

2010

# Feathers as Bioindicators of PCB Exposure in Clapper Rails

Jay W. Summers, *Eastern Illinois University*

Karen F. Gaines, *Eastern Illinois University*

N. Garvin, *University of Georgia*

Warren L. Stephens, Jr., *University of Georgia*

James C. Cumbee, Jr., *USDA APHIS Wildlife Services*, et al.

# Feathers as bioindicators of PCB exposure in clapper rails

J. W. Summers · K. F. Gaines · N. Garvin ·  
W. L. Stephens Jr. · J. C. Cumbee Jr. · G. L. Mills

Accepted: 1 March 2010 / Published online: 11 March 2010  
© Springer Science+Business Media, LLC 2010

**Abstract** In this study we used feathers to biomonitor exposure to the polychlorinated biphenyl (PCB) Aroclor 1268 congener mixture in clapper rails (*Rallus longirostris*). This species has been used as an indicator species of environmental damage for the LCP superfund site located in Brunswick, GA, USA which is contaminated with Aroclor 1268, a congener mixture that has been used in limited amounts elsewhere and therefore can be used as a contaminant marker. The Aroclor 1268 congener mixture, including congener profiles, were quantified in feathers using gas chromatography (GC). Concurrently, each sample was quantified for the total Aroclor 1268 congener mixture using an enzyme-linked immunosorbant assay (ELISA) and compared to the GC results to determine if ELISA was an efficient method for quantifying or

qualifying PCBs in feathers. ELISA consistently quantified PCB loads over an order of magnitude lower 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 more suitable for qualitative exposure assessment for this particular Aroclor. Moreover, since all clapper rails from the LCP site had the Aroclor 1268 congener mixture in their feathers, this experiment showed that birds were returning to the site to breed despite the adverse effects experienced by this population from the contamination revealed in previous studies. This study also supports the utility of feathers as a non-lethal mechanism by which to biomonitor PCBs in the environment.

**Keywords** Aroclor 1268 · ELISA · Clapper rail · Organochlorine · LCP · PCBs

---

J. W. Summers · K. F. Gaines (✉)  
Department of Biological Sciences, Eastern Illinois University,  
600 Lincoln Avenue, Charleston, IL 61920-3099, USA  
e-mail: kfgaines@eiu.edu

J. W. Summers  
e-mail: jaysummers@hotmail.com

N. Garvin · W. L. Stephens Jr. · G. L. Mills  
University of Georgia's Savannah River Ecology Laboratory,  
P. O. Drawer E, Aiken, SC 29802, USA  
e-mail: ngarvin@uga.edu

W. L. Stephens Jr.  
e-mail: cub@srel.edu

G. L. Mills  
e-mail: mills@srel.edu

J. C. Cumbee Jr.  
USDA APHIS Wildlife Services, Room 200 Thompson Hall,  
100 Stone Blvd., Mississippi State, MS 39762, USA  
e-mail: James.C.Cumbee@aphis.usda.gov

## Introduction

Persistent organochlorine pollutants (POPs) have become a concern as an environmental contaminant because of their slow degradation, bioaccumulative nature and chronic detrimental effects on wildlife as well as humans. Polychlorinated biphenyl's (PCBs) tend to be highly persistent and therefore do not degrade once they enter a system. By nature, POPs are lipophilic and hydrophobic and partition strongly to organic matter in aquatic sediments. POPs become stored in adipose tissue and persist in biota because the metabolism of lipids is relatively slow and therefore may accumulate in food chains (Jones and de Voogt 1999). This paper focuses on the bioavailability and monitoring of the PCB Aroclor 1268 in a Superfund site in Brunswick, GA, USA.

From 1968 to 1994, waste sludge (used in paints, transmission fluids and other coating compounds) containing mostly mercury (Hg) and PCBs were disposed of into surface impoundments constructed along the tidal marsh next to 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 mixture to lubricate graphite electrodes used in process equipment (Kannan et al. 1998), from the sole manufacturer, Monsanto Corporation, which produced only a limited amount. Based on its extremely restricted geographic distribution, the congener mixture of Aroclor 1268 (in its unique proportion), can be used as a chemical marker to determine whether biota have resided in LCP (Novak et al. 2006). Also due to the fact that adsorption to organic materials, sediments and soils will typically increase with PCB chlorine content (Callahan et al. 1979), the high chlorination of Aroclor 1268 (68%) makes it extremely persistent. This PCB mixture has been reported to have a relatively low toxic potential (TCDD equivalents); however, the great proportion of higher chlorinated congeners reduces the mobility of the Aroclor 1268 mixture from a physico-chemical standpoint (e.g. exceptionally low aqueous solubilities and vapor pressures) and makes it highly resistant to biotransformation (high degree of chlorine substitution, including *ortho* locations; Kannan et al. 1997).

