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We demonstrate the previously undocumented transport of a rare PCB mixture (Aroclor 1268) from a Superfund site in Georgia, and compare mercury loads among sample types, using least tern samples.

Environmental Impacts

This work demonstrates the dispersion of a rare, extremely hydrophobic PCB mixture (Aroclor 1268), derived from a single location at the LCP (Linden Chemical Plant) EPA Superfund Site near Brunswick, GA, USA, in piscivorous seabirds (least terns) across 180 km of coastal Georgia, USA. Because Aroclor 1268 has not been reported in sediments anywhere else along the Southeastern coast, this study demonstrates the extensive dispersal of this PCB mixture via bioaccumulation and trophic transfer. Due to Aroclor 1268's specificity to LCP, the presence and abundance of its unique signature in high-level consumers in the region has important implications for understanding the accumulation and transport of extremely hydrophobic, persistent organic contaminants, including highly chlorinated PCBs, years after their release.

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Exposure to mercury and Aroclor 1268 congeners in least terns (*Sternula antillarum*) in coastal Georgia, USA

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Concentrations of total mercury and the rare PCB mixture Aroclor 1268 (in dry weight ppb) in least terns (*Sternula antillarum*), a colonially-nesting, piscivorous seabird, in the Turtle River estuary and other coastal sites in Georgia, USA, were investigated. The Turtle River estuary is the location of the Linden Chemical Plant (LCP) Superfund site, a site prioritized by USA law for immediate remediation, where industries released effluent containing these contaminants until 1994. Aroclor 1268 is a highly-chlorinated PCB mixture that was used and released exclusively at the LCP site and nowhere else in the south eastern USA. High concentrations of Aroclor 1268 congeners and mercury have been documented in biota local to LCP, but no studies report concentrations in high trophic level, piscivorous birds such as least terns. We collected feathers and feces from chicks, and eggs from adults, at nesting colonies along the Georgia coast to analyze contaminant loads. Mean Aroclor 1268 mixture concentrations in eggs (≤16,329 ppb) were highest at colonies in and just outside LCP, and decreased with increasing distance (up to 110 km) from LCP, but the Aroclor 1268 signature congener mixture was present at all sites. Mercury concentrations in eggs (≤3,370 ppb), feathers (≤5,950 ppb), and feces (≤417 ppb), were present at all sites, but did not vary significantly among sites. This report further confirms the extensive dispersal of Aroclor 1268 congeners (approximately 110 km north and 70 km south of its point source) via bioaccumulation and trophic transfer.

Introduction

Contaminant exposure in seabirds

Persistent pollutants like polychlorinated biphenyls (PCBs) and methylmercury are not easily metabolized by most animal species and are often stored over time in adipose, or proteinaceous organ and muscle tissue, respectively[1,2]. Seabirds are often used as sentinel species for contamination of the aquatic habitats in which they live, and they tend to harbor some of the highest concentrations of contaminants, in part due to their high trophic status (where biomagnification results in peak exposure rates) and their relatively long lifespans (often decades-long)[3]. Even though PCBs are no longer produced and their environmental concentrations continue to decline, mercury and PCBs' longevity, bioavailability, and documented harmful effects on living organisms make their persistent presence in the environment a public health concern and a threat to

Figure 1. Location of Linden Chemical Plant (LCP) Superfund site and the Andrew's dredged-material island in the Turtle River estuary in Brunswick, Georgia, USA.

wildlife[4]. For example, effects of PCB and methylmercury exposure in birds include decreased egg production, abnormal incubating behavior, reduced egg volume and weight, thinning of eggshells, lowered hatch success due to embryonic death and decreased fertility of eggs, physical and physiological abnormalities in offspring, decreased growth rate, abnormal development of sex organs, immune system disorders, and high nestling mortality[5-10]. However, the extensive geographical ranges of many seabird species can often confound the determination of contaminant origin. Given relatively short distances of travel in the foraging adults and their prey species, measuring contaminant loads in flightless chicks of a colonial-nesting species can reflect local contamination relatively well, because chicks are hatched, fed, and raised on the colony site[3]. Contaminant origin can be most easily determined in chicks of a colonial species that forages close to the colony site when rearing young.

Least terns (*Sternula antillarum*) are seabirds that breed along the U.S. Atlantic Coast, including locations in coastal Georgia. Least terns are migratory, colonially-nesting, relatively long-lived, typically lay 2-3 eggs per clutch, and forage on small fish (<1.5 cm body depth) close (≤5 km in Georgia[11]) to their breeding colony[12]. Because of their high trophic position, localized foraging habits during the nesting season, and relative longevity, least terns are likely to have increased exposure to environmental contaminants local to their nesting grounds. Least terns are listed as 'rare' in Georgia and in many other eastern states, and the Central and Pacific populations of the least tern are federally protected in the USA by the Endangered Species Act, making the negative impacts of contaminants to least tern survival and recruitment a conservation concern.

