ORGANOCHLORINE CONTAMINANTS IN DOUBLE-CRESTED CORMORANTS FROM LEWIS AND CLARK NATIONAL WILDLIFE REFUGE IN THE COLUMBIA RIVER ESTUARY

FINAL REPORT

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ABSTRACT

The Columbia River receives numerous contaminants from permitted municipal and industrial discharges, nonpoint pollution, accidental spills, and hazardous waste sites. Studies by the U.S. Fish and Wildlife Service and others have documented bioaccumulation of organochlorine contaminants in fish and wildlife in or along the river, and these compounds have been associated with poor reproductive success in resident bald eagles (*Haliaeetus leucocephalus*). Elsewhere in the United States, double-crested cormorants (*Phalacrocorax auritus*) have been used as indicators of exposure to organochlorine compounds. The Columbia River estuary supports an estimated 6,620 breeding cormorant pairs which are exposed to, and potentially harmed by, organochlorine contaminants. Between 1990 and 1995, we collected cormorant eggs from two islands along the lower Columbia River, and in 1993 from a reference colony off the coast of southern Oregon. Eggs were chemically analyzed for organochlorine pesticides, total mercury, polychlorinated biphenyls (PCBs), dioxins, and furans. Extracts from some eggs were also used in a H4IIE rat hepatoma bioassay to assess exposure to planar halogenated hydrocarbons (dioxin-like compounds). Concentrations of p,p -DDE and total PCBs in eggs from the reference colony were lower than in eggs from the Columbia River colonies. Concentrations of dioxins and furans in 1993 eggs were elevated in the lower Columba River colonies, but below detection limits at the reference colony. Concentrations of nearly all chemical constituents in the lower Columbia River eggs appeared higher at the upriver colony at Rice Island than at the colony located near the mouth of the river on East Sand Island, and some contaminants were significantly higher. Some eggs of lower Columbia River cormorants exhibited eggshell thinning, and concentrations of p,p -DDE in eggs were correlated (r= -0.47, P=0.001, n=46) to eggshell thickness. The toxicity of the dioxin-like compounds, measured as toxic equivalents (TEQs), in some Columbia River cormorant eggs approached or exceeded threshold effects levels or concentrations associated with reproductive impacts in some sensitive species. Results of the H4IIE-extract bioassay conducted on eggs in 1993 and 1994 indicated as much as 24% egg mortality could occur at Columbia River colonies when compared to concentration-response relationships for cormorants in the Great Lakes. Results indicated that concentrations of organochlorine chemicals could impact developing embryos or elicit egg mortality in some individuals, but concentrations were near estimated no-effect levels and did not approach levels considered to impact the population.

INTRODUCTION

The Lewis and Clark National Wildlife Refuge (NWR) encompasses a group of islands in Cathlamet Bay within the lower Columbia River. The refuge is a satellite of the Willapa National Wildlife Refuge Complex. The islands in Cathlamet Bay and nearby (downstream) Baker Bay provide essential habitat for migratory birds and several threatened and endangered species listed under the Endangered Species Act of 1973. The refuge and associated islands provide nursery areas for juvenile salmonids, and serve as a freshwater to saltwater transition zone for anadromous fish. The area serves as a critical link for migratory birds in the Pacific Flyway, and supports the largest currently active colony of double-crested cormorants (*Phalacrocorax auritus*) on the Pacific coast (Carter et al. 1995). An estimated 6,620 breeding pairs nest at five active colonies along the lower Columbia River, including two of the largest colonies at Rice and East Sand Islands.

East Sand and Rice Islands are exposed to numerous environmental contaminants from the Columbia River. The river drains an area encompassing 260,000 square miles, and receives contaminants through municipal and industrial permitted discharges, urban and industrial nonpoint pollution, agricultural runoff, accidental spills of oil and hazardous materials, and atmospheric deposition. Exposure to contaminants carried by the river threatens the viability of fish and wildlife in the lower Columbia River and on the refuge, and previous studies have documented a variety of environmental contaminant problems affecting fish and wildlife resources.

Several contaminants including dioxins, furans, polychlorinated biphenyls (PCBs), and organochlorine pesticides and their metabolites have been found in fish and wildlife from areas on or near the Lewis and Clark NWR at potentially hazardous concentrations. Elevated concentrations of PCBs and p,p -DDE (a metabolite of the pesticide DDT) have been found in fish, in eggs of fish-eating birds, and in mammals from the Columbia River (Anthony et al. 1993, Henny et al. 1996, Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999). The dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been found in fish at concentrations that exceed guidelines for protection of human health (U.S. Environmental Protection Agency 1986a; U.S. Fish and Wildlife Service unpubl. data). Because of elevated TCDD concentrations, the U.S. Environmental Protection Agency (EPA) restricted the concentration of allowable dioxin in the river in 1991 through the establishment of a Total Maximum Daily Load (TMDL) for TCDD to protect aquatic resources.