LCP Chemicals is a relatively small industrial facility surrounded by approximately 500 to 600 acres of tidal marshlands (Fig. 1). The marshlands surrounding the

industrial site 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). In 1989, the U.S. Environmental Protection Agency (USEPA) performed a field investigation of 7 LCP impoundments, and the Aroclor 1268 congener mixture was found in the majority of sources. In 1991, a Georgia Environmental Protection Division/Department of Natural Resources investigation discovered extremely high levels of the Aroclor 1268 congener mixture in both sediment and crab tissue (USEPA 2002). By 1996, 25,000 tons of contaminated soil/sediment had been recovered and shipped to off-site disposal grounds (USEPA 2002). Since then, however, little has been done in terms of remediation. The investigation presented here is the continuation of a long-term study, using clapper rails (*Rallus longirostris*) as an indicator species (Cumbee et al. 2008; Gaines et al. 2003; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006), to investigate the ecological integrity of the marshes surrounding this Superfund site.

Clapper rails are opportunistic feeders that typically prey on invertebrates in tidal marshes (Heard 1982). In southeastern coastal salt marshes, home ranges of clapper rails typically range from 0.04 to 1.66 ha during the February–August nesting season, and 0.10–2.00 ha in winter months (Roth et al. 1972; Cumbee et al. 2008). This makes them ideal species to study the effects of site specific contamination such as that of 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).

Since high levels of the Aroclor 1268 congener mixture have been found in clapper rail adults, chicks and eggs from the marshes associated with LCP, and Aroclor 1268 is a direct fingerprint to the source of the pollution at the LCP site (Novak et al. 2006; Cumbee et al. 2008), many questions can be asked regarding how this superfund site is effecting local populations of wildlife species. Recently, feathers have been used successfully as a non-lethal tool to detect PCB concentrations (Dauwe et al. 2005; Jaspers et al. 2006, 2007b). Although these keratinous tissues tend not to accumulate high levels of lipophilic chemicals such as PCBs due to their low fat content, methods have been refined to detect trace amounts (Covaci et al. 2002). Endogenous deposition in bird feathers happens exclusively in the course of feather growth, when the feather is joined to the bloodstream for a limited time. Because of predictable molting patterns, feathers offer the opportunity to examine phylogeny and population structure of both migratory and resident species, and therefore can be useful in understanding the consequences of human activities



**Fig. 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

(Smith et al. 2003). Specifically, since clapper rails only molt after their nesting season (post-nuptial molt) and young of the year grow their adult plumage at the same time (Eddleman and Conway 1998), new recruits that did not reside in LCP the year prior would have no Aroclor 1268 in their feathers. Having this information would allow the exploration as to whether the marshes associated with LCP are acting as an ecological trap (Robertson and Hutto 2006). That is, if a large proportion of birds did not have Aroclor 1268 in their feathers, then one explanation would be that birds are not surviving and coming back to breed at LCP, despite the fact that it is ideal clapper rail nesting habitat (Gaines et al. 2003).

The purpose of this study was to quantify the Aroclor 1268 congener mixture burdens (as expressed by the specific congeners and relative proportions) in clapper rail feathers from birds residing in and near the LCP superfund site in Brunswick, GA. The specific objectives were to: (1) expose clean chicken feathers to Aroclor 1268 contaminated sediment from the LCP superfund site and wash them using various methods to determine if feather contaminant loads could be from exogenous sources; (2) quantify the Aroclor 1268 congener mixture in clapper rail feathers from birds residing in and near the LCP Superfund site using both Gas Chromatography (primary congener profile) and Enzyme Linked Immunoassay (ELISA) techniques (antibody with specificity for the Aroclor 1268 mixture).

Feathers can become contaminated exogenously in multiple ways: constant contact with the contaminant, oil residues in the environment, introduction from the uropygial gland, or from the contamination being trapped by outside oils or from the uropygial gland oils through preening. Removing these residues has been primarily performed and proven to be successful using a non-polar solvent (Burger 1993; Hobson and Wassenaar 1997; Jaspers et al. 2007a, 2008). To further ensure that our washing methods would remove the Aroclor 1268 congener mixture, we incubated chicken feathers with contaminated mud. Chicken feathers are of similar structure to clapper rails and these birds, in a similar fashion to rails, will preen using the uropygial gland. Chicken feathers obtained directly from local farms in east-central Illinois were used as feather blanks (i.e. birds had no prior exposure to the Aroclor 1268 congener mixture).

No remediation actions have taken place at LCP during the collection period and therefore due to the persistent nature of the PCB, congener profiles or relative contamination in the sediments should not have markedly changed during this relatively short time period. This is also evident when previous studies documenting the congener profiles and total contaminant loads of Aroclor 1268 in sediments and the biota from this estuary are compared (Kannan et al. 1997, 1998, 1999; Cumbee et al. 2008). Moreover, given

that the purpose of this study is to use the Aroclor 1268 congener mixture as a marker, any temporal diminishment of this PCB mixture due to weathering or natural attenuation does not confound the study. 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.

