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Environmental Toxicology and Chemistry, Vol. 20, No. 3, pp. 608–617, 2001
Printed in the USA
0730-7268/01 $9.00 + .00

PERSISTENT ORGANOCHLORINE POLLUTANTS IN EGGS OF COLONIAL WATERBIRDS FROM GALVESTON BAY AND EAST TEXAS, USA

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(*Received* 27 *March* 2000; *Accepted* 27 *July* 2000)

Abstract—Eggs of neotropic cormorants (*Phalacrocorax brasilianus*), black-crowned night herons (*Nycticorax nycticorax*), and great egrets (*Ardea alba*) nesting on several locations in Galveston Bay (TX, USA) and at two control sites outside the bay were collected during April–May 1996 and analyzed for chlorinated pesticides, PCBs, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans. Additionally, concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQs) were determined by use of relative potency factors (TEQs) or the H4IIE-luc bioassay TCDD-EQs. Concentrations of 1,1,-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) were greater in eggs of neotropic cormorants from Alexander Island (mean = 1,040 ng/g wet wt) in the Houston Ship Channel (Houston, TX, USA) and in those from Telfair Island (mean = 1,460 ng/g wet wt), a reference location outside the bay, than in most locations inside the bay (mean range = 119–453 ng/g wet wt). Mean PCB concentrations were greater in eggs of neotropic cormorants from Alexander Island (mean = 5,720 ng/g wet wt) than in eggs of cormorants from areas farther away from the ship channel, including two reference sites outside the bay (mean range = 404–3,140 ng/g wet wt). The TCDD was the main dioxin congener detected in eggs from all locations within Galveston Bay. Instrumental TEQs in eggs ranged from 67 pg/g wet weight at control sites to 452 pg/g wet weight at Alexander Island. Concentrations of TCDD-EQs determined in the H4IIE assay were correlated with instrumental TEQs and were greater in eggs of cormorants from islands within the bay, although these were farther away from the ship channel. Overall, concentrations of DDE, PCBs, TCDD, and TCDD-EQs were less than the threshold levels known to affect reproduction. However, some eggs contained concentrations of total PCBs or DDE greater than what would elicit adverse effects on birds. No identifiable deformities or abnormalities were detected in embryos collected from all sites.

Keywords—Galveston Bay, Texas, USA Polychlorinated biphenyls Dioxins Chlorinated pesticides Colonial waterbirds

INTRODUCTION

Galveston Bay and the Houston Ship Channel (TX, USA) are important transportation arteries for the Port of Houston, the third largest in the world [1]. Galveston Bay receives much of the local drainage from the City of Houston via Buffalo Bayou and the Houston Ship Channel. Treated wastewater from more than 1,400 industry and municipal point source discharges and runoff from agriculture through the San Jacinto and Trinity rivers (Fig. 1) contribute many of the contaminants to the bay.

Despite its significance as a natural resource and the great potential for the presence of contaminants, to our knowledge, few studies of Galveston Bay have been conducted, and most have focused on sediments, invertebrates, and fish [2–6]. Polycyclic aromatic hydrocarbons (PAHs), PCBs, chlorinated pesticides, metals, and metalloids have been reported in sediments and biota of Galveston Bay [2]. From 1986 to 1987, concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in edible fish tissue collected downstream from bleached kraft pulp and paper mill discharges in the Houston Ship Channel were greater than 0.7 ng/kg wet weight, the U.S. Environmental Protection Agency (EPA) criterion for human consumption [3]. Seafood consumption advisories were issued during 1990 for the upper bay and Houston Ship Channel because of concentrations of PCDDs and PCDFs in hardhead catfish (*Arius felis*) and blue crabs (*Callinectes sapidus*) [7]. The PCDDs, PCDFs, PCBs, and other persistent organochlorine pollutants (POPs) have been associated with potential adverse effects on wildlife [8–11]. Some of these adverse effects include decreased fertility and hatching success, embryonic malformations and behavioral anomalies in breeding adult birds, and reductions in vitamin A and thyroid (i.e., triiodothyronine and thyroxine) hormones in bird plasma [12,13].

Until now, the accumulation and effects of PCDDs and PCDFs on birds nesting in Galveston Bay have not been investigated, and only a few earlier studies have addressed the effects of other POPs, such as pesticides and PCBs [14]. One recent study suggested declines in the concentrations of 1,1,-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and PCBs in birds from Galveston Bay [15]; however, increased exposure to PAHs and genetic damage in black-crowned night herons (*Nycticorax nycticorax*) from the Houston Ship Channel have been reported [16]. Double-crested cormorants (*Phalacrocorax auritus*) from the same area also contained elevated concentrations of polychlorinated styrenes and other aromatic hydrocarbons [14]. Several colonial waterbirds nest on Alexander Island in the Houston Ship Channel, and birds nesting on this island possibly may have greater exposure to halogenated and

