek et al. (1996, 2000) showed success in quantifying PCBs in fish and specifically demonstrated the need for the complete removal of the non-polar solvent and quantitative dissolution of the PCB residues with methanol prior to dilution in water. ELISA determination of PCBs in biological tissue extracts can be problematic because the PCBs must be separated from

non-polar biogenic compounds such as lipids which have limited compatibility with the polar solvents and the immunochemical reagents used in ELISA techniques (Zajicek et al. 1996). Again, our extraction methods did remove these residues, but lower quantification via ELISA methods still remained. Therefore, although ELISA may be an efficient and cost effective way to screen samples for PCB residues, caution should remain when using hydrophobic solvents. Moreover, ELISA has to our knowledge never been used as a tool for quantifying PCBs within feathers and residues not removed by the extraction process could very well be interfering with the diluents or enzymatic processes of the assay.

These results suggest that our washing techniques removed exogenous sources of PCBs from the feather and that the Aroclor 1268 detected are from endogenous deposits originating when the feather was grown. Clapper rails found within the marshes near LCP are likely year round residents due to its favorable habitat. Therefore, it is fair to assume that the birds that were collected for this study from LCP either hatched or nested in LCP the previous year. Aroclor 1268 is extremely persistent in the environment and is comparatively less mobile than other PCBs due to its low aqueous solubility and vapor pressure. High degrees of chlorination, including the *ortho* position, and low water solubility may cause Aroclor 1268 to be highly resistant to biotransformation and subsequently more likely to bioaccumulate (Sajwan, et al. 2008). This is consistent with our findings showing that clapper rails harvested from the LCP site had Aroclor 1268 in similar proportions of the 6 major congeners found in the standard in all of the tissues measured as well as soil and chick whole body (Figure 3).

Recently, there has been increasing advancement in using keratinous tissue such as hair and feathers to detect organic pollutants (Covaci, et al. 2002; Dauwe, et al. 2005). Testing for POPs in these tissues offers a non-lethal approach when compared to traditional tissues such as muscle or liver. Because of its non-invasive nature, this form of testing can be applied to endangered or otherwise compromised species such as endangered clapper rail populations residing in the San Francisco Bay area that is a focus of restoration and conservation actions. Specifically, testing with feathers offers the opportunity to monitor long-term exposures of both migratory and resident species (Smith, et al. 2003).

Conclusions

Clapper rails in Georgia do not appear to be migratory and reside close (within 5-10 km) to their breeding home-ranges (Gaines et al. 2003; Meanley 1985). Since feathers from these birds can be used as a measure of Aroclor 1268 exposure, this monitoring tool further expands the use of clapper rails as indicator species especially for the LCP Superfund site. Further, monitoring feathers from birds residing in compromised ecosystems for POPs will provide a mechanism by which to prioritize conservation and clean-up efforts. However, as demonstrated in this study, establishing that contaminant loads are truly endogenous and understanding the life history such as feather growth chronology, eating habits and habitat use patterns is essential if birds can really be used to monitor POP bioavailability. Our results also suggest that more research is needed into the best extraction procedures if an ELISA is being considered to quantify Aroclor 1268 in feathers.

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