## Methods

### Sample collection

Clapper rails were collected using a shot gun from the LCP estuary and also nearby control sites Frederica and Troupe Creek ( $n = 23$ ) in the months of November and December 1999 and January 2000 (Fig. 1; tissues were also used in other toxicological studies; Rodriguez-Navarro et al. 2002; Gaines et al. 2003; Novak et al. 2006; Cumbee et al. 2008). Additional clapper rails were collected in September 2006 ( $n = 35$ ) and the following spring in May–June 2007 ( $n = 21$ ). Birds were stored at  $-20^{\circ}\text{C}$  until dissection.

### Sample preparation and analysis

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. Previous studies from LCP have indicated that this is the only Aroclor mixture found in elevated levels (Kannan et al. 1997, 1998, 1999; Cumbee et al. 2008). Therefore, hereafter, these primary congeners of the Aroclor 1268 mixture will be collectively referred to as the Aroclor 1268 mixture. Aroclor 1268 standards (Supelco, Inc.) were used as spikes to determine procedural recovery rates, as well as to determine analytical accuracy and precision.

Four washing methods and two incubation periods were used to determine if duration of outside exposure and washing method affected the amount of the Aroclor 1268 congener mixture left on the outside of the feather. Feathers ( $n = 24$ ) were incubated in contaminated sediment ( $1672 \text{ ng g}^{-1}$  Aroclor 1268, Cumbee et al. 2008) taken from close proximity to the LCP superfund site (LCP estuary) at room temperature (6–12 h) or in an oven at  $70^{\circ}\text{C}$  (overnight  $\sim 12$ –16 h). Feathers were removed from the sediment and dried completely in a drying oven at  $23^{\circ}\text{C}$ . After drying, four feathers (two from each incubation period) were either (1) washed with Distilled De-Ionized water (DDI) only; (2) DDI/1% liquinox (a concentrated, anionic liquid laboratory detergent designed to easily rinse off in running water to yield completely residue free surfaces); (3) DDI/1% liquinox and methanol (GC grade); or (4) DDI/1% liquinox, methanol, and

*n*-hexanes (GC grade). For the latter procedure, feathers were shaken and rinsed with DDI then shaken in liquinox, rinsed with DDI, shaken in methanol, rinsed with DDI, shaken in hexane and then rinsed with DDI. For the other 3 washing techniques, the wash ended with a final DDI wash after the final removal agent. Initial washing with DDI and/or liquinox was performed for at least 1 min or until the sample was rinsed clean from any visual external contamination. Feathers were then agitated for approximately 30 s in methanol and/or hexane as described above. This experiment provided information regarding the persistence of PCBs to adhere to the feather, even with high heat conditions where the contaminant is more likely to mix with oil deposits on the feather mimicking environmental conditions.

The chicken feather experiment described above showed that all four washing procedures removed exogenous contamination (see [Results](#) below). Although possibly redundant, to ensure that all exogenous contamination was removed including oil, feathers from the LCP area were rinsed with DDI, washed with a 1% liquinox solution, rinsed with DDI, and washed again in GC grade methanol, rinsed with DDI and washed one last time in *n*-hexanes to remove external contamination. Methods to extract PCB congeners were adopted from (Covaci et al. 2002; Dauwe et al. 2005). Specifically, 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 8 ml 4 M HCl and 5 ml dichloromethane (DCM) then incubated overnight in a 40°C H<sub>2</sub>O water bath. The DCM layer was transferred to a 20 ml vial with a Teflon-lined cap. The extraction was repeated with an additional 3 ml 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 Na<sub>2</sub>SO<sub>4</sub>. The DCM was then concentrated to 1 ml 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 concentrated (98%) sulfuric acid to 50 g of Silica Gel 60 (70–230 mesh; EM Science) while stirring to maintain homogeneity (Method 3065A). The mixture was stirred for another 60 min following the acid addition. Approximately 780 mg of acidified silica was weighed out for each sample and added to a 6 ml glass column lined with a Teflon frit. The columns were conditioned with 2 ml hexane:DCM and 2 ml hexane, respectively. One milliliter 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 silica 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 determined in the GC analysis, a second cleanup step was necessary. A second set of silica columns were prepared as previously described and the samples were eluted with 4 ml hexane. The final eluate was blown completely dry with a gentle nitrogen stream and then raised to the volume of 1 ml with 100% GC grade methanol. Methanol was used as the final eluate so that matched samples could be run on both the GC and ELISA.