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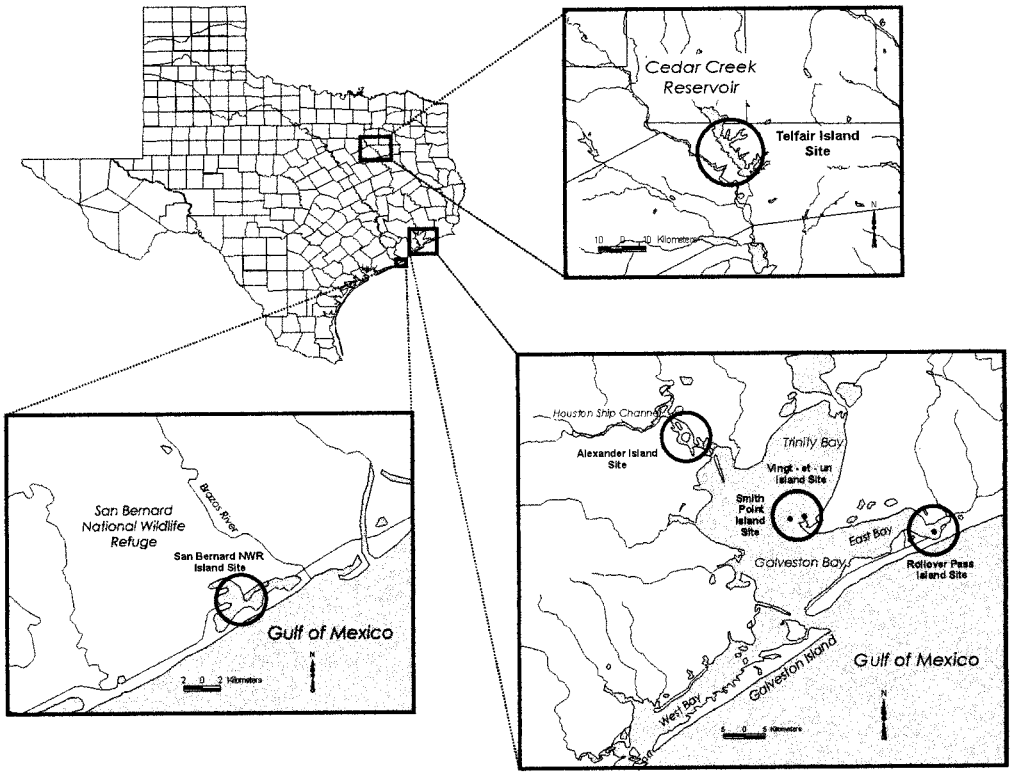

Fig. 1. Map of Galveston Bay, Texas, USA, and two reference sites in east Texas.

petroleum hydrocarbons than birds nesting on other islands in Galveston Bay.

We investigated the potential effects of POPs on birds nesting in Galveston Bay and two reference sites because of the fish consumption advisories resulting from the presence of PCDDs and PCDFs in fish and other aquatic organisms of the Houston Ship Channel and Galveston Bay. Our study aimed to determine concentrations of chlorinated pesticides, PCBs, PCDDs, and PCDFs in eggs of colonial waterbirds to assess the potential hazards these chemicals present to nesting waterbirds in the Galveston Bay area; to measure total aromatic hydrocarbon receptor (AhR)–mediated activity of complex mixtures with the H4IIE-luc bioassay and to correlate the concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQs) with concentrations of POPs; and to increase our understanding of the ecological condition of the bay, with implications for wildlife management and environmental pollution control policies.

MATERIALS AND METHODS

Study area

Galveston Bay, Texas, USA, covers an area of approximately 1,550 km^2 and lies southeast of the Houston metropolitan area in the northwest Gulf of Mexico [1]. Galveston Bay is divided into four major sub-bays: Trinity Bay, Galveston Bay, East Bay, and West Bay (Fig. 1). Four collection sites were selected in the Galveston Bay area: Alexander Island, in the lower portion of the Houston Ship Channel in Harris County; Smith Point and Vingt-et-un islands in Chambers County; and Rollover Pass Island in Galveston County, at the eastern end of Galveston Bay (Fig. 1). Two reference sites outside Galveston Bay were at the San Bernard National Wildlife Refuge in Brazoria County, south of Galveston Bay, and Telfair Island at the Cedar Creek Reservoir in Henderson County, approximately 100 km east of Dallas, Texas (Fig. 1).

Sample collection

Sixty-two eggs were collected opportunistically during April–May 1996 from three colonial waterbird species nesting in Galveston Bay and two reference sites outside the bay. Eggs of neotropic cormorants (*P. brasilianus*, $n = 10$), black-crowned night herons ($n = 9$), and great egrets (*Ardea alba*, $n = 7$) were collected from Alexander Island. Eggs of neotropic cormorants also were collected from Rollover Pass ($n = 4$), Smith Point ($n = 4$), Vingt-et-un ($n = 10$), San Bernard National Wildlife Refuge ($n = 7$), and Telfair Island ($n = 11$).

Chemical analyses

Eggs were cleaned and rinsed with water, then allowed to dry. Each egg was weighed, and the egg volume was determined by water displacement. Egg contents were transferred to chemically cleaned jars, and embryos at advanced stages of development were checked for abnormalities.

Eggs were analyzed individually. Sample extraction was based on the National Oceanic and Atmospheric Administration status and trends method for pesticides, PCBs, and PAHs [17]. Egg contents were homogenized with a tissuemizer (Tek-Mar, Cincinnati, OH, USA), and approximately 2 g of homogenate in sodium sulfate were extracted with dichloromethane. Sample extracts were fractionated by elution of an alumina:silica (1:2) column with a 1:1 mixture of dichloromethane and pentane. The extracts were further purified by a SP800 ternary high-performance liquid chromatography pump (Spectra-Physics, Freemont, CA, USA) and two size-exclusion columns connected in series (22.5 × 250 mm Phenogel 100-

Å columns). Extracts were concentrated to a final volume of 1.0 ml in hexane for gas chromatographic analysis.

Concentrations of 29 pesticides, 94 PCB congeners, and three non-*ortho*-substituted PCB congeners were determined in each sample extract. Pesticides and PCBs were analyzed by gas chromatography with an electron-capture detector (Ni^{63}) using a HP5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) in splitless mode [18] and a 0.25-μm DB-5 (i.d. = 30 m × 0.25 mm) fused silica capillary column (J&W Scientific, Folsom, CA, USA). High-resolution gas chromatography/mass spectrometry was used for confirmation of pesticides and PCBs.

Pesticides and PCBs were quantified with the aid of a computer-generated software package (Xchrom® version 1.5; Fisons Instruments, Beverly, MA, USA). Analytes in the extracts were identified based on the retention times of the authentic standards. Spike recoveries for pesticides averaged 83%, and recoveries for PCBs averaged 96%. Method detection limits for pesticides and PCB congeners were calculated as established by U.S. EPA guidelines [19] and were 1 ng/g wet wt or less.