A highly-contaminated estuary in Georgia, USA

The Linden Chemical Plant (LCP) in Brunswick, Georgia, USA, closed in 1994 and became a Superfund site (i.e., one designated by USA federal law for remediation). The 220-ha site is at the mouth of the Turtle River that empties into the Atlantic Ocean (Fig. 1). From 1919-1994, various industrial enterprises operated at the LCP site, including a coal- and later oil-fired petroleum refinery, an oil-fired power plant, a paint and varnish manufacturer, a chlor-alkali facility, and for most of the latter half of the last century, a chemical plant (LCP)[13]. Industrial activity at the LCP site resulted in the release of various persistent pollutants into waterways of the Turtle River estuary, primarily PCBs and 150-200 tons of mercury[14]. LCP used a highly-chlorinated PCB mixture known as Aroclor 1268, purchased for industrial use from its sole producer, Monsanto Chemical Company. LCP is one of the only sites in the eastern U.S. where this specific PCB signature was used[14]. Available records indicate Aroclor 1268 likely accounted for ≤ 1% of PCBs sold[15]. Aroclor 1268 is the most highly chlorinated PCB mixture that was manufactured and used commercially. Its composition is dominated by hepta, octa, and nona-, and deca-chlorobiphenyls (45%, 35%, 10%, and 4.8 % by wt., respectively). Because of its physico-chemical properties, including extreme hydrophobicity and low water solubility, the congeners comprising the Aroclor 1268 mixture (rank order of abundance PCB 206>199>203/196>209) are nearly entirely bound to sediment particles. Consequently, its physical transport from the LCP site is restricted to sediment transport in and near the Turtle River estuary during tidal flows. Except for sediments in the Taunton River and Narragansett Bay in New England[15] and the Sapelo National Estuarine Research Reserve[16], there are no reports of Aroclor 1268 contamination in sediments from the East coast of the USA. Thus, the origin of Aroclor 1268 mixture recovered in biota is unambiguous, and a hallmark of LCP pollution in the southeastern USA[14].

Despite remediation efforts at LCP, Aroclor 1268 congeners and mercury are still found in marsh sediments and organisms near LCP[17,18]. Aroclor 1268 signature congeners have been documented in reptiles[19-21], cetaceans[22], invertebrates[21,23], plants[24], and fish[21,25], near LCP. Bioaccumulation[21] and the trophic transfer of Aroclor 1268 congeners between fish species[25], between bottlenose dolphins (*Trusiops truncatus*) and their prey fish[26], and between clapper rails (*Rallus longirostris*) and their invertebrate prey[27], have been demonstrated. In addition, Aroclor 1268 congener concentrations in the blubber of bottlenose dolphins near LCP were higher than PCB concentrations found previously in any cetacean worldwide, suggesting biomagnification implications for other high trophic level consumers such as piscivorous birds. However, while PCBs were quantified in several lower level consumer avian species at LCP, including red-winged blackbirds (*Agelaius phoeniceus*), mottled ducks (*Anas fulvigula*), and boat-tailed grackles (*Quiscalus major*)[21], PCB concentrations have not yet been reported in any piscivorous bird species. Furthermore, the transport of Aroclor 1268 congener mixture has only recently been documented as far as 50 km from the point source[16], but its presence in biota across the rest of the approximately 160 km of Georgia coastline is unknown. Because of Aroclor 1268's congener mixture specificity to the LCP site, the presence and abundance of its unique signature in high trophic level organisms in the region has important implications for understanding the transport and accumulation of PCBs years after their release.

Study aims and expectations

We aimed to determine the degree to which least terns nesting on the coast of Georgia are exposed to mercury and Aroclor 1268 congeners, and how concentrations among various sample types (eggs, feathers, and feces) compare to one another. We expected that least terns would harbor lower concentrations of mercury as distance to LCP from the breeding colony increased. We also hypothesized that because the Aroclor 1268 mixture was released at LCP and no other point source in the

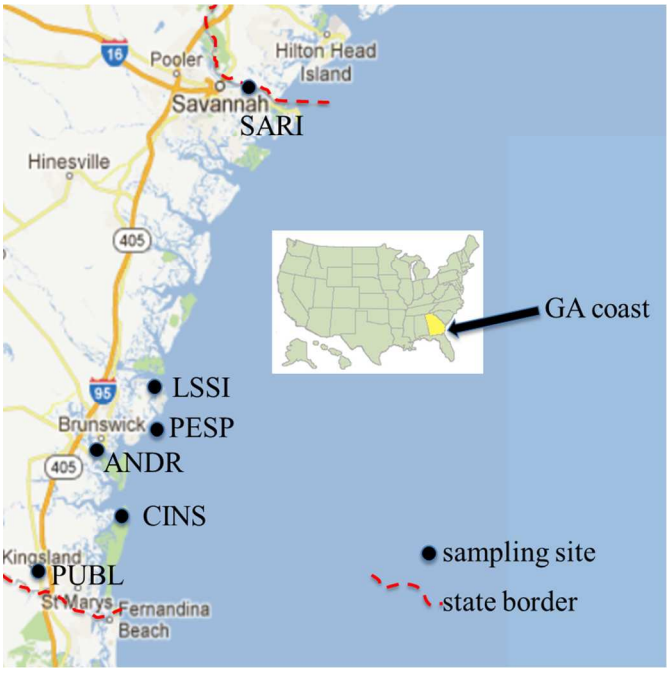

Figure 2. Locations of least tern nesting colonies along the 150-km of Georgia (GA), USA coastline: Savannah River confined disposal facility in the Savannah River estuary (SARI), Little St. Simon's barrier island (LSSI), Pelican Spit sandbar (PESP), Andrew's dredged-material island in the Turtle River estuary (ANDR), Cumberland Island National Seashore barrier island (CINS), and a grocery store rooftop in Kingsland, GA (PUBL). Locations of the state borders Georgia shares with South Carolina (to north) and Florida (to south) are also shown.