GC analyses (EPA Method 8082A) 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 µm film thickness; J&W Scientific, 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 1 min, 54–160°C at 20°C min<sup>-1</sup>, isothermal for 5 min, then 160–270°C at 3°C min<sup>-1</sup>, held isothermal for 3 min; 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 (Supelco; CAS# 11100-14-4) 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). All of the 12 congeners in the Aroclor 1268 mixture were determined in the GC–MS analysis, with a minimum detection of 17.53 ng g<sup>-1</sup>. Ten percent of the samples were replicated.

Detection of the Aroclor 1268 congener mixture with ELISA was performed using the Abraxis PCB Magnetic Particle Assay Kit specific for Aroclor 1268 (Catalog #: PN 530003). For this kit, specificity QA/QC analysis reported that Aroclor 1268 demonstrated a 16.5% cross-reactivity with other Aroclor mixtures with similar congener profiles (the specific Aroclor mixtures were not defined by the kit manufacturer; Lawruk et al. 1996) which is expressed as the least detectable dose estimated at 90% B/B<sub>0</sub>. The ELISA assay manufacturer QA/QC demonstrated no reactivity in the PCB RAPID Assay for Biphenyl, 2,5-Dichlorophenol, 2,3,5-Trichlorophenol, or Di-*n*-octyl-phthalate. Concentrations of 0.00, 0.50, 2.50, 10.0, and 50.0 ng g<sup>-1</sup> supplied with the kit in a methanolic solution with preservative and stabilizers were used to calibrate the photometric analyzer. Calibration curves (ln/linear) were accepted if coefficient of determination values were >0.990 with replicate coefficient of variation differences of <10%. The ELISA test kit detection range was 0.22–50 ng ml<sup>-1</sup> based on the



calibration standards. Test kit procedures were followed for water samples. Therefore, feather samples were diluted with DDI water (1:1) prior to ELISA analysis based on the kit instructions. One hundred percent of the feather samples were replicated for quality assurance purposes for each ELISA (due to precision problems discussed below) including a certified Aroclor 1268 standard solution (Supelco; 1000  $\mu\text{g ml}^{-1}$ , CAS# 11100-14-4) diluted in methanol to 50  $\text{ng ml}^{-1}$  and chicken feathers spiked with the 50  $\text{ng ml}^{-1}$  Aroclor 1268 standard solution.

A paired  $t$  test was used to test the hypothesis that there was no difference in the Aroclor 1268 congener mixture concentrations in samples run using the GC versus the ELISA. A simple linear regression was used to determine the relationship between samples analyzed with the GC to the matched samples using the ELISA method. Pearson's correlation coefficients and associated  $p$ -values were used to compare feather data with matched samples from a previous study (Cumbee et al. 2008).

## Results

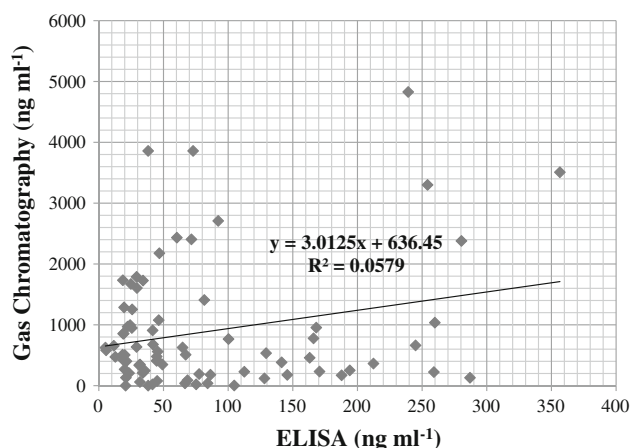
No PCB congeners were detected in any of the 24 chicken feathers based on GC analysis, regardless of washing technique or incubation method. However, ELISA results from chicken feathers cleaned by the methods listed above ranged from 1.5 to 7.1  $\text{ng ml}^{-1}$ . There was no validation to determine if these methods could have removed some proportion of endogenous contamination. However, based on the results below the possibility of endogenous PCB removal from the washing methods did not weaken the ability to detect PCB congeners in the feather.

The two internal spikes used to assess the recovery rates of our PCB extraction procedure yielded differing results based on instrumentation (GC vs. ELISA). Each 50  $\text{ng ml}^{-1}$  Aroclor 1268 spike recovered 38.5 and 38.8  $\text{ng ml}^{-1}$ , respectively (78% recovery) as determined by the GC. ELISA quantified these 2 spikes (each ran 4 times due to lack of precision) from 19.2 to 84.1  $\text{ng ml}^{-1}$  (Chicken 1:  $n = 4$ ,  $\bar{x} = 43.89$ ,  $\text{SD} = 30.12$   $\text{ng ml}^{-1}$ ; Chicken 2:  $n = 4$ ,  $\bar{x} = 41.81$ ,  $\text{SD} = 24.05$   $\text{ng ml}^{-1}$ ) or a 38–168% estimated recovery. The Chicken 1 and Chicken 2 spikes within ELISA runs were between 0.2 and 20.8% of each other. Replicate clapper rail samples run on the GC ranged from 2 to 3% of each other ( $n = 7$ ). In contrast, clapper rail samples run on the ELISA were consistently less than the same extracts run on the GC with the replicates run on the ELISA ranging from 49 to 365% of each other ( $n = 78$ ,  $\bar{x} = 108.62\%$ ,  $\text{SD} = 68\%$ ).