Seven PCDDs, 10 PCDFs, and three non-*ortho*-substituted PCB congeners were analyzed in 15 eggs according to the National Oceanic and Atmospheric Administration status and trends method as described by Gardinali et al. [6]. Two grams of tissue were extracted with dichloromethane after homogenizing with sodium sulfate. Extracts were concentrated with hexane and treated with 40 g of a mixture of 44% concentrated sulfuric acid/silica gel slurry to remove lipid interferences. The extracts were further purified by elution through a mixed-bed silica column with hexane, an alumina column (i.d. = 250 × 13 mm, 200-mL reservoir) with hexane, and a 2.3:1 mixture of hexane and dichloromethane.

The final cleanup and fractionation was conducted with a charcoal column that was made by cutting both ends of a clean, disposable serological pipette and combusting it for 4 h at 440°C. The column was packed with glass wool, 1.0 cm of activated silica gel, 1.0 g of AX-21 charcoal/silica gel, and glass wool again at the top. The extracts were eluted with mixtures of 1:1 dichloromethane/cyclohexane, 15:4:1 dichloromethane/methanol/toluene, and 100% toluene, and the final volume was concentrated into tetradecane. A HP5890 Series II gas chromatograph (Hewlett-Packard) fitted with a DB-5 MS fused silica capillary column (i.d. = 60 m × 0.25 mm) and film with a thickness of 0.25 μm (J&W Scientific) was used for this analysis. Two microliters of the sample extracts were injected in a splitless mode using a CTC-2000S programmable autosampler. Helium was used as the carrier gas. The injection port was kept at 280°C and directly interfaced by the analytical column to a VG Autospec Ultima double-focusing, high-resolution mass spectrometer (Fisons Instruments). The electron-impact ionization detector was set at 35 eV using selected ion monitoring at a resolution power of 10,000 or greater [20]. Recoveries of internal standards ranged from 84 to 134% for PCDDs and PCDFs and from 62 to 102% for non-*ortho* PCBs. Recoveries of spikes in blanks were greater than 90%.

H4IIE-luc bioassay

Concentrations of TCDD-EQ were determined by use of the H4IIE-luc bioassay [21]. Briefly, H4IIE rat hepatoma cells were stably transfected with an AhR-controlled luciferase reporter gene construct (H4IIE-luc) under a humidified 95:5 air/carbon dioxide atmosphere at 37°C. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% defined fetal bovine serum (Hyclone, Logan, UT, USA). For the bioassay, 250 μl of cell suspension at a density of 6×10^4 cell/ml were used to seed 96-well culture ViewPlates (Packard Instruments, Meriden, CT, USA). Cells were dosed in triplicate with TCDD in iso-octane (0.1–30 pg/well) or with the tissue extract dissolved in iso-octane. Three days after dosing, cell viability was measured with a Live/Dead™ kit (Molecular Probes, Eugene, OR, USA) and quantified with a Cytofluor 2300 fluorescence measurement system (Millipore, Bedford, MA, USA). Luciferase activity was measured by incubating cells with LucLite reagent (Packard Instruments) for 20 min at room temperature. A Dynatech ML 3000 luminometer (Dynatech Labs, Chantilly, VA, USA) at 30°C was used to measure light production, which is a measurement of luciferase activity. Cell confluency was verified microscopically; therefore, protein normalization was not necessary. Luciferase activity was reported as either relative light units or as a percentage of solvent control. Because of the amount of sample available, a standard dose–response curve for TCDD (six concentrations tested in triplicate per plate) was compared with only a single concentration of tissue extract performed in triplicate tests.

Statistical analyses

Concentration values were logarithmically transformed to approximate a normal distribution. Concentrations of pesticides, PCBs, PCDDs, and PCDFs were compared with general linear models [22]. Tukey-Kramer multiple comparisons were used to determine which means were significantly different. The probability of a type I error (α) was set a priori to 0.05. Principal component analyses were conducted by use of the PRINCOMP® procedure [22] to compare PCB congener patterns in eggs of species nesting on Alexander Island and in eggs of cormorants nesting on other locations. Multiple linear regression was used to test for relationships between bioassay results and concentrations of TCDD; total PCBs; PCBs 77, 126, 169, 105, 118, 156, 167, and 189; TCDD-EQs; and relative potency factors.

Determination of TCDD-EQs

The TCDD-EQs were measured in two ways. The total equivalents were calculated by summing the product of toxic equivalent factors established by the World Health Organization [23] and concentrations of individual PCDDs, PCDFs, and PCB congeners. The concentrations calculated from an additive model were designated TEQs. Alternatively, the total concentrations of equivalents were determined by subjecting the entire extract containing PCDDs, PCDFs, and PCBs to the H4IIE bioassay. The equivalents determined by the H4IIE assay were designated TCDD-EQs.

The TCDD-EQs were calculated from the bioassay relative light unit values by fitting the TCDD standard curve to a logistic equation and using the transform and nonlinear regression function of SigmaPlot® (version 3.0; Jandel Scientific, San Rafael, CA, USA). The estimated TCDD-EQ values (pg/μl) were then divided by 1.25 (i.e., the sample added per well in microliters). Concentrations of TCDD-EQ expressed as picograms per gram wet weight were then determined by dividing the TCDD-EQ (pg/ml) by sample weights. Differences between TCDD treatments were tested for significance by use of a Student's *t* test ($p \leq 0.05$). For all samples tested, the coefficient of variation was between 5 and 10%. The relative

Table 1. Dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB; geometric means and ranges, ng/g wet wt) in eggs of colonial waterbirds from Galveston Bay and two reference sites (TX, USA)[a]