south eastern U.S., least terns in the Turtle River estuary would have Aroclor 1268 congeners in their tissues.

Experimental

Sites and field sampling

Samples were collected from six least tern nesting colonies along the Georgia, USA, coast from May-August 2011 and 2012: Andrews Island (ANDR), the Savannah River confined disposal area for dredged material (SARI), a flat gravel rooftop of a large building (PUBL), Cumberland Island National Seashore (CINS), and Little St. Simon's Island (LSSI) in both years, and a nesting colony on Pelican Spit (PESP) which was only active and sampled in 2011 (Fig. 2). The CINS and LSSI colonies were located on natural barrier islands, remote from urban development. The ANDR and SARI colonies were located on manmade dredged-material sites, in Brunswick and Savannah, Georgia, respectively. The PUBL colony was located on top of a flat, gravel roof in the city of Kingsland. The PESP colony was located on a sand bar ~1 km offshore from St. Simon's Island.

All research activities were performed in compliance with, and approved by, the relevant state (Georgia Department of Natural Resources) and institutional (University of Georgia Institutional Animal Care and Use Committee) laws and guidelines. Least tern colonies were monitored every three days throughout the nesting season. Eggs were collected throughout the nesting season in both years, usually following nest abandonment due to random events such as partial predation, flooding and nest over-wash, or nighttime evacuation of the nesting site by adults trying to avoid predation by nocturnal predators (e.g., *Bubo virginianus*). Collected eggs were wrapped in aluminum foil and stored frozen in 50-ml low-density polyethylene (LDPE) centrifuge tubes until processed. Chicks were individually identified upon hatching using a uniquely numbered aluminum leg band, and monitored until fledging. Chicks were regularly monitored for health and growth rate for a separate health study, and during routine handling of chicks fecal samples were opportunistically collected and stored frozen in 20-ml LDPE centrifuge tubes. The second primary feather was collected from both wings of each chick when primaries were >50% grown, typically within a few days of the chick fledging, about 15-18 days old. We chose the second primary because it is one of the largest feathers and one of the first flight feathers to develop, making it a major deposition site for mercury during nestling development[27]. Chicks were opportunistically collected when found dead (usually depredated) for whole body contaminant analysis.

Sample Preparation

Eggs, feathers, feces, and whole bodies were analyzed for total mercury, while only eggs and whole bodies provided enough material for PCB analysis. Chick carcasses were cleaned with a 1% liquinox solution, preceded and followed by a Milli-Q H_2O rinse (18 MΩ deionized water). Feathers were cleaned once with a 1% liquinox solution and again with GC-grade methanol and hexane, with each cleaning being preceded and followed by a rinse with Milli-Q H_2O. Feathers were cut into fragments to fit into the mercury analyzer boats. Chick carcasses, feathers, whole eggs, and fecal samples were lyophilized to a constant weight in small plastic containers which were tightly coverd with a Kimwipe filter and placed in 1 L glass freeze-drying vessels (Labconco Freeze Dry System, Labconco, USA). The Eggs and fecal samples were ground and homogenized using solvent-cleaned glass rods, and a stainless steel coffee grinder was used to grind the carcasses. Each egg sample was divided into separate plastic tubes for subsequent PCB and total Hg (THg) analysis.

Total mercury analysis

A subsample was separated from each homogenized sample for analysis. Mean (and range in parentheses) subsample dry weights used for each sample type were: 0.0618 g (0.0128 – 0.1680 g) for eggs, 0.0180 g (0.0003 – 0.1689 g) for feces, 0.0085 g (0.0003 – 0.0196 g) for feathers, and 0.0945 g (0.0139 – 0.1826 g) for carcasses. Subsamples were analyzed for THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA), according to U.S. Environmental Protection Agency (EPA) method 7473. For quality assurance, each group of 10 samples included a replicate, blank, and two standard reference materials (SRM; TORT-2 lobster hepatopancreas and DOLT-4 dogfish liver, National Research Council of Canada, Ottawa, ON.) The DMA 80 was calibrated using the solid SRMs (TORT-2 and DOLT-4). Method detection limits (MDLs; threefold the standard deviation of procedural blanks) averaged 0.0004 ppm (0.3679 ppb) dry mass. Mean percent recoveries (± standard deviation)

Table 1. Mean concentrations (ppb dry wt.) of Aroclor 1268 congeners in least tern eggs among nesting colony sites in Georgia, USA in 2011 and 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant Superfund site in Brunswick, GA, were Andrew's dredged-material island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), grocery store rooftop (PUBL), and Savannah River confined disposal facility (SARI). *Sample size (*n*) is in parentheses following each site name.