Elevated concentrations of p,p -DDE, PCBs, dioxins, and furans have also been found in lower Columbia River bald eagles. High concentrations of p,p -DDE and PCBs have been associated with significant eggshell thinning and low productivity in bald eagles nesting on the refuge and along the estuary. Based on mean five-year productivity, Columbia River eagles nesting below river mile (RM) 60 produce half as many young as other eagles in Oregon and Washington (U.S. Fish and Wildlife Service 1999). Eggs of fish-eating birds collected from Lewis and Clark NWR in 1990 and 1991 also exhibited elevated concentrations of dioxins and furans, p,p -DDE, and PCBs (U.S. Fish and Wildlife Service unpubl. data). Contaminant concentrations in some of

these birds were were similar to concentrations found in fish-eating birds from Michigan which exhibited poor reproduction (Kubiak et al. 1989).

In the Great Lakes region, double-crested cormorants have been used as indicator species to describe risk, assess hazards, and monitor trends of organochlorine compounds in tissues over time (Gilbertson et al. 1991, Tillitt et al. 1992, Yamashita et al. 1993, Custer et al. 1999). Cormorants in this region were nearly extirpated in the 1970s, primarily due to reproductive failure associated with eggshell thinning (Peakall 1988). Cormorant populations rebounded following restrictions in the use of the pesticide DDT (Scharf and Shugart 1981). Dioxin-like compounds, particularly the planar PCBs, have also been implicated in causing egg mortality, deformities, and developmental anomalies such as beak defects, edema, and incomplete skeletal ossification in cormorants and other fish-eating birds (Kubiak et al. 1989, Fox et al. 1991, Tillitt et al. 1992, Yamashita et al. 1993). However, Custer et. al. (1999) reported that DDE concentrations and not PCBs were the primary factor associated with reproductive and other impacts in double-crested cormorants from Green Bay Wisconsin. Recently, concentrations of TCDD and dioxin-like compounds at low levels have been implicated in causing neural disorders in developing cormorant embryos (Henshel 1998). Fish-eating birds along the lower Columbia River exhibit concentrations of dioxin-like compounds similar to some areas of the Great Lakes (U.S. Fish and Wildlife Service unpubl. data). Cormorants in this area could be experiencing reproductive or other problems associated with exposure to dioxin-like and other organochlorine compounds.

This investigation built upon previous studies (Anthony et al. 1993, Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999) along the lower Columbia River designed to evaluate the possible impacts in fish and wildlife from exposure to organochlorine compounds. Specifically, the investigation objectives were to determine concentrations of organochlorine contaminants in eggs of double-crested cormorants nesting on East Sand and Rice Islands; compare concentrations between the islands and to a reference area; evaluate the toxicological significance of the compounds; and examine the accumulation pattern of contaminants in the eggs. As these birds are useful indicators of contaminant conditions in upper trophic level species, information gathered in this study can be used to assess possible effects to other fish-eating birds such as bald eagles.

METHODS

Study Sites

Double-crested cormorant colonies were monitored along the lower Columbia River and off the coast of southern Oregon (Figure 1). The largest nesting colonies of double-crested cormorants along the Columbia River are on Rice and East Sand Islands. The third colony on Hunters Island, located just off the coast of southern Oregon, served as a reference colony.