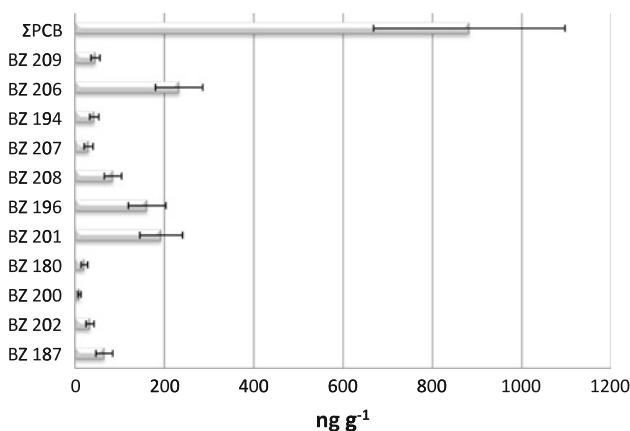
The six clapper rail samples taken from individuals that did not reside in the LCP marsh (e.g. reference locations: Frederica and Troupe Creeks located within 10 km of the

LCP site; Fig. 1) all fell below detection limit (BDL) of the GC. However, these same samples analyzed for the Aroclor 1268 congener mixture using ELISA yielded concentrations between 20 and 75  $\text{ng g}^{-1}$ .

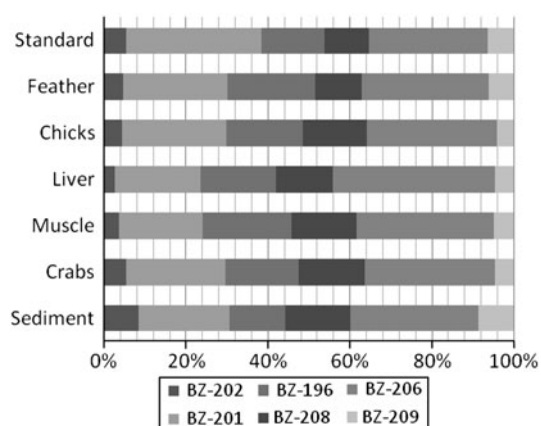
The majority of samples taken from LCP contained the Aroclor 1268 congener mixture (Figs. 2, 3, 4). Concentrations as determined by GC ( $\bar{x} = 869.40$ ,  $\text{SD} = 948.72$ ) were significantly higher ( $p < 0.0001$ ,  $t = 1.66$ ,  $\text{df} = 150$ ) than those quantified using the ELISA methods ( $\bar{x} = 83.89$ ,  $\text{SD} = 82.56$   $\text{ng ml}^{-1}$ ). Specifically, values from ELISA results showed almost a 10 fold reduction from results obtained from the GC (Fig. 2).



**Fig. 2** Regression plot comparing Aroclor 1268 congener mixture detected using gas-chromatography (GC) and enzyme linked immunosorbent assay (ELISA) methods in clapper rail feathers ( $\text{ng g}^{-1}$  dry weight). Feathers were taken from birds harvested at and near the contaminated LCP superfund site in Brunswick, GA, USA



**Fig. 3** Mean (with associated 95% confidence limits) Aroclor 1268 congener concentrations ( $\text{ng g}^{-1}$ ) for clapper rail feathers as determined by gas chromatography (GC). Congeners are presented in the Ballschmiedt–Zell (BZ) numbering system. Samples whose individual congener quantification fell below detection limits were omitted for the purposes of congener summation



**Fig. 4** 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 in 2000, 2002, 2006, and 2007. The percent compositional breakdown for Aroclor 1268 (standard) is shown as a reference

Eleven congeners of Aroclor 1268 were quantified by the GC (Figs. 3, 4). Of these eleven, six congeners (BZ 202, 201, 196, 208, 206 and 209) comprised 85% of the sample. The percent profile of these 6 congeners was similar to the congener profile of the Aroclor 1268 standard (Fig. 4). 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, subsequently 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; Fig. 4).

The summation of Aroclor 1268 congeners in clapper rail feathers was compared to the liver and muscle tissue values of those same clapper rails ( $n = 8$ ; Cumbee et al.