Site*	[PCB-187]	[PCB-202]	[PCB-201]	[PCB-180/193]	[PCB-200]	[PCB-196]	[PCB-208]	[PCB-207]	[PCB-194]	[PCB-206]	[PCB-209]
ANDR (23)	895	364	75	149	796	640	302	82	196	681	85
PESP (20)	1,009	446	80	176	839	707	316	82	228	788	89
LSSI (16)	533	175	49	93	445	322	123	38	97	347	35
CINS (17)	496	232	76	99	540	541	401	58	131	621	64
PUBL (9)	210	109	24	231	172	148	62	19	61	142	15
SARI (9)	135	80	20	64	149	135	50	20	52	232	19

of THg for the SRMs TORT-2 and DOLT-4 were 103.2 ± 5.7, and 98.9 ± 4.1, respectively.

PCB analysis

Prior to extraction, 100 ng of 2,4,5,6-Tetrachloro-m-xylene was added to each sample extract as a surrogate standard (Supelco, USA) Lyophilized egg samples were extracted (0.5-1.0 g) by sonication in 75 ml of acetone: hexane (1:1v/v) using a Tekmar Sonic Disruptor (Tekmar, Inc. USA) operated at 100% power in the pulsed mode with a 50% duty cycle for 3 min.. The mixture was filtered and the extraction repeated twice with fresh solvent. The solvent extracts were combined and residual water removed using Na_2SO_4. The extract was solvent-exchanged into 100% hexane and concentrated to about 5 mL. Extensive sample cleanup was performed prior to analysis using EPA Methods 3665A and 3630C. These procedures, described below, removed or destroyed co-extracted lipids and nearly all other organic contaminants. Residual co-extracted lipid material was removed by adding 8 mL of concentrated H_2SO_4 (Aristar –Trace metal free grade, Sigma-Aldrich, USA) analytical grade), mixing on a Vortex automatic electric mixer (Thomas Scientific, USA) , and allowing the mixture to cool for 30 min. Ultrapure water was added until two phases were clearly evident, and the acid-water phase was then removed with a Pasteur pipet. This step was repeated until the remaining hexane solvent extract was nearly clear following the acid addition. The hexane was concentrated to 1 ml using nitrogen evaporators (either TurboVap II, Zymark Corp.USA or N-Vap Model 111, Organomation, USA). All extracts were processed through columns of acidified (H_2SO_4) fully activated silica gel for a final cleanup step.

For quantification of PCB congeners, an Agilent (Atlanta, GA) 6890 gas chromatograph was used equipped with a DB-5 capillary column (30 m × 0.25 mm I.D., 0.25 um film thickness; J&W Scientific, Folsom, CA), electronic pressure control (EPC), and an electron capture detector (ECD). Samples were quantified using the internal surrogate standard (2,4,5,6-tetrachloro-m-xylene) and a response factor was calculated using a six-point calibration curve derived from dilutions of certified standards for the congeners (Ultra Scientific, USA) and Aroclor 1268 (Supelco,(USA); CAS# 11100-14-4) mixture standards. A total of 12 congeners were identified in the Aroclor 1268 mixture, based on retention time and elution order relative to the standards and included (PCB 180, 187, 194, 196, 199, 200, 201, 202, 206, 207, 208, 209), with a minimum detection of 17.5 ng g^{-1}. Congener peaks selected for quantitation (PCB 196, 199, 206, 208, 209) were at least 25% of the highest Aroclor component. We refer to the sum of these congeners concentrations as total Aroclor 1268 (Taro). The mean recovery of the internal surrogate standard (2,4,5,6-Tetrachloro-m-xylene) was 78% for all samples. Mean recoveries of Aroclor 1268 spikes was 82% (Range 78-86%; *n*=5). Sample blanks for all analysis were < 17 ppb. When concentrations were sufficient, identification of congeners was supported by GC-MS (Agilent 6890 GC coupled with a 5973 MSD) using the selected ion monitoring (SIM) acquisition mode. Ions monitored for the SIM analysis were selected using the mass spectrum (NIST Spectral Library) of each of the Aroclor 1268 congeners. The selected ions were, in most cases, the most abundant isotope mass within the spectra of the molecular ion and the most abundant fragment ions.

Statistical analysis

Contaminant concentrations were not normally-distributed for any of the sample types, but all contaminant distributions were successfully transformed to fit a normal distribution, a standard procedure[28]. Transformations for each sample type were chosen based on which best normalized a histogram of the samples, as well as showed homogeneity of variance using a Levene's test. A square-root transformation was used to normalize egg mercury, egg Aroclor 1268 congeners, and chick whole body mercury concentrations. A $\log_{10}$ transformation was used to normalize fecal mercury concentrations. A reciprocal transformation was used to normalize feather mercury concentrations, and a cube root transformation was used to normalize chick whole body Aroclor 1268 concentrations. A post-hoc Tukey's Honestly Significant Difference (HSD) test was used to detect pairwise differences (α = 0.05) in mean contaminant concentrations among sites and years. R statistical computing program was used for all statistical analyses[30].