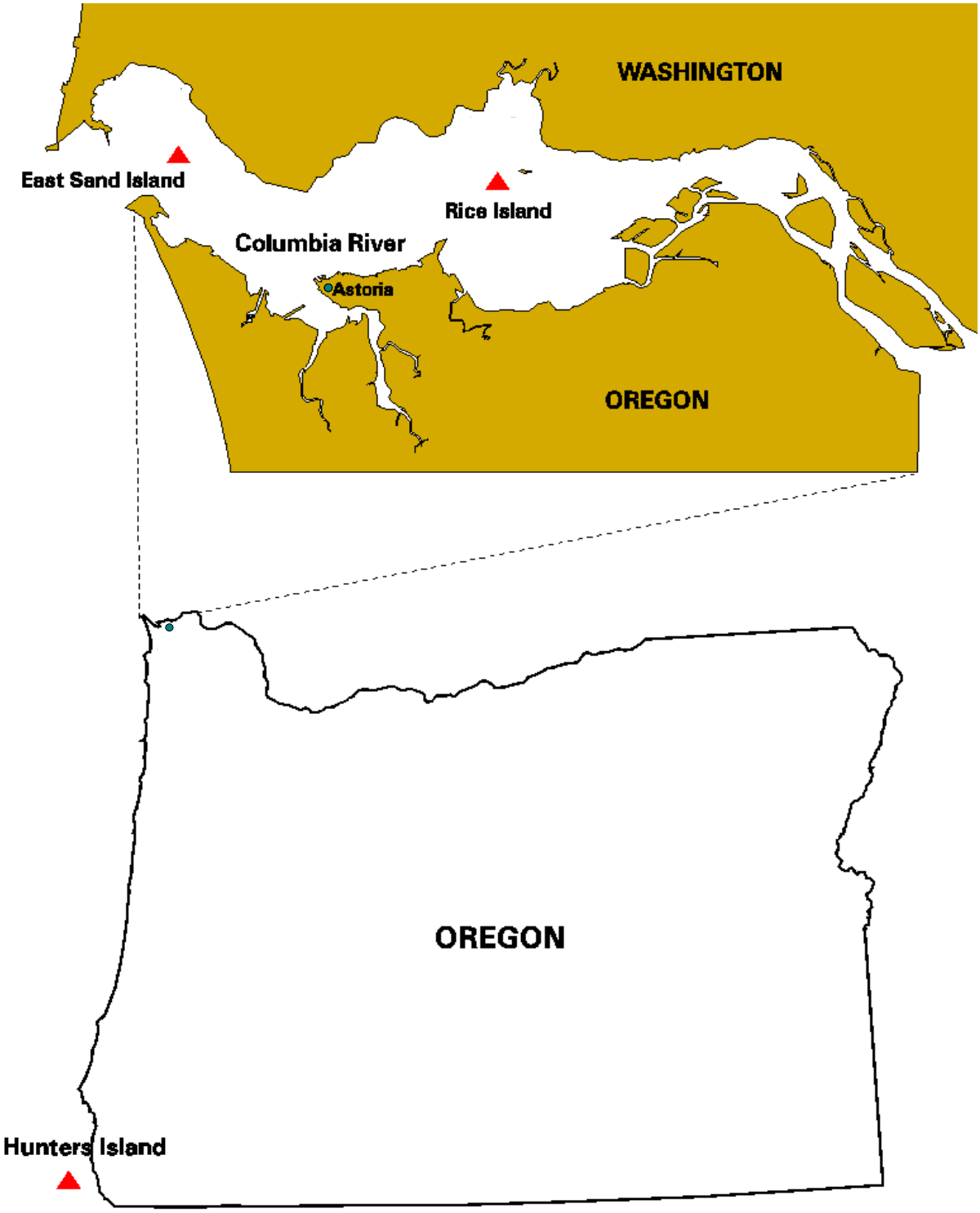

Figure 1. Location of double-crested cormorant colonies in the lower Columbia River, and off the coast of southern Oregon.

Rice Island is located across from Cathlamet Bay in the lower Columbia River at RM 22, and is currently owned by the Oregon Division of State Lands (Figure 1). The island was part of the Lewis and Clark NWR until 1995, when a management agreement with the Division of State Lands lapsed. The 128-hectare island was created from sandy dredge spoils from the Columbia River in the 1960s. The habitat on the island is predominantly bare sand due to frequent dredge spoil deposition. Grasses, chiefly European beachgrass (*Ammophila arenaria*) and dunegrass (*Elymus mellis*), various forbs, willows (*Salix spp.*), and cottonwood (*Populus balsamifera*) grow in the debris zone ringing the island and at the eastern and western tips, where dredge spoil has not been placed for several years. A variety of waterbirds nest on the island, including mallard (*Anas platyrhynchos*), Canada geese (*Branta canadensis*), western (*Larus occidentalis*) and glaucous-winged (*L. glaucescens*) gulls, Caspian terns (*Sterna caspia*), and double-crested cormorants. All species nest on the ground in sand (terns and gulls), in grasses (waterfowl), or in stick nests (cormorants). An estimated 2,422 cormorant pairs have nested on Rice Island in the past (Carter et al. 1995). However, no nesting occurred in 1999, possibly due to increased human disturbance (Al Clark, Julia Butler Hansen NWR, pers. comm.).

East Sand Island is another dredge-spoil island located at RM six near the mouth of the river in Baker Bay (Figure 1). Ownership is by the U.S. Army Corps of Engineers (COE). The 20-hectare island is mostly vegetated with grasses (European beachgrass and dunegrass), willows, red alder (*Alnus rubra*), Scotch broom (*Cytisus scoparius*), and gorse (*Ulex europaeus*). Nesting birds on the island include western and glaucous-winged gulls, Caspian terns, and double-crested cormorants. Cormorants on the island build large stick nests on the ground, predominantly on a rock jetty at the southwest tip of the island. The cormorant colony on East Sand Island numbers at least 4,000 breeding pairs (Carter et al. 1995) and has likely increased in recent years due to cormorants shifting from Rice Island.