Species	Location	*n*	% Moisture ± SD[b]	% Lipid ± SD	DDE[c]	HCB[c]
Neotropic cormorant	Alexander Island	10	83.4 ± 1.0	3.5 ± 1.2	1,040AB (423–3,020)	324A (72–1,570)
	Vingt-et-un	10	84.4 ± 2.0	3.1 ± 0.6	390C (115–943)	33B (3–380)
	Smith Point	4	83.1 ± 0.9	3.6 ± 0.6	205CD (42–547)	8B (2–20)
	Rollover Pass	4	84.1 ± 1.5	2.9 ± 0.7	119D (77–201)	6B (2–103)
	San Bernard NWR[d]	7	83.4 ± 0.7	3.1 ± 0.7	453BC (186–1,050)	40B (22–75)
	Telfair Island	11	82.5 ± 1.6	3.3 ± 0.7	1,460A (753–4,600)	10B (2–20)
Black-crowned night heron	Alexander Island	9	80.3 ± 0.9	5.1 ± 1.7	401A (35–12,900)	59B (2–975)
Great egret	Alexander Island	7	81.2 ± 0.8	4.1 ± 0.5	372A (126–1,850)	7B (2–36)

[a] Comparisons for herons and egrets are only with cormorants from Alexander Island, Texas, USA.
[b] SD = standard deviation.
[c] Means not sharing the same uppercase letter are significantly different ($p < 0.05$).
[d] NWR = National Wildlife Refuge.

potencies reported are the averages of two or three independent determinations, with three replicates per treatment.

RESULTS

Chlorinated pesticides

Hexachlorocyclohexane isomers (α, β, γ, and δ), chlordane-related compounds, and dieldrin were detected in eggs of the three species from all locations at low concentrations (generally <100 ng/g wet wt). Only hexachlorobenzene and DDE, a metabolite of DDT, were detected in bird eggs at concentrations greater than 100 ng/g wet wt. Therefore, only concentrations of these two organochlorines are shown in Table 1. The concentration of hexachlorobenzene was significantly greater ($p < 0.05$) in eggs of cormorants from Alexander Island than in those of cormorants from other islands both within and outside the bay. Concentrations of DDE were greater in eggs of cormorants from Telfair Island and similar to those in eggs of cormorants from Alexander Island (Table 1). The mean concentration of DDE in eggs of cormorants from Alexander Island was not significantly different from those in eggs of black-crowned night herons and great egrets nesting on Alexander Island; however, it was significantly greater ($p < 0.05$) than those in eggs of cormorants from other sites in Galveston Bay (Table 1). The two highest DDE levels were in one egg from a black-crowned night heron from Alexander Island (12,900 ng/g wet wt) and one from a neotropic cormorant from Telfair Island (4,600 ng/g wet wt). The rest were all less than 3,000 ng/g wet wt.

Polychlorinated biphenyls

Total concentrations of PCBs were significantly greater ($p < 0.05$) in eggs of cormorants from Alexander Island than in eggs of cormorants from Rollover Pass, San Bernard, and Telfair Island, but they were similar to those in eggs from Vingt-et-un and Smith Point islands in Galveston Bay (Table 2). Within Alexander Island, total PCBs were also significantly greater ($p < 0.05$) in eggs of neotropic cormorants than in eggs of great egrets, but they were similar to those in eggs of black-crowned night herons (Table 2).

The most abundant of the PCB congeners was 153, followed by PCB congeners 138 and 180. These three congeners contributed from 37 to 43% to total PCBs in all species (Table 2 and Fig. 2). Six congeners (PCBs 153, 138, 180, 118, 99, 187, and 170) contributed from 56 to 68% to total PCBs. Polychlorinated biphenyl congener patterns were similar in eggs of all three waterbird species from Alexander Island and in all cormorant eggs from various locations (Fig. 2).

Planar chlorinated hydrocarbons

Concentrations of PCDDs, PCDFs, non-*ortho* PCBs, TCDD-EQs, and TEQs are given in Table 3. Concentrations of mono-*ortho* PCBs that were used for the calculation of TEQs also are provided (Table 3). Except for one sample, TCDD was detected in all the eggs analyzed. This was the major dioxin isomer detected in all the samples, except for one sample that also contained penta- and hexachloro-dibenzo-*p*-dioxins. Too few samples were available with which to make comparisons of TCDD concentrations among species within Alexander Island or among locations for cormorants; however, TCDD was approximately twofold greater in eggs of cormorants and black-crowned night herons from Alexander Island than in eggs of great egrets from the same location. On average, TCDD concentrations were nearly ninefold less in cormorants from Vingt-et-un and Smith Point islands in east Galveston Bay than in eggs of birds nesting on Alexander Island near the Houston Ship Channel. The furan isomer 2,3,7,8-tetrachlorodibenzofuran was detected in only three black-crowned night heron eggs collected from Alexander Island.

The non-*ortho*-substituted PCBs 77, 126, and 169 were detected in all 15 eggs analyzed (Table 3). Concentrations of PCB congeners 77, 126, and 169 were similar in cormorants from Alexander Island, Ving-et-un, and Smith Point, but they were somewhat less in one egg from Telfair Island, one of the reference sites. Concentrations of non-*ortho*-substituted PCBs also were similar among eggs of different species from Alexander Island, except for PCB congener 77, which was nearly fivefold greater in concentration in eggs of black-crowned night herons than in those of the other two species. Concen-

Table 2. PCBs in eggs (geometric means and ranges, ng/g wet wt) of colonial waterbirds from Galveston Bay and two reference sites (Texas, USA)