Results

PCBs in egg samples

Total Aroclor 1268 congener concentration (TAro), and individual Aroclor 1268 congeners (Table 1), reported in dry weight (ppb), were measured from 104 least tern eggs across six sites in two consecutive years. Because least terns did not

Table 2. Total mercury concentration (THg) and total Aroclor 1268 concentration (TAro), reported in ppb dry wt., in least tern eggs among nesting colony sites in Georgia, USA, in 2011 and 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant Superfund site in Brunswick, GA, were: Andrew's dredged-material island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), grocery store rooftop (PUBL), and Savannah River confined disposal facility (SARI). *Sample size (*n*) is listed in parentheses beside each sample mean.

Site	Distance from LCP (km)	Egg THg (ppb) RANGE	Egg THg (ppb) MEDIAN	Egg THg (ppb) MEAN*	Egg TAro (ppb) RANGE	Egg TAro (ppb) MEDIAN	Egg TAro (ppb) MEAN*
2011							
ANDR	5	710 – 1,390	904	983 (10)	2,183 - 5,054	3,468	3,611 (10)
PESP	30	351 - 2,290	964	1,080 (20)	1,310 - 14,580	2,869	4,758 (20)
LSSI	40	404 - 1,340	911	1,037 (16)	619 - 7,062	1,364	2,257 (16)
CINS	42	350 - 1,860	1,030	1,081 (19)	13 - 9,125	1,991	2,624 (19)
SARI	110	362 - 3,370	857	1,127 (21)	13 – 1,838	303	481 (6)
2012							
ANDR	5	488 - 2,190	991	1,079 (33)	1,294 - 16,329	3,962	4,769 (13)
LSSI	40	404 - 2,810	1,160	1,425 (3)	--	--	--
CINS	42	372 - 1,040	497	602 (4)	--	--	5,525 (1)
PUBL	70	338 - 1,780	697	830 (18)	445 - 2,158	1,303	1,193 (9)
SARI	110	433 – 1,880	787	887 (26)	192 - 3,395	284	538 (10)

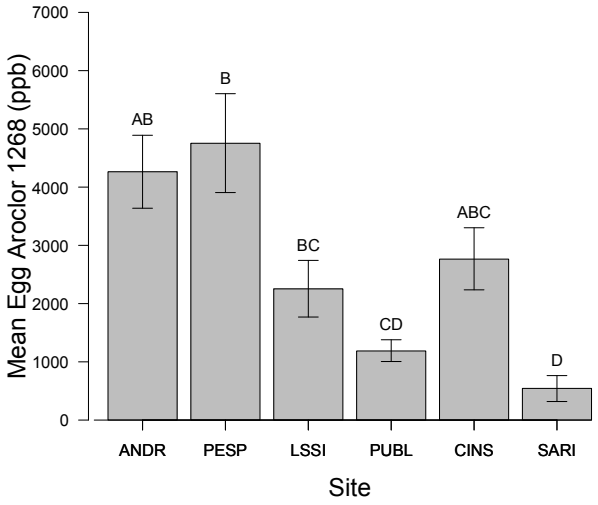

Figure 3. Mean total Aroclor 1268 congener concentrations (ppb dry wt). in least tern eggs among colony sites in Georgia, USA, in 2011 and 2012. Colony sites, shown in order of increasing distance to the Linden Chemical Plant Superfund site in Brunswick, GA, were Andrew's dredged-material island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), grocery store rooftop (PUBL), and Savannah River confined disposal facility (SARI). Standard error bars are shown, and means that do not share a common letter are

nest successfully at every site each year and sample sizes among sites were not equal, and due to possible differences in contaminant availability to least terns each year, results were sorted by year (Table 2). However, an analysis of variance (ANOVA) did not detect a year effect on TAro ($F_{1,102} = 2.01$, $p = 0.16$). Thus, TAro data were pooled over years, and in ascending order of distance from LCP, mean and median TAro in eggs, respectively, were 4,266 ppb and 3,843 ppb at ANDR; 4,758 ppb and 2,869 ppb at PESP; 2,257 ppb and 1,364 ppb at LSSI; 2,769 ppb and 2,037 ppb at CINS; 1,193 ppb and 1,303 ppb at PUBL; and 544 pbb and 284 ppb at SARI. Because ANOVA detected an effect of collection site on egg TAro ($F_{5,98} = 12.80$, $p < 0.01$), a follow-up Tukey's HSD was conducted to define pairwise differences between sites. PESP had greater egg TAro than PUBL ($p = 0.01$) and SARI ($p < 0.01$); and ANDR had greater egg TAro than both PUBL ($p = 0.01$) and SARI ($p < 0.01$). Egg TAro was higher at CINS ($p < 0.01$) and LSSI ($p = 0.01$) than at SARI, but CINS and LSSI had similar egg TAro to all other sites (Fig. 3).