Egg Collection and Processing

Double-crested cormorant eggs were collected during incubation from Rice Island in 1990, and from both Rice and East Sand Islands in 1991, 1993, 1994, and 1995. In 1993, additional eggs were collected from a reference colony at Hunters Island off the southern coast of Oregon. One egg was collected per nest. Eggs were collected opportunistically by hand from unmarked nests at numerous locations within the colonies except in 1993, when eggs were collected from each of five marked nests on each Columbia River island.

Cormorant eggs were measured for length, width, whole egg mass, and volume. Each egg was cut along the equator, emptied into a chemically cleaned glass jar, staged for embryonic development, observed for gross deformities, weighed, and frozen at -13°C. The five eggs collected from the reference colony were measured as described above, then separated into pipped embryo and yolk sac samples for storage. A composite embryo and yolk sac homogenate was later prepared for use in chemical analysis. All double-crested cormorant egg samples were shipped overnight on dry ice to laboratories contracted directly by the U.S. Fish and Wildlife Service, Oregon State Office in Portland, Oregon (1990 and 1991 samples), or by the Patuxent Analytical Control Facility in Maryland (1993 to 1995 samples).

Eggshell thickness (membranes included) was measured on 68 eggs which were collected intact. Each eggshell half was rinsed with water and air-dried for a minimum of 30 days after harvesting. Eggshell thickness was measured using a dial micrometer with rounded contacts at five sites along the equator. Eggshell thinning was determined as the percent difference in thickness between each eggshell and the mean eggshell thickness (0.432 mm) determined for museum specimens collected in the Northwest prior to 1947, when DDT was not in widespread use (Henny et al. 1982).

Sample Analysis

In 1990, 10 cormorant egg samples collected from Rice Island were submitted to three different laboratories for quantification of contaminant concentrations (Table 1). Alta Analytical Labs (El Dorado Hills, California) analyzed four eggs for organochlorine pesticides, total PCBs, and total mercury. Radian Analytical Services (Radian; Austin, Texas) analyzed three eggs for TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF). The remaining three eggs were analyzed by Mississippi State Chemical Laboratory (MSCL; Mississippi State, Mississippi) for organochlorine pesticides and total PCBs.

In 1991, 22 egg samples were collected from Rice and East Sand Islands and analyzed at 1) Pacific Analytical (PA; Carlsbad, California) and Triangle Laboratories (TRI; Research Triangle Park, North Carolina) for TCDD and TCDF; 2) Geochemical and Environmental Research Group (GERG; College Station, Texas) for organochlorine pesticides and total PCBs; and 3) Environmental Trace Substances Laboratory (ETSL; Rolla, Missouri) for total mercury concentrations (Table 1). Eggs from both islands were analyzed for TCDD and TCDF at PA and TRI in order to identify any differences in results that could be attributed to contract laboratories.

Ten cormorant eggs were collected in 1993 from Rice and East Sand Islands and analyzed at GERG for organochlorine pesticides, total PCBs, select non- and mono-ortho-substituted PCBs (planar PCBs), seven polychlorinated dibenzo-*p*-dioxins (PCDDs), and 10 polychlorinated dibenzofurans (PCDFs; Table 1). Five egg samples collected from the Hunters Island reference colony in 1993 were analyzed at GERG for DDT and its metabolites, total PCBs (measured as the sum of individual congeners), dioxins, and furans. In 1994, 11 egg samples were collected from Rice and East Sand Islands and 10 samples were analyzed for organochlorine pesticides and total PCBs at Hazleton Environmental Services, Inc. (HES), Madison, Wisconsin. The Environmental Contaminant and Research Center (ECRC), U.S. Geological Survey, Columbia, Missouri analyzed one of the 11 egg samples for organochlorine pesticides and total PCBs, and analyzed five of the 11 eggs for planar PCBs, eight PCDDs, and 10 PCDFs (Table 1). The 1995 egg samples from Rice and East Sand Islands (14 eggs) were analyzed by ECRC for organochlorine pesticides, planar PCBs, eight PCDDs, and 10 PCDFs. ECRC also quantified planar PCBs, PCDDs, and PCDFs in six of the 11 egg samples collected but not analyzed in 1994 (Table 1). These 1994 egg samples were analyzed concurrently at ECRC with the 1995 samples.

Table 1. Percent matrix spike or surrogate recoveries and detection limits (DL) from contract laboratories conducting analytical chemistry on double-crested cormorant eggs from the lower Columbia River.