**Table 1** Feather, muscle, and liver Aroclor 1268 congener mixture concentrations ( $\mu\text{g g}^{-1}$  dry weight) measured in clapper rails collected from the Linden Chemical Plant (LCP), Brunswick, Georgia during 1999–2007

Id	Feather	Muscle <sup>a</sup>	Liver <sup>a</sup>
LCP 104	0.088	1.7	8.3
LCP 107	0.209	18	56
LCP 106	0.246	5	20
LCP 358	0.336	19	157
LCP 103	0.346	9.4	N/A
LCP 108	0.625	49	499
LCP 567	2.176	18	126
LCP 337	2.708	71	568

All samples were analyzed by gas chromatography (GC)

<sup>a</sup> Cumbee et al. (2008)

**Table 2** Correlation coefficient ( $r$ -value, lower matrix) and associated  $p$ -values (upper matrix) for paired feather, muscle, and liver Aroclor 1268 congener mixture concentrations ( $\mu\text{g g}^{-1}$  dry weight) measured in clapper rails collected from the Linden Chemical Plant (LCP), Brunswick, Georgia during 1999–2007

	Feather	Muscle <sup>a</sup>	Liver <sup>a</sup>
Feather	.	0.060	0.271
Muscle	0.686	.	0.001
Liver	0.538	0.975	.

All samples were analyzed by gas chromatography (GC)

<sup>a</sup> Cumbee et al. (2008)

2008) revealing that rail feathers contained approximately 2–3 orders of magnitude less the Aroclor 1268 congener mixture than muscle and liver tissues from the same bird (Table 1). These birds were also followed during a telemetry study to ensure that they were full time residents of the contaminated site (Cumbee et al. 2008). The trend of minimum versus maximum feather load did not always correspond to the PCB loads in the matching tissues. However, correlations of feather to muscle ( $r = 0.686$ ,  $df = 7$ ,  $p = 0.60$ ) and feather to liver ( $r = 0.538$ ,  $df = 6$ ,  $p = 0.271$ ) explained some of the variation in the dataset (Table 2).

## Discussion

### ELISA techniques

Cross-reactivity with other PCBs is a potential problem when using the ELISA method. The discrepancies between the amount of the Aroclor 1268 congener mixture quantified based on the ELISA versus the GC methods, however, do not seem to be from this potential source of error. The quantitative magnetic ELISA that was used combines an antibody specific for the Aroclor 1268 congener mixture with an enzyme-labeled PCB. The enzyme-labeled Aroclor 1268 has been reported to exhibit a 16.5% cross-reactivity with other Aroclor mixtures that share similar congener profiles (Lawruk et al. 1996) which may cause an over estimation due to the presence of such compounds. However, based on the congener profiles detected by the GC, only the Aroclor 1268 congener mixture was present in the sample. Also, most samples were under estimated by the ELISA compared to the GC making this phenomena unlikely. Moreover, although some reduction of Aroclor 1268 has occurred over time from natural attenuation or perhaps chemical weathering (GDNR 2000), this PCB mixture is still present in very high concentrations in the LCP marsh (Cumbee et al. 2008) and any other Aroclor mixture would

be from global distribution. Not only were most of the feather Aroclor 1268 congener mixture concentrations consistently under estimated using the ELISA method compared to the GC, these estimates were consistently an order of magnitude less. Although ELISA quantified samples were consistently less than the same extracts run on the GC, the replicates run on the ELISA ranged from 49 to 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 (Zajicek et al. 2000). This is not a likely explanation for the ELISA under-detection because the congener profiles from our feathers were almost identical to 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. These studies demonstrated a strong reaction if when using other solvents such as isooctane and hexane which remained in the extract even in trace amounts (Zajicek et al. 1996, 2000). In contrast, these same studies have reported that the evaporative removal of such hydrophobic solvents with subsequent dissolution in 100% methanol can give near quantitative recovery of model PCB congeners. Samples from this study were blown down completely 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 residues from non-polar solvents used in the extraction (requiring an additional clean-up step), although none were visible to the naked eye. This can cause the PCB to not distribute homogeneously in the methanol, providing an inaccurate reading by the photometer. Therefore, to avoid this potential contamination, our extraction methods would need to be further refined to make the final extract less susceptible to the presence of such residues. 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.

#### Feather PCB concentrations

Our experiment using chicken feathers suggest that the washing techniques removed exogenous sources of PCBs from the feather and that the Aroclor 1268 mixture detected is from endogenous deposits originating when the feather was grown. The ability to successfully remove exogenous