Mercury concentrations in egg samples

THg was analyzed for 170 eggs from all six study sites in 2011 and 2012 (Table 2). An ANOVA detected no effect of year on THg ($F_{1,168} = 2.48$, $p = 0.12$), so data were pooled over years. For pooled years, in ascending order of distance from LCP, mean and median THg concentrations (respectively) in eggs were 1,057 ppb and 991 pbb at ANDR; 1,080 ppb and 964 ppb at PESP; 1,098 ppb and 936 ppb at LSSI; 965 ppb and 943 ppb at CINS; 830 ppb and 697 pbb at PUBL; and 994 ppb and 829 pbb at SARI. There was no effect of collection site on THg ($F_{5,164} = 1.03$, $p = 0.40$).

Table 3. Summary of total mercury concentrations (THg), reported in ppb dry wt., in least tern chick feces and second primary feathers (P2) among nesting colony sites in Georgia in 2011 and 2012. Distance from each nesting colony to the Linden Chemical Plant Superfund site in Brunswick, GA, is listed. Colony sites were Andrew's dredged-material island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), grocery store rooftop (PUBL), and Savannah River confined disposal facility (SARI). *Sample size (*n*) is listed in parentheses beside each sample mean. **Mean values in bold font with double asterisks are greater than other mean values of the same sample type within that sampling year ($\alpha = 0.05$).

Site	Distance from LCP (km)	P2 THg (ppb) RANGE	P2 THg (ppb) MEDIAN	P2 THg (ppb) MEAN*	Fecal THg (ppb) RANGE	Fecal THg (ppb) MEDIAN	Fecal THg (ppb) MEAN*
2011							
PESP	30	1,030 - 2,260	1,540	**1,581**** (17)	22 - 358	49	81 (10)
CINS	42	--	--	--	6 - 9	7	7 (2)
PUBL	70	--	--	--	23 - 52	33	35 (4)
SARI	110	832 - 1,660	1,115	1,152 (20)	34 - 203	56	80 (20)
2012							
ANDR	5	715 - 5,950	3,220	3,190 (11)	23 - 417	81	103 (21)
PUBL	70	873 - 4,360	1,830	2,105 (20)	14 - 175	66	84 (25)
SARI	110	1,570 - 2,280	1,820	1,841 (29)	14 - 158	58	67 (20)

Table 4. Summary of total mercury concentrations (THg) and total Aroclor 1268 concentrations (TAro), reported in ppb dry wt., in least tern chick whole body (carcasses) among nest colony sites in Georgia in 2012 ; *n* is the sample size in number of carcasses analyzed; and distance from each nesting colony to the Linden Chemical Plant Superfund site in Brunswick, GA, is listed. Colony sites were Andrew's dredged-material island (ANDR), grocery store rooftop (PUBL), and Savannah River confined disposal facility (SARI). *Mean values in bold font with an asterisk were greater than mean values at other sites ($\alpha = 0.05$).

Site	Distance from LCP (km)	*n*	body THg (ppb) RANGE	body THg (ppb) MEDIAN	body THg (ppb) MEAN	body TAro (ppb) RANGE	body TAro (ppb) MEDIAN	body TAro (ppb) MEAN
ANDR	5	5	820 - 1,090	939	937	2,643 - 3,906	3,465	**3,299***
PUBL	70	5	294 - 1,260	569	631	308 - 574	443	418
SARI	110	5	598 - 1,000	823	789	0 - 2,255	0	484

Mercury concentrations in fecal samples from chicks

THg was analyzed for 102 chick fecal samples from five different sites (Table 3). There was no effect of year on fecal THg ($F_{1,100} = 3.47$, $p = 0.07$); thus, data were pooled over years, and in order of increasing distance to LCP, mean and median fecal THg (respectively) were 103 ppb and 81 pbb at ANDR, 81 ppb and 49 ppb at PESP, 7 ppb and 7 ppb at CINS, 78 ppb and 62 ppb at PUBL, and 74 ppb and 57 pbb at SARI. Due to predation and flooding of nests, LSSI ($n = 0$) and CINS ($n = 2$) had inadequate chick fecal sample sizes and were omitted from the analysis. There was no difference in fecal THg among colony sites ($F_{3,96} = 1.00$, $p = 0.40$).

Mercury concentrations in feathers from chicks

The second primary feather (P2) was analyzed for THg from 97 chicks from four different sites (Table 3). An ANOVA detected differences in P2 THg between years ($F_{1,95} = 30.67$, $p < 0.01$); therefore, data were sorted by year for statistical analysis. Only PESP and SARI produced chicks that survived long enough to grow primary feathers in 2011, and P2 THg was greater in PESP chicks than SARI chicks ($F_{1,35} = 20.78$, $p < 0.01$). P2 THg did not differ between sites (ANDR, PUBL, and SARI) in 2012 ($F_{2,57} = 2.07$, $p = 0.14$).