		Total Mercury		Organochlorine pesticides & total PCBs		Dioxins and Furans		non-ortho/mono-ortho PCBs	
Year and lab[a]	n[b]	Recov.	DL (µg/g)	Recov.	DL (ng/g)	Recov.	DL (pg/g)	Recov.	DL (pg/g)
1990	10								
	3			58 - 100	10/50[c]				
MSCL	4	91.9	0.07	46 - 110	10/50/100[d]				
Alta	3					77-103[e]	5.7		
Radian									
1991	22								
ETSL	6	95-99	0.08						
GERG	6			57-93[f]	10/50[c]				
TRI	6					42 - 87	0.01-1.0		
PA	4					46 - 95	1.0-2.0		
1993	15								
GERG	15			75 - 165	2.0/20[c]	89 - 150	2.0/10-24[g]	95-149	20-50
1994	11								
HES	10			67 - 130	17/86/86[d,h]				
ECRC	5					23-66	0.2-0.5	48-147	1.0-2.0/200-500[i]
1995	14								
ECRC	20[j]			62-92[k]	0.05-0.30/30[c]	47-84	0.1-1.7	25-108	1.0

[a] Laboratories: MSCL=Mississippi State Chemical Laboratory, ETSL=Environmental Trace Substances Laboratory, GERG=Geochemical and Environmental Research Group, TRI=Triangle Laboratories, PA=Pacific Analytical, HES=Hazleton Environmental Services , ECRC=Environmental Contaminant and Research Center.

[b] n=number of samples. Number across from year indicates the total number of samples collected, and the number across from the lab represents the number of total samples analyzed by the lab. Prior to 1993, each lab analyzed separate egg samples for a group of contaminants. Starting in 1993, multiple labs analyzed different aliquots of the same egg samples for different compound groups.

[c] Detection limit for organochlorine pesticides/total PCBs.

[d] Detection limit for organochlorine pesticides/total PCBs/toxaphene.

[e] Value listed = surrogate recovery. Insufficient sample material was available to perform matrix spikes on sample tissue.

[f] Recoveries for beta-BHC and HCB were below 50% and were reported as estimates in Table 2.

[g] Detection limits for TCDD and TCDF/all other dioxins and furans.

[h] Detection limits raised due to insufficient sample material.

[i] Detection limits for non-ortho PCBs/mono-ortho PCBs.

[j] Includes six 1994 eggs analyzed for dioxins, furans, and planar PCBs, and one 1994 egg analyzed for organochlorine pesticides and total PCBs.

[k] Matrix spike recoveries for HCB, heptachlor, o,p DDE, mirex, and trans-nonachlor ranged from 36-50% and were reported as estimates in Table 2.

Organochlorine pesticide and total PCB extraction and cleanup followed the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Method (MacLeod et al. 1985) or U.S. Environmental Protection Agency (1986b) method 3540, with minor revisions (Brooks et al. 1989, Wade et al. 1988). Following Soxhlet extraction under a solvent, samples were seperated by silica gel chromatography and/or florisil column chromatography to isolate the

aliphatic and polyaromatic hydrocarbon (PAH)/pesticide/PCB fractions. High performance liquid chromatography (HPLC) was used to remove interfering lipids in these fractions. Quantitation of analytes was performed by capillary gas chromatography (CGC) with electron capture detector (ECD) for pesticides and PCBs. A mass spectrometer in the selected ion monitoring (SIM) mode was used for confirmation (Wade et al. 1988). Sample preparation for mercury analysis included nitric-reflux digestion and determination by cold vapor atomic absorption (Hatch and Ott 1968).

Methods for extraction, cleanup, and fractionation for dioxins, furans, and planar PCBs followed contract specifications outlined by U.S. Environmental Agency (1990 and 1991 samples) or PACF (1993 to 1995 samples) and were similar at all laboratories conducting analyses. Analytical procedures followed U.S. Environmental Protection Agency (1990) method 1613 at GERG and Pacific Analytical, EPA method 8290 at Triangle Laboratories, and EPA method SW8280 at Radian. In general, egg extracts were cleaned-up by column chromatography on alumina, silica gel, and activated carbon on silica. Analytes were quantified using high-resolution gas chromatography (HRGC)/ high-resolution mass spectrometry (HRMS) in the SIM mode (U.S. Environmental Protection Agency 1990, Tondeur 1987). The ECRC prepared and analyzed egg samples for PCDDs, PCDFs, and planar PCBs according to Feltz et al. (1995), using two-stage reactive cleanup with sulfuric acid silica gel/potassium silicate column and a column of sulfuric acid silica gel/potassium silicate/silica gel. Extracts were purified with high performance gel phase chromatography. Analytes were separated on a C-18/PX-21 carbon column HPLC system. Extracts were analyzed by CGC/ECD to measure mono ortho-PCB concentrations (Schwartz and Stalling 1991). The PCDD/PCDF and non ortho-PCB fractions were determined by HRGC/HRMS and by monitoring selected ions during the chromatographic separation (Kuehl et al. 1991). Nomenclature for the planar PCB congeners discussed in this report follow International Union of Pure and Applied Chemists (IUPAC) numbers (Ballschmiter and Zell 1980).