	Neotropic cormorant						Black-crowned night heron	Great egret
	Alexander (n = 10)	Vingt-et-un (n = 10)	Smith Point (n = 4)	Rollover Pass (n = 4)	San Bernard (n = 7)	Telfair (n = 11)	Alexander (n = 9)	Alexander (n = 7)
PCB28	56 (19–174)	24 (2–143)	9 (1–40)	5 (2–27)	3 (2–5)	5 (1–30)	17 (1–72)	12 (3–64)
PCB74	78 (2–226)	51 (4–265)	22 (2–118)	9 (4–39)	5 (2–10)	9 (3–35)	31 (2–130)	17 (4–63)
PCB66	147 (66–377)	64 (5–416)	26 (3–148)	9 (4–45)	7 (3–13)	8 (3–28)	33 (3–163)	24 (5–68)
PCB99	331 (168–756)	159 (16–571)	79 (8–386)	23 (14–41)	28 (12–62)	18 (9–42)	81 (8–312)	49 (7–259)
PCB118	418 (194–1,130)	213 (27–726)	123 (15–552)	40 (23–73)	49 (22–120)	41 (20–87)	146 (18–421)	95 (14–332)
PCB146	182 (99–554)	114 (10–425)	56 (5–265)	12 (8–22)	11 (5–28)	12 (7–40)	62 (6–157)	42 (3–183)
PCB153	905 (487–2,610)	540 (60–2,260)	333 (36–1,390)	96 (61–226)	92 (40–167)	88 (57–192)	338 (36–879)	233 (24–963)
PCB105	94 (45–241)	45 (6–116)	21 (2–101)	7 (3–17)	7 (3–17)	8 (4–19)	26 (3–86)	19 (2–70)
PCB138	639 (316–2,200)	347 (31–1,450)	185 (23–950)	38 (28–56)	41 (16–93)	45 (26–124)	250 (23–678)	168 (19–535)
PCB158	72 (27–333)	34 (3–122)	15 (1–86)	3 (2–6)	3 (1–8)	4 (2–10)	26 (2–74)	22 (2–65)
PCB187	269 (152–794)	165 (15–708)	93 (9–429)	17 (13–30)	15 (6–28)	20 (12–76)	96 (9–291)	66 (5–283)
PCB183	107 (54–329)	64 (7–359)	33 (3–180)	5 (4–8)	5 (2–9)	8 (5–23)	44 (4–90)	34 (2–160)
PCB128	99 (46–362)	51 (5–152)	24 (2–127)	7 (5–10)	6 (2–17)	6 (4–17)	24 (2–74)	23 (3–81)
PCB156/171	98 (43–497)	48 (4–173)	28 (2–170)	7 (4–13)	6 (3–15)	8 (4–19)	24 (2–75)	18 (2–49)
PCB180	561 (268–1,820)	324 (30–1,870)	187 (19–868)	38 (25–74)	28 (12–53)	57 (36–175)	210 (18–583)	161 (14–555)
PCB170	219 (130–560)	162 (15–872)	80 (10–334)	17 (11–34)	14 (6–25)	26 (15-76)	84 (8–191)	67 (6–225)
PCB194	102 (51–245)	64 (6–322)	34 (3–171)	8 (5–20)	6 (3–11)	13 (8–32)	32 (3–74)	23 (2–98)
PCB209	118 (42–299)	27 (2–341)	12 (4–22)	6 (2–19)	11 (4–20)	3 (2–4)	46 (11–125)	14 (5–129)
Σ PCBs[a]	5,720A (2,729–16,210)	3,140A (299–10,684)	1,640AB (180–7,250)	446B (259–880)	404B (171–782)	464B (304–1,158)	2,100AB (242–5,576)	1,510B (165–4,782)

[a] For ΣPCBs, means not sharing the same uppercase letter are significantly different ($p < 0.05$).

trations of PCB congener 126 were, on average, nine- and 22-fold greater than the concentrations of PCBs 169 and 77, respectively (Table 3).

Comparisons of TCDD-EQs and TEQs

Concentrations of TCDD-EQs were, on average, 30% lower than concentrations of TEQs. Concentrations of total PCBs were significantly correlated with concentrations of TCDD-EQ values when all samples and controls were included (n = 84, R^2 = 0.51, $p < 0.001$); however, this correlation was insignificant when only the 15 eggs that were analyzed for planar chlorinated hydrocarbons (PCHs) were compared (n = 15, $p > 0.05$). Concentrations of TCDD-EQs and TEQs were positively and significantly correlated (n = 15, p = 0.02). Total equivalents also were positively correlated with concentrations of all PCHs, particularly with PCB congener 126 ($p < 0.001$), which was the congener that contributed the most to total TEQs. Dioxin equivalents were positively correlated with TCDD concentrations; however, this correlation was significant only with concentrations of the non-*ortho*-substituted PCB congener 169 ($p < 0.05$).

Concentrations of TCDD-EQs and TEQs were similar among species from Alexander Island and in cormorants from Galveston Bay. The contribution of PCB congener 126 to total TEQs in eggs of colonial waterbirds ranged from 46 to 91%, whereas the contribution of TCDD ranged from 26 to 51%.

DISCUSSION

Chlorinated pesticides

Except for DDE, concentrations of most organochlorine pesticides were relatively low in eggs of waterbirds from all sites in Galveston Bay and the two reference sites outside the bay. Greater concentrations of DDE in eggs of neotropic cormorants from Telfair Island and Alexander Island than in eggs from other areas may be explained, perhaps, because the bird colony in Telfair Island was closer to farmland and rural areas, where DDT may have been used in the past. Alexander Island also is closer to the mouth of the San Jacinto River, which could be bringing relatively great concentrations of DDE from agricultural areas.