Mercury and Aroclor 1268 congener concentrations in the whole bodies of chicks

In 2012, 15 chick carcasses were collected from three colony sites (Table 4). Chicks were between 7-12 days old, except one that was 15-16 days old. Chicks were found dead within, or on the perimeter of the colony, from unknown causes, predation, or falling from the roof (at PUBL only). Mean chick Aroclor

1268 congener concentration differed among sites ($F_{2,12}$ = 15.08, $p < 0.01$) with concentrations at ANDR (3,299 ppb) exceeding those at PUBL (418 ppb, $p = 0.01$) and SARI (484 ppb, $p < 0.01$). Total mercury in chick carcasses did not differ among sites ($F_{2,12} = 2.41$, $p = 0.13$).

Discussion

Transport of a unique PCB mixture

Aroclor 1268 is a unique, highly-chlorinated PCB mixture that is exclusively linked to LCP pollution in the south eastern U.S. The sole producer of Aroclor 1268 was Monsanto, and the only place in the southeastern U.S. where it was used and released into the environment was the LCP Chemical Company in Georgia. Our findings are the first to document the presence of Aroclor 1268 congeners in biota as far north as the South Carolina border (SARI colony) and as far south as Kingsland, Georgia (PUBL colony) and Cumberland Island National Seashore (CINS colony), which borders the state of Florida. While several studies since the 1990s have investigated bioaccumulation of Aroclor 1268 congeners in biota in the Turtle River estuary, only within the last few years has the Aroclor 1268 congener pattern been identified in organisms beyond the Turtle River estuary. Concentrations of Aroclor 1268 congeners recently found in fish[16] and in the blubber of dolphins with feeding ranges both in Brunswick and in the Sapelo Island National Estuarine Research Reserve (SINERR), exceed any previous reports of PCB concentrations found in any cetacean worldwide[31]. The SINERR has long been thought of as a relatively pristine habitat, and used as a reference site in many ecological studies. Sapelo Island is about 40 km north of Brunswick, and here we report the presence of Aroclor 1268 in the food web at least as far as 110 km north of Brunswick, in Savannah; and 70 km south of Brunswick, in Kingsland. Aroclor 1268 concentrations in eggs were >2,000 ppb at PUBL, >3,000 ppb at SARI, and >9,000 ppb at CINS, our three study sites farthest from Brunswick. We also report Aroclor 1268 concentrations in least tern eggs at Cumberland Island National Seashore, one of the premier national seashores in the eastern U.S., also regarded as a relatively pristine reference site for many biological studies.

Implications for least terns and other birds

The Aroclor 1268 congeners and mercury concentrations found in least tern samples along the Georgia coast reach exceed those associated with adverse effects in other studies. Much variability in sensitivity to PCBs and mercury exists among bird species, and even sometimes within individuals of the same species. Mean concentrations of mercury and Aroclor 1268 congeners may not warrant concern at all sites in our study, but the range of concentrations of these contaminants at our study sites are cause for concern. For example, several studies found that the total PCB concentration in eggs associated with reduction in hatch success in terns was 7,000 – 10,000 ppb[32-34]. Egg TAro ranges in our study exceed 7,000 ppb (in ten different egg samples) at all sites except PUBL and SARI, the two sampling sites farthest from LCP. The upper range of egg TAro at our two sampling sites closest to LCP was over 14,000 ppb at PESP, and over 16,000 ppb at ANDR. A review by Eisler[35] highlights that egg mercury concentrations as low as 1,500 ppb, and feather mercury concentrations as low as 5,000 ppb, have been associated with adverse effects. Ranges of least tern egg THg exceeded 1,500 ppb (in 18 different egg samples) at all sites in either 2011 or 2012 (Table 2.3), and the range of feather THg exceeded 5,000 ppb (in two chicks) at ANDR. Further research is needed to identify sublethal health and reproductive effects in birds posed by the elevated exposure to these contaminants along the Georgia coast.

Comparison of sample types for gauging local contamination

Female birds sequester mercury and PCBs into eggs[36-37], while feather growth is a major depuration pathway for mercury in both sexes[1]. Mercury is also excreted in feces[2]. Contaminant concentrations found in seabird chicks (with the exception of newly-hatched chicks) are attributed primarily to ingestion of contaminated food, while contribution from the parent (egg) is negligible[28,40]. It has been suggested that feathers are ideal samples for reflecting mercury exposure in birds. There is a constant flow of blood to growing feathers, and thus mercury in the blood is sequestered in a feather throughout its development, making feather growth one of the primary mercury depuration pathways in birds. Thus, many studies have demonstrated that feathers developed at the sampling site can provide a fairly accurate reflection of local mercury pollution at that site[38,41-45]. Because feather growth in chicks is ongoing from hatching to fledging, chick feathers represent an archive of mercury contamination throughout the chick's entire nestling period[46]. In contrast, eggs are the only sample type we collected that come directly from the adult bird. Studies in gulls (closely related to terns) found that 40% of the total body burden of mercury is deposited in an egg just before it is laid[47]. Egg samples are often used to determine the degree of local mercury exposure[3,48-52], and eggs are an ideal sample type because they are easy to collect and do not require the capturing or handling of the study species. Species like least terns that are migratory and relatively long-lived accumulate contaminants like mercury and PCBs in tissues over time, and thus could be introducing contaminants into their eggs that have been acquired elsewhere. However, egg samples could still provide an adequate proxy for local pollution in some cases, especially in those where the pollutants have a very limited geographic range and history of release, as is the case with Aroclor 1268 congeners.