H4IIE Rat Hepatoma Bioassay

Extracts from each egg sample collected from 1993 to 1995 was used in a H4IIE rat hepatoma cell bioassay to assess exposure to planar halogenated compounds, including planar PCBs, dioxins, and furans. The H4IIE bioassay measures the potency of egg tissue extracts compared to the potency of TCDD, the most toxic known dioxin congener. The results are expressed as TCDD-equivalents (TCDD-EQs), which reflects the overall dioxin-like potency found in the sample, inclusive of TCDD and all the other planar compounds in the samples (Tillitt et al. 1991). The H4IIE-extract bioassay is a screening tool; the relationship between the potency of the planar compounds in the bioassay and the overall potency of these compounds to cause embryo lethality is poorly understood. However, the potency of planar compound mixtures in the H4IIE cells has been correlated to the hatching success in double-crested cormorants from the Great Lakes (Tillitt et al. 1992).

The bioassay was conducted at the ECRC following the methods of Tillitt et al. (1991) as modified for 96-well microtitre plates (Tysklind et al. 1994). The H4IIE cells were seeded at 7,000 cells/well in 250 µL of D-MEM culture media. After a 24-hour incubation period, the cells were dosed with sample extracts or standards in a 5 µL volume of isooctane. The cells were

exposed to six different concentrations of the samples in a 25% dilution series. The samples were calibrated against TCDD for the determination of TCDD-EQ in the samples. TCDD standards were dosed at eight concentrations and, following a 72-hour incubation period, the plates were washed and the cells lysed. 20 µL of Tris-sucrose (0.05-0.2M) with dicumerol (80 µM) and 20 µL of 5 µM 7-ethoxyresorufin were added to each well. The reactions were initiated with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the microtitre plates were placed in a fluorometric plated reader where resorufin production was measured kinetically (530 nm excitation, 590 nm emission). The relative fluorescence intensity of the samples was then compared to a twelve point resorufin standard curve and converted to picomole (pmol) resorufin. A linear regression was performed to determine ethoxyresorufin-o-deethylase (EROD) activity rate (pmol/min). The doses of each sample or TCDD-standard were plotted against EROD activity to develop dose-response curves, which were in turn used to compare the relative potencies of the samples with that of the standard, TCDD. A slope ratio assay was used for the determination of TCDD-EQ (Finney 1980). Replication and subsequent performance checks were performed during the H4IIE bioassay procedure. Sample extracts were dosed at eight serial dilutions and each dose was replicated in quadruplicate. Eight-point TCDD standard curves with four replications at each dose were analyzed simultaneously with the samples. The limit of detection for the bioassay was <0.043 pg TCDD-EQ/g.

Quality Assurance/Quality Control

Due to financial and contractual restrictions, egg samples were shipped to many different contract laboratories for quantification of contaminant concentrations over the duration of the project. Sample sizes were insufficient to assess variance among the different laboratories that quantified chemical concentrations. Therefore, quality assurance/quality control (QA/QC) results from procedural blanks, duplicates, and matrix spike samples were evaluated for each individual laboratory. Accuracy and precision of analytical chemistry data were measured by matrix spike recovery and analysis of duplicate samples. Average spiked matrix recoveries considered acceptable were between 80 to120% for organochlorine pesticides, total PCBs, and mercury, and between 25 to 125% for dioxins, furans and planar PCBs. Recoveries were only considered valid if the spike to background ratio was above one. Duplicate results were considered valid if the average, relative percent difference between duplicates was 1) 200% for average analyte concentrations at zero to two times the detection limit; 2) 17.3% for concentrations at two to 10 times the detection limit; or 3) 8.6% for concentrations >10 times the detection limit.

Matrix spikes and duplicate results for most analytes at each laboratory were within the specified limits for this study, with few exceptions. In general, analyte concentrations in egg samples with corresponding matrix spike recoveries outside specified boundaries were below or near detection limits or reported as estimated results. For three 1990 egg samples analyzed at Radian, insufficient sample material was available for performing matrix spikes, and surrogate recoveries were poor for some dioxin and furans other than the tetra congeners. Therefore, only the TCDD and TCDF concentrations for these three samples were reported. In 1991 samples, four eggs exhibited unacceptable detection limits for TCDD and TCDF, so the samples were re-analyzed following an additional clean-up or re-extraction step. Results from two eggs receiving additional clean-up did not coincide with earlier results or the results from the other two eggs

receiving re-analysis, and were not reported in this study. In 1994 egg samples, insufficient sample material was available and detection limits were elevated for the organochlorine pesticide and total PCB analysis. Surrogate recovery for two samples was poor in the 1994 egg batch analyzed for organochlorine pesticides and total PCBs, and results for the two eggs were considered estimates and not reported. Concentrations of HCB, heptachlor, o,p -DDE , mirex, and transnonachlor in 1995 eggs, and HCB and beta-BHC in 1991 eggs, exhibited poor matrix spike recoveries ranging from 36 to 50%. Results for these compounds were reported as estimates (Table 2).