organic residues with detergent and/or non-polar solvents is supported by other studies focusing on endogenous materials in feathers (Burger 1993; Hobson and Wassenaar 1997; Jaspers et al. 2007a, 2008). Therefore, since the Aroclor 1268 mixture was detected in all of the feathers collected from LCP, it is fair to assume that these birds either hatched or nested in LCP the previous year. Clapper rails found within the marshes near LCP are likely year round residents due to its favorable habitat. Aroclor 1268 is extremely persistent in the environment and is comparatively less mobile than other PCB mixtures 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 similar proportions of the 6 major Aroclor 1268 congeners found in the standard in all of the tissues measured as well as sediment and chick whole body (Fig. 4). However, concentrations in feathers did not necessarily follow the same trends in other tissues of the same birds (e.g. highest vs. lowest concentrations). Although the sample size was limited, some variation was explained in the correlation analysis. Since contaminant burden of the feather is indicative of what was in the blood when the feather was grown, the residence time of PCBs in the tissue or compartment must be considered. Clapper rails grow their feathers after the breeding season and are most likely still being exposed to contaminated food items, thus explaining for the correlation. Jaspers and colleagues (2007b) did find a significant correlation between tail feathers, muscle and liver contaminant loads in European buzzards (*Buteo buteo*), but not in other raptors or waterbirds. European buzzards have a very different molting chronology than rails, where the former tends to molt over an entire year (Zuberogoitia et al. 2005) versus one yearly molt in the clapper rail (and other waterbirds). It should also be mentioned that feather lipid extraction in this study or those referenced to, was not performed during sample preparation due to the fact that the feather weight is too low to quantify lipids gravimetrically.

Clapper rails in Georgia do not appear to be migratory and reside close (within 5–10 km) to their breeding home-ranges (Meanley 1985; Gaines et al. 2003). Since feathers from these birds can be used as a measure of exposure to the Aroclor 1268 mixture, this monitoring tool further expands the use of clapper rails as indicator species especially for the LCP Superfund site. Moreover, monitoring feathers from birds residing in compromised ecosystems for POPs will provide a mechanism to prioritize conservation and clean-up efforts. However, as demonstrated in



this study, establishing that contaminant loads are truly endogenous and understanding feather growth chronology, eating habits and habitat use patterns is essential if birds are to be useful in monitoring POP bioavailability. Our results also suggest that more research is needed into the best extraction procedures if an ELISA is being considered to quantify the Aroclor 1268 congener mixture in feathers.

**Acknowledgments** The Savannah River Ecology Laboratory (based upon work supported by the Department of Energy under Award Number DE-FC-09-075R22506) and Eastern Illinois University provided funding and access to equipment for analyses. We would like to thank Stacey Stephens and Nhilven DeChavez for their outstanding contributions in the laboratory. This manuscript benefited from the editorials of 6 anonymous reviewers.