Mean DDE concentrations in eggs of colonial waterbirds in our study were similar to those reported earlier for Galveston

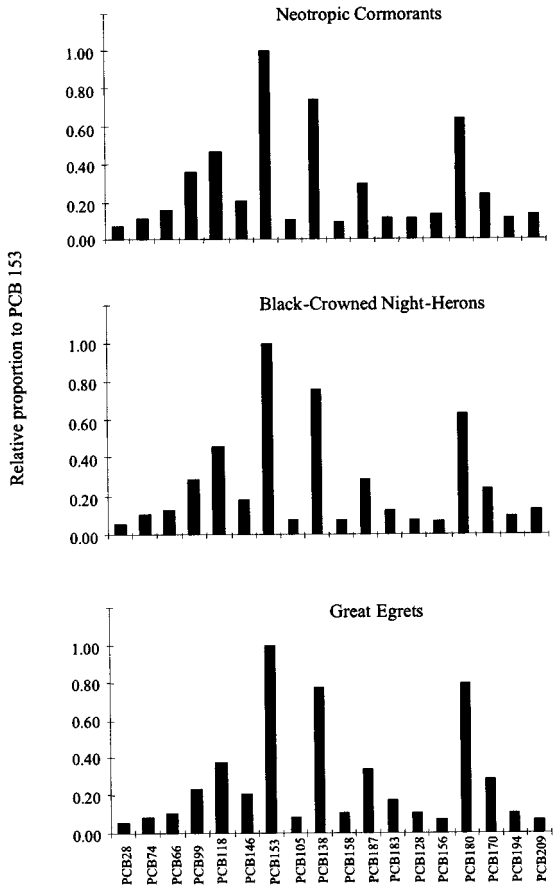

Fig. 2. Contribution of the most common PCB congeners to total PCBs (relative to PCB congener 153) in eggs of colonial waterbirds nesting on Alexander Island, Texas, USA.

Bay [24]. Olivaceous (renamed neotropic) cormorant eggs collected during 1970 and 1976 in Sabine Lake, Texas, USA, contained DDE concentrations of 6,000 and 400 ng/g wet weight, respectively [25]. Olivaceous cormorant eggs collected in 1980 and 1981 in Galveston Bay contained mean DDE concentrations of 1,700 and 700 ng/g wet weight, respectively [24]. Similarly, double-crested cormorant carcasses collected in 1982 and 1983 contained DDE concentrations of 700 and 900 ng/g wet weight, respectively [14]. This suggests that DDE concentrations in birds of Galveston Bay may not have declined as previous data indicated [15]. Current DDE concentrations in birds of Galveston Bay may be attributed to heavy use of DDT in the past and to atmospheric deposition from other areas. Concentrations of DDE as low as 4,000 ng/g wet weight have been reported to cause 5% eggshell thinning in cormorants [26]. Concentrations of approximately 8 μg/g wet weight in eggs have been associated with reproductive failures in black-crowned night herons [27]. Overall, however, DDE concentrations in most eggs were less than those that are associated with adverse effects on wildlife.

Polychlorinated biphenyls

Concentrations of total PCBs were significantly greater in eggs of cormorants from Alexander Island than in those from most locations, except for Vingt-et-un and Smith Point islands in Galveston Bay (Table 2). Alexander Island is closer to the Houston Ship Channel and to industrial areas; thus, colonial waterbirds nesting on Alexander Island probably feed closer to the Houston Ship Channel and may accumulate greater concentrations of contaminants than birds feeding in other areas of Galveston Bay. Foraging locations of the different species were not recorded, but we assume that birds foraged closer to their colony during the breeding season. Concentrations of total PCBs in eggs of neotropic cormorants nesting in Galveston Bay during 1996 were similar to those reported for earlier years. This suggests that concentrations of PCBs in birds of Galveston Bay have not decreased during the last 15 years. The concentrations of PCBs in olivaceous cormorant eggs from Galveston Bay averaged 3,600 ng/g wet weight in 1980 and 5,000 ng/g wet weight in 1981 [24]. Carcasses of double-crested cormorants collected during 1982 and 1983 averaged 1,500 ng/g wet weight [14].

Within Alexander Island, total concentrations of PCBs were greater in eggs of cormorants than in eggs of great egrets. The greater concentrations of PCBs in eggs of cormorants than in those of the other species could be explained by differences in diet or in the size of fish ingested. Cormorants usually eat larger fish than egrets and herons, and larger fish usually have greater concentrations of contaminants than smaller fish [28]. Also, cormorant diets consist mainly of fish and shrimp, whereas those of the other two species are more varied, consisting of smaller fish, aquatic insects, and crustaceans [29,30]. Mean concentrations of PCBs in eggs of cormorants from Alexander and Vingt-et-un islands in Galveston Bay were near or greater than the concentrations (4,000 ng/g wet wt) at which some detrimental effects (e.g., lethality and deformities) have been reported in double-crested cormorants in the Great Lakes [31]. One great blue heron (*Ardea herodias*) egg from Alexander Island contained a concentration of PCBs of 9,150 ng/g wet weight. The maximum concentration of PCBs measured in one cormorant egg from Alexander Island was 16,210 ng/g wet weight.

As demonstrated in other studies, the most common PCB congeners in colonial waterbird eggs from Galveston Bay were 153, 138, 180, and 118. This pattern seems to be the most commonly observed in fish-eating birds [32–34]. We did not observe any differences in PCB congener profiles or in degree of chlorination among eggs of the three species studied. This suggests that the PCB congeners in these species' diets may be similar, or that the three species metabolize PCBs in a similar way. Polychlorinated biphenyl patterns may be determined more by individual dietary factors than by species differences in the bioaccumulation of PCBs [28,33]. Similar PCB congener patterns among cormorants from different locations also suggest that metabolism may be responsible for the common pattern observed, or that the PCB sources in the diet are similar, despite differences in locations.

Of the non-*ortho*-substituted PCBs, congener 126 occurred at the greatest concentration. Concentrations of PCBs 126 and 169 were within the range reported in double-crested cormorants from the Great Lakes [31], but they were much lower than the median lethal dose (177 μg/kg egg) reported for double-crested cormorants from the Great Lakes [35]. The contribution of PCB congener 126 to total PCBs in our study ranged from 0.02 to 0.2%, whereas in Great Lakes cormorants, this contribution was approximately 0.02% [31].