Replicate and positive control results from the H4IIE-extract bioassay were acceptable for all eggs collected in 1993, and for five eggs collected in 1994. However, the remaining six eggs collected in 1994 were analyzed alongside the 15 eggs collected in 1995, and all results were substantially lower than results from the earlier analyses of 1993 and 1994 eggs. Results of the six 1994 eggs (collected from both lower Columbia River islands) were noticeably different compared to the five eggs collected in the same year but analyzed earlier in a different batch. In addition, results from the positive control material analyzed alongside the 1994/1995 batch was unusually low. Because of the discrepancies in 1994 egg results between islands and the low positive control values, the results from the 1994/1995 egg samples were censored from the data set.

Data Analysis

Contaminant values for all eggs were adjusted for moisture and lipid loss using volume measurements and presented as fresh weight (Stickel et al. 1973). Volume was estimated by measuring water displacement on the whole egg for eggs collected from 1993 to 1995. Displacement volume was not determined on eggs collected in 1990 and 1991, so volume of these eggs was estimated based on length and breadth measurements (Stickel et al. 1973). Natural log transformation of nearly all concentration data improved linearity, so all individual chemical concentrations and toxic equivalent values were log transformed prior to statistical analysis. Chemical concentrations below detection limits were assigned a value of one-half the detection limit for determining geometric means, provided the majority of values were above detection limits.

Geometric means and ranges of contaminant concentrations in eggs collected during all years of the study were tabulated and compared to effect-level thresholds estimated for the species in laboratory or field studies conducted elsewhere. Mean differences in selected egg-contaminant concentrations between islands within each year were compared with a two-tailed t-test. One-way Analysis of Variance (ANOVA) was used to determine differences in DDT and p,p -DDE concentrations between Columbia River colonies and the reference colony for eggs collected in 1993. Differences in egg concentrations among the three islands in 1993 were identified using Bonferroni separation. Mean differences in contaminant concentrations among years were not compared due to differences in collection techniques and the contract laboratories used from year to year. Egg and shell measurements were compared between islands for all years of the study. Mean contaminant concentrations computed using more than one value below detection limits were excluded from statistical comparison. Egg samples exhibiting poor recoveries or other

QA/QC problems were also excluded from statistical tests. All statistical tests were performed at the 0.05 level of significance using the software program SYSTAT 8.0 (SPSS 1998).

The overall dioxin-like potency of planar chlorinated compounds in double-crested cormorant egg tissues was summarized as TCDD toxic equivalents (TEQs). TEQs were determined by normalizing concentrations of individual dioxin-like compounds (including planar PCBs), relative to the potency of TCDD, using toxic equivalency factors (TEFs) proposed by Safe (1990) and Ahlborg et al. (1992,1994) as modified by the World Health Organization (WHO-TEFs; van den Berg et al. 1998). The concentration of each planar chlorinated hydrocarbon in an egg sample was multiplied by its corresponding WHO-TEF value. The values obtained for each planar compound were then summed, which resulted in a single TEQ value of dioxin-like potency for each sample. The geometric mean of these sums were then used to represent the cormorants at each colony.

Various TEF values have been suggested for use in risk assessment to better represent exposure to multiple dioxin-like compounds in a range of animal groups (Safe 1990, Ahlborg et al. 1992, 1994, Bosveld et al. 1995). The WHO recommended three TEFs based on mammalian, avian, and fish toxicological endpoints (van den Berg et al. 1998), and Tillitt et al. (1991; 1993) determined TEFs based on responses from egg extracts used in the H4IIE bioassay. We calculated three TEQ values for the cormorant eggs and presented the results as A-TEQ for the avian-based TEFs, M-TEQ for the mammalian-based TEFs, and H4IIE-TEQ for the bioassay-derived TEFs. We choose these three models to better evaluate risks directly to avian receptors or mammalian egg predators, and to compare our data with other studies that only used the mammalian-based models to represent bird species.

TEFs have been determined for many dioxin-like compounds. However, authors of previous studies have not included the planar PCBs when determining TEQs. In order to compare our data to those from other authors, we determined two sets of A-TEQs, M-TEQs, and H4IIE-TEQs that included 1) PCDDs, PCDFs, and non-ortho PCBs (TEQ_{nPCBs}; and 2)PCDDs, PCDFs, non-ortho and mono-ortho PCBs (TEQ_{nmPCBs}).