Aroclor 1268 congeners and mercury in eggs and chicks

In our study Aroclor 1268 congener concentrations in least tern eggs decreased with increasing distance from LCP. However, mercury in egg samples was consistent across all sites. This difference could be explained by the low probability of least terns in our study being exposed to Aroclor 1268 congeners anywhere other than at our study sites, while birds could have been exposed to mercury from other geographic locations where they bred or over-wintered in previous years. However, the same trend in mercury and Aroclor 1268 congeners concentrations in eggs (derived from adults) existed for samples derived directly from chicks (which would have been exposed to contaminants predominantly through the local food source). That is, Aroclor 1268 congeners were higher in chick whole bodies located in close proximity to LCP, while mercury in chick whole bodies and chick fecal samples was consistent across sites. This indicates that mercury exposure in at least chicks, if not adults, was local and comparable at all sampling sites, where mercury intake of chicks came directly from the surrounding habitat. However, Aroclor 1268 congeners, while present at all sites were higher in both chick whole body samples at sites in close proximity to its point source, LCP.

Mercury present and varied in all sample types

Both fecal and feather mercury from chicks show variability within sites, but for the most part did not vary among sites. The one exception was that in 2011 feather mercury was higher at PESP than at SARI. Least terns did not nest at PESP in 2012 but did nest at ANDR, and there were no differences in feather mercury among chicks at ANDR, PUBL, and SARI in 2012. Because successful, chick-fledging colony sites did not overlap between 2011 and 2012 (with lack of success at colony sites mainly attributed to intense predation) comparisons of feather mercury could only be made among sites within a given year. Thus, we can only say that PESP chicks had higher feather mercury than SARI chicks (2011), but that feather mercury at SARI did not differ from ANDR or PUBL (2012). It is possible that other factors involving prey movement and availability may have been at play causing variation in mercury exposure among sites or years. More than 50 species of fish have been documented as prey items of least terns[53], with the most common prey species in the mid-Atlantic and south eastern USA being anchovy (*Engraulis eurystole*), menhaden (*Brevoortia tyrannus*), mummichog (*Fundulus heteroclitus*), and silverside (*Menidia* spp.)[54]. A high diversity of prey species, coupled with unaccounted for changes in availability of these species to least terns among sampled sites and years, could have had effects on mercury content in chicks in this study.

Variation in contaminant load within broods

In our study, eggs from the same brood/adult did not always have similar contaminant concentrations, especially in the case of the Aroclor 1268 congener mixture. For example, a 2-egg nest at LSSI had Aroclor 1268 congener concentrations of 5,307 ppb in one egg, and 704 ppb in the other; at a SARI nest 1,838 ppb and 13 ppb; and at a CINS nest 9,125 ppb and 5,302 ppb. Mercury, by contrast, was within a few hundred ppb between eggs in almost all cases where both eggs in a clutch were analyzed. While there may be between-clutch variation in PCB sequestration into eggs due to diminishing body fat throughout a nesting season[55], several studies have shown that there is little within-clutch variation in PCB concentrations[56-58]. While we do note these large within-clutch variations in PCB load in several samples from our study, we do not have a large enough sample size of nests where both eggs were analyzed to expound upon these disparities statistically. Regardless, investigators should consider these disparities, particularly when interpreting results where contaminant concentrations in a collected egg are used to explain effects (e.g., hatch success, observable adverse effects), or lack thereof, in other eggs or chicks from the same brood.

Conclusions

While mercury and PCBs are concentrated near the LCP Superfund site, we found that they are also pervasive along the Georgia coast. In addition, the transport of Aroclor 1268 congeners at least 110 km north and 70 km south from its point source (LCP) is more extensive than previously thought. The Aroclor 1268 congener mixture has only been reported in marsh or estuarine sediments in one location along the southeastern U.S. coast, this study demonstrates the extensive dispersal of this PCB mixture via bioaccumulation and trophic transfer. The presence of elevated concentrations of mercury and Aroclor 1268 congeners is important information for resource managers, as well as for researchers at preserves like Little St. Simon's Island and Cumberland Island National Seashore that are often considered reference sites in ecological research projects staged there each year. Because least terns are piscivorous birds at the top of their local food web, elevated exposure to, and adverse effects of, contaminants in this species indicates exposure and possible adverse effects in lower trophic order species that may warrant further investigation, especially in species of conservation concern.

Concentrations of both mercury and PCBs in Georgia least tern samples reached or exceeded those levels documented in the literature to cause adverse effects in birds. Least terns are threatened or endangered across most of their range, and habitat loss and subsequent mitigation often results in suitable habitat occurring in urbanized and polluted areas, such as dredge spoil sites in Georgia. Thus, the implications of mercury and PCB exposure on least tern health and reproduction demands further attention, in order to effectively manage the conservation of this species both in Georgia and beyond.

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Notes and references

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