American oysters (*Crassostrea virginica*), blue crabs, and hardhead catfish collected in Galveston Bay during the late 1980s and early 1990s had concentrations of non-*ortho*-substituted PCB congeners 77, 126, and 169 in livers of 920, 9,269, and 1,852 pg/g dry weight, respectively [5,20]. Eggs

Table 3. Dioxins, furans, non-*ortho* and mono-*ortho* PCB, and instrumental and bioassay derived 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin toxic equivalents (individual and arithmetic means, pg/g wet wt) in eggs of colonial waterbirds nesting in Galveston Bay and two reference sites (Texas, USA)

		PCDDs/PCDFs[a]				Non-*ortho* PCBs			Mono-*ortho* PCBs						
Species[b]	Location	TCDD	PeCDD	HxCDD	TCDF	PCB77	PCB126	PCB169	PCB105	PCB118	PCB156	PCB167	PCB189	Instrumental TEQs[c]	Bioassay TCDD-EQ
NC	Alexander	59	—	—	—	70	1,112	115	56,533	300,244	65,739	29,032	3,652	190	169
NC	Alexander	126	—	—	—	68	1,259	138	74,945	412,916	70,321	30,717	5,606	275	147
NC	Alexander	132	—	—	—	159	2,523	255	216,266	827,755	287,212	71,727	16,411	452	174
	Mean	106				99	1,632	169	115,915	513,638	141,091	43,825	8,556	305	163
NC	Vingt-et-un	23	—	—	—	39	1,286	159	45,896	141,840	54,134	25,305	5,986	166	121
NC	Vingt-et-un	8	—	—	—	54	3,452	666	62,859	316,355	141,851	89,112	21,246	381	184
NC	Vingt-et-un	11	—	—	—	76	3,342	463	94,499	726,214	172,683	75,480	17,602	384	437
	Mean	14				57	2,693	429	67,751	394,803	122,890	63,299	14,945	310	247
NC	Smith Point	13	—	—	—	104	3,330	551	100,826	376,130	169,956	75,825	16,680	383	413
NC	Smith Point	7	—	—	—	71	3,257	313	79,870	552,261	144,518	57,678	16,530	365	109
	Mean	10				87	3,293	432	90,348	464,195	157,237	66,751	16,605	374	261
NC	Telfair	ND[d]	—	—	—	30	614	91	18,571	80,041	16,254	8,147	266	67	59
BCNH	Alexander	179	25	26	6	293	1,307	137	85,793	420,944	74,924	31,505	4,798	376	229
BCNH	Alexander	174	—	—	12	563	1,200	142	38,920	344,368	33,625	19,323	5,028	345	290
BCNH	Alexander	136	—	—	8	502	1,104	110	52,773	177,730	49,284	25,513	5,427	291	173
	Mean	163			9	453	1,204	130	59,162	314,348	52,611	25,447	5,085	337	231
GrEg	Alexander	33	—	—	—	102	908	88	26,526	134,898	22,305	14,760	3,146	136	79
GrEg	Alexander	122	—	—	—	118	1,030	118	30,503	161,309	40,085	22,279	2,366	240	320
GrEg	Alexander	12	—	—	—	70	1,122	50	70,497	331,683	49,020	25,031	5,592	143	60
	Mean	56				97	1,020	85	42,509	209,296	37,137	20,690	3,702	173	153

[a] HxCDD = 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, PCDD = polychlorinated dibenzo-*p*-dioxin, PCDF = polychlorinated dibenzofuran, PeCDD = 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDF = 2,3,7,8-tetrachlorodibenzofuran.

[b] BCNH = black-crowned night heron, GrEg = great egret, NC = neotropic cormorant.

[c] Toxic equivalency factors (TEFs) for birds from [23] as follows: TCDD = 1, PeCDD = 1, HxCDD = 0.01, TCDF = 1, PCB77 = 0.05, PCB126 = 0.1, PCB169 = 0.001, PCB105 = 0.0001, PCB118 = 0.00001, PCB156 = 0.0001, PCB167 = 0.00001, and PCB189 = 0.00001. The toxic equivalents (TEQs) were calculated by multiplying individual concentrations by the TEF values and adding the results.

[d] ND = not determined.

of colonial waterbirds from Alexander Island had total non-*ortho* PCB concentrations ranging from 6,550 to 15,800 pg/g dry wt, an average of 1.5- to 14-fold greater than those concentrations observed in oysters, crabs, and catfish. Oysters, blue crabs, and hardhead catfish livers collected at Todd's Dump in Galveston Bay, across from Vingt-et-un and Smith Point islands, had total non-*ortho* PCB concentrations of 305, 4,856, and 552 pg/g dry weight, respectively. Mean total concentrations of non-*ortho*-substituted PCBs in eggs of cormorants from Vingt-et-un and Smith Point islands were 21,450 pg/g dry wt, approximately four- to 70-fold greater than those in oysters, crabs, and catfish. In contrast, concentrations of total non-*ortho*-substituted PCBs in oysters, crabs, and catfish livers from Hanna's Reef in East Bay (Fig. 1) were only 1, 2, and 11 pg/g dry weight, respectively. Overall, concentrations of total and non-*ortho* PCBs in oysters, crabs, catfish, and birds, to some extent, clearly show a pattern of decreasing concentrations from the Houston Ship Channel toward the Gulf of Mexico.