## References

- Burger J (1993) Metals in avian feathers: bioindicators of environmental pollution. *Rev Environ Contam T* 5:203–311
- Callahan MA, Slimak MW, Gabel NW, May IP, Fowler CF, Freed JR, Jennings P, Durfee RL, Whitmore FC, Maestri B, Mabey WR, Holt BR, Gould C (1979) Water-related environmental fate of 129 priority pollutants. USEPA report no. EPA-440/4-79-029a
- Covaci A, Tutudaki M, Tsatsakis AM, Schepens P (2002) Hair analysis: another approach for the assessment of human exposure to selected persistent organochlorine pollutants. *Chemosphere* 46:413–418
- Cumbee JC, Gaines KF, Mills GL, Garvin N, Stephens WL Jr, Novak JM, Brisbin IL Jr (2008) Clapper rails as indicators of mercury and PCB bioavailability in a Georgia saltmarsh system. *Ecotoxicology* 17:484–494
- Dauwe T, Jaspers V, Covaci A, Schepens P, Eens M (2005) Feathers as a nondestructive biomonitor for persistent organic pollutants. *Environ Toxicol Chem* 24:442–449
- Eddleman WR, Conway CJ (1998) Clapper Rail (*Rallus longirostris*). In: Poole A, Gill F (eds) *The Birds of North America*. The Birds of North America, Inc., Philadelphia, PA, USA
- Gaines KF, Cumbee JC, Stephens WL (2003) Nest characteristics of the Clapper Rail in coastal Georgia. *J Field Ornithol* 74:152–156
- GDNR (2000). Total maximum daily load (TMDL) development for fish consumption guidelines due to PCBs in Gibson Creek, Terry Creek, Purvis Creek, and the Turtle River system, Georgia and commercial fishing ban due to PCBs in Purvis Creek (HUC 03070203), USEPA Region 4 Serving the Southeast Report July 2, 2001
- Heard RW (1982) Observations on the food and food habits of clapper rails from tidal marshes along the east and gulf coast of the eastern United States. *Gulf Res Rep* 7:125–135
- Hobson KA, Wassenaar LI (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109:142–148
- Jaspers VLB, Voorspoels S, Covaci A, Eens M (2006) Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? *Biol Lett* 2:283–285
- Jaspers VLB, Covaci A, Van den Steen E, Eens M (2007a) Is external contamination with organic pollutants important for concentrations measured in bird feathers? *Environ Int* 33:766–772
- Jaspers VLB, Voorspoels S, Covaci A, Lepoint G, Eens M (2007b) Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: a comparative and meta-analytical approach. *Environ Int* 33:328–337
- Jaspers VLB, Covaci A, Deleu P, Neels H, Eens M (2008) Preen oil as the main source of external contamination with organic pollutants onto feathers of the common magpie (*Pica pica*). *Environ Int* 34:741–748
- Jones KC, de Voogt P (1999) Persistent organic pollutants (POPs): state of the science. *Environ Pollut* 100:209–221
- Kannan K, Maruya KA, Tanabe S (1997) Distribution and characterization of polychlorinated biphenyl congeners in soil and sediments from a superfund Site contaminated with Aroclor 1268. *Environ Sci Technol* 31:1483–1488
- Kannan K, Nakata H, Stafford R, Masson GR, Tanabe S, Giesy JP (1998) Bioaccumulation and toxic potential of extremely hydrophobic polychlorinated biphenyl congeners in biota collected at a superfund site contaminated with Aroclor 1268. *Environ Sci Technol* 32:1214–1221
- Kannan K, Kawano M, Kashima Y, Matsui M, Giesy JP (1999) Extractable organohalogenes (EOX) in sediment and biota collected at an estuarine marsh near a former chloralkali facility. *Environ Sci Technol* 33:1004–1008
- Lawruk TS, Lachman CE, Jourdan SW, Fleeker JR, Hayes MC, Herzog DP, Rubio FM (1996) Quantitative determination of PCBs in soil and water by a magnetic particle-based immunoassay. *Environ Sci Technol* 30:695–700
- Meanley B (1985) *The marsh hen: a natural history of the Clapper Rails of the Atlantic coast salt marsh*. Tidewater Publications, Centreville, MD
- Novak JM, Gaines KF, Cumbee JC, Mills GL, Rodriguez-Navarro AB, Romanek CS (2006) Clapper Rails as indicator species of estuarine marsh health. *Stud Avian Biol* 32:270–281
- Robertson BA, Hutto RL (2006) A framework for understanding ecological traps and an evaluation of existing evidence. *Ecology* 87:1075–1085
- Rodriguez-Navarro AB, Gaines KF, Romanek CS, Masson GR (2002) Mineralization of clapper rail eggshell from a contaminated salt marsh system. *Arch Environ Contam Toxicol* 43:449–460
- Rodriguez-Navarro AB, Romanek CS, Alvarez-Lloret P, Gaines KF (2006) Effect of in ovo exposure to PCBs and Hg on clapper rail bone mineral chemistry from a contaminated salt marsh in coastal Georgia. *Environ Sci Technol* 40:4936–4942
- Roth RR, Newsom JD, Joanen T, McNease LL (1972) The daily and seasonal behavior patterns of the clapper rail in the Louisiana coastal marshes. In: *Proceedings from the annual conference of the Southeastern Association of Fish & Wildlife Agencies*, vol 26, pp 136–159
- Sajwan KS, Kumar KS, Weber-Goeke MA, Weber-Snapp S, Gibson C, Loganathan BG (2008) Extremely hydrophobic Aroclor 1268 and residues of polybrominated diphenyl ethers (PBDEs) in marsh sediment collected from Superfund Site in Brunswick, Georgia, USA. *Mar Pollut Bull* 56:1371–1376
- Smith TB, Marra PP, Webster MS, Lovette I, Gibbs HL, Holmes RT, Hobson KA, Rohwer S (2003) A call for feather sampling. *Auk* 120:218–221
- USEPA (1990) Guidance on remedial actions for superfund sites with PCB contamination. Report no.: EPA/540/G-90/007
- USEPA (2002) Georgia NPL/NPL Caliber Cleanup Site Summaries: LCP Chemicals Georgia Inc. Report no.: EPA GAD099303182
- Zajicek JL, Tillitt DE, Huckins JN, Petty JD, Potts ME, Nardone DA (1996) Application of enzyme-linked immunosorbent assay for the measurement of polychlorinated biphenyls from hydrophobic solutions. Extracts of fish and dialysates of semipermeable membrane devices. In: Van Emon JM, Gerlach CL, Johnson JC (eds) *Environmental immunochemical methods*

- ACS symposium series. American Chemical Society, Washington DC, pp 307–325
- Zajicek JL, Tillitt DE, Schwartz TR, Schmitt CJ, Harrison RO (2000) Comparison of an enzyme-linked immunosorbent assay (ELISA) to gas chromatography (GC)—measurement of polychlorinated biphenyls (PCBs) in selected US fish extracts. *Chemosphere* 40:539–548
- Zuberogitia I, Martinez JA, Zabala J, Martinez JE, Castillo I, Azkona A, Hidalgo S (2005) Sexing, ageing and moult of buzzards (*Buteo buteo*) in a southern European area. *Ring. Migr* 22:153–158