PCDDs and PCDFs

Of all the dioxin and furan congeners analyzed, TCDD and TCDF were the most commonly detected. Only black-crowned night herons had detectable amounts of other dioxins and furans. Concentrations of TCDD were greater in eggs of cormorants from Alexander Island than in eggs of cormorants from other locations, perhaps because Alexander Island is located in the lower portion of the Houston Ship Channel, downstream from pulp and paper processing plants. This idea is further supported by the fact that TCDD concentrations were similar in eggs of species nesting on Alexander Island, which suggests that they all feed at locations near the ship channel. Catfish and oysters collected in the Houston Ship Channel at Morgan's Point, and blue crabs at the San Jacinto Monument near Alexander Island during 1986 and 1987, contained concentrations of TCDD that exceed the consumption advisory criteria for seafood and fish [1]. This resulted in consumption advisories for catfish and blue crabs for the upper bay system of Galveston Bay [7]. Concentrations of TCDD in American oysters, blue crabs, and hardhead catfish collected in the Houston Ship Channel in 1994 averaged 16, 138, and 100 pg/g dry weight, respectively [20]. Concentrations of TCDD in oysters, crabs, and catfish livers from Todd's Dump and Hanna's Reef in Galveston Bay were lower than those from the ship channel. Furthermore, concentrations of PCDDs and PCDFs in sediment samples from various locations in the Houston Ship Channel and Galveston Bay suggested that the Houston Ship Channel, from the Port of Houston to Alexander Island, was the most contaminated area, and that contamination decreased toward the Gulf of Mexico [20].

Great blue herons have been observed to reproduce normally with TCDD concentrations in eggs of 92 pg/g wet weight [36]. However, reproduction was impaired by TCDD concentrations of 252 pg/g wet weight. Reproduction of wood ducks (*Aix sponsa*) was significantly impaired when concentrations of TEQs were between 20 and 50 pg/g wet weight [37]. The median lethal dose (LD50) for TCDD injected in double-crested cormorants from the Great Lakes was 4 μg/kg egg [35]. Concentrations of TCDD in eggs of birds from Alexander Island in our study were less than those associated with reproductive impairment of great blue herons or double-crested cormorants but as much as fivefold greater than mean concentrations of TCDD associated with reproductive impairment of wood ducks. The greater TCDD concentrations in samples from the Houston Ship Channel could be attributed to the greater density of industrial and municipal discharges along the channel in addition to urban runoff and atmospheric deposition.

Comparison of concentrations of TCDD-EQs and TEQs

Polychlorinated biphenyl congener 126 contributed the most to total TEQs in waterbird eggs. Because of its persistence and significant contribution to total TEQs, current concentrations of this congener pose a greater threat to wildlife than TCDD [38,39]. The great contribution of PCB congener 126 to TEQs explains why the bioassay-derived TEQs were better correlated with this congener ($p = 0.07$) than with concentrations of TCDD ($p = 0.5$). It also explains why TCDD-EQs were similar in cormorant eggs among locations or among species of birds from Alexander Island, whereas concentrations of TCDD were higher in samples from Alexander Island than in samples from locations away from the ship channel. Concentrations of TEQs and TCDD-EQs were significantly correlated and differed only by 30%, indicating that the bioassay was a good measure for the activity of PCHs and their additive toxic effects. Greater concentrations of TEQs than of TCDD-EQs have been reported previously, indicating that in vitro activities of PCHs may be less than additive due to antagonistic effects [40,41]. However, Koistinen et al. [42] reported that bioassay TEQs were 20% greater than the instrumental TEQs in eggs and muscle of white-tailed sea eagles (*Haliaetus albicilla*) from the Baltic Sea, possibly due to the presence of other dioxin-like chemicals that were not analyzed. Due to the limited sample size available for the bioassay procedure, full dose–response curves were not evaluated to estimate precise TCDD-EQs, but this study provides a reasonable estimate of TCDD-EQs and AhR-activity in the extracts.

Toxic equivalents in eggs of birds from Galveston Bay were within the range observed in the Great Lakes; however, we did not observe any anomalies or deformities in the eggs collected or in those inspected at the colonies. Dioxin equivalents in eggs of double-crested cormorants from the Great Lakes ranged from 19 to 344 pg/g wet weight [9,43]. Neotropic cormorants nesting on Alexander Island produced approximately one chick per nest in 1996, which was considered to be normal [44]. The last year that any birds were observed nesting on Alexander Island was 1996, perhaps because of the invasion of predators to the island (J. Woodrow, personal communication).

CONCLUSIONS

Our results indicate that colonial waterbirds nesting on Alexander Island in the Houston Ship Channel are at greater risk of exposure to and effects of PCHs than birds nesting farther off Galveston Bay and outside the bay. However, concentrations of DDE also were great at the Telfair Island colony in the Cedar Creek Reservoir, which has more surrounding farmland. Black-crowned night herons, great egrets, and neotropic cormorants nesting on Alexander Island had high concentrations of PCBs and dioxins; however, cormorants contained the greatest concentrations of PCBs. Cormorants tend to feed more in open water along the ship channel and in the bay, whereas herons and egrets look more for shallow water outside of the ship channel but also feed along the channel. Black-crowned night herons contained slightly greater concentrations of dioxins than cormorants, which suggests that the prey of herons

is as large as that of cormorants, or that herons feed along the same environmental source of dioxins. Some eggs of cormorants nesting on Alexander Island contained PCH concentrations in eggs similar to those that have been associated with lethalities and developmental abnormalities in double-crested cormorants in the Great Lakes.

Birds nesting on Alexander Island were exposed to greater concentrations of TEQs than birds nesting on other sites. Until recently, two pulp and paper processing plants had been operating near the Houston Ship Channel, upstream from the nesting colony. Some dioxin residues likely are still present in the sediments and biota of the Houston Ship Channel, as has been reported previously [5,20]. Our results show that birds nesting on Alexander Island may be at greater risk of some reproductive failures due to high levels of PCHs than birds nesting farther off the Houston Ship Channel or outside the bay; however, sampling at other locations along the Gulf of Mexico would be needed to address potential effects of PCHs on other bird populations.

Acknowledgement—We greatly appreciate the assistance of B. Cain, P. Gardinali, M. Kennicut, J. Ramirez, R. Speer, T. Wade, J. Whitehead, and J. Woodrow. This manuscript has been improved by comments from G. Cobb, K. Echols, and two anonymous reviewers.

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