RESULTS

Organochlorine Pesticide, Total PCBs, and Total Mercury Concentrations

Total PCBs and p,p -DDE were the most elevated contaminants in eggs from the Columbia River Islands during all years of the study (Table 2). Total PCBs were detected in all samples,

Table 2. Geometric mean and range (parenthesis) of organochlorine pesticides, total polychlorinated biphenyls (PCBs), and total mercury in double-crested cormorant eggs collected from East Sand and Rice Islands (Columbia River) and from a reference colony on Hunters Island (south Oregon Coast).

Year	1990	1991		1993			1994		1995	
Island	Rice	East Sand	Rice	East Sand	Rice	Hunters	East Sand	Rice	East Sand	Rice
n	7	3	3	5	5	5	5	4	8	6
Total PCBs, DDE, and Mercury (µg/g fresh weight)										
Total PCBs	3.35 (1.57-6.53)	1.66A[a] (1.26-2.25)	6.07B (4.34-10.8)	2.54A (0.90-8.14)	3.33A (1.36-8.81)	0.37[b] (0.16-1.13)	0.86A (0.56-1.33)	1.82B (1.16-2.25)	0.82A (0.36-2.38)	2.13B (0.71 -3.87)
p,p -DDE	0.50 (<0.01[c]-2.85)	1.88A (1.27-2.67)	5.31A (3.66-9.88)	2.58A (1.06-12.0)	3.02A (2.06-4.23)	0.45B (0.20-1.51)	1.57A (0.72-3.42)	2.49A (1.06-4.82)	0.83A (0.26-2.29)	3.41B (1.36-9.88)
Total mercury	0.40[d] (0.29-0.63)	1.95A (1.25-3.19)	1.58A (1.38-1.95)							
Other Organochlorine Pesticides (ng/g fresh weight)										
p,p -DDT	NC[e] <10[f]	NC (8.64- <10)	11.2 (9.08-17.0)	12.5A (3.85-22.5)	6.57A (4.84-13.9)	1.12B (0.36-4.09)	NC (4.8 - <17)	NC (<17-18)	0.94A (<0.06 -11.6)	9.05B (4.72-29.7)
p,p -DDD	NC (9.20 - <10)	NC <10	11.2 (9.08-17.0)	12.1A (4.67-51.7)	12.9A (5.40-31.3)	NC (<0.48-0.76)	NC <17	NC <17	0.88A (<0.06-13.0)	2.76A (<0.06-36.6)
o,p -DDT	NC[g] <10	NC <10	8.91 (8.52-9.14)	15.8 (6.41-42.0)	22.9 (12.4-57.3)		NC <17	NC <17	<0.06[h]	<0.06[h]
o,p -DDE	NC[g] <10	NC <10	NC <10	NC (<1.99-4.74)	NC (<1.87-2.46)		NC <17	NC <17	<0.06	NC (< 0.14-6.10)
o,p -DDD	NC[g] <10	NC <10	NC <10	4.04 (<1.71-11.9)	3.54 (1.99-8.60)		NC <17	NC <17	<0.06	<0.06
dieldrin	15.8 (<20-27.6)	25.2 (17.3-52.6)	44.7 (18.3-179)	45.8A (9.85-147)	31.2A (13.7-60.5)		NC (11- <17)	NC (<17-19)	9.41A (2.74-44.1)	28.1A (12.6-124)
endrin	NC (<50-55.7)	NC <10	NC (<10-11.9)	15.0A (5.09-48.6)	13.3A (<1.88-41.5)		NC <17	NC <17	NC (< 0.05-0.16)	NC (< 0.05-3.73)
gamma-chlordane	NC[g] <10	NC <10	NC <10	3.48 (<1.86-15.4)	2.05 (<1.88-4.61)		NC <17	NC <17	NA	NA
trans-chlordane									NC (<0.14- 0.33)	NC (<0.06-0.56)
cis-chlordane /octa-chlordane									1.06A (0.47-4.85)	4.44B (1.19-18.3)

Table 2. Continued.

Year	1990	1991		1993			1994		1995	
Island n	Rice 7	East Sand 3	Rice 3	East Sand 5	Rice 5	Hunters 5	East Sand 5	Rice 4	East Sand 8	Rice 6
alpha-BHC	NC <10	NC <10	NC <10	1.96 (<2.00-3.11)	2.14 (<1.88-4.27)		NC (1.6 - <17)	NC <17	1.38A (0.82-2.11)	0.83B (0.62-1.23)
beta-BHC	NC (9.20- <10)	NC[h] (9.08-<10)	NC[h] <10	2.54 (<2.00-4.91)	4.04 (2.36-11.6)		NC (4.0 - <17)	NC <17	5.18A (1.51-13.1)	3.68A (2.08-7.33)
gamma-BHC (lindane)	NC <10	NC <10	NC <10	NC (<2.00-2.76)	NC (<1.74 - 2.76)		NC (0.37 - <17)	NC <17	0.32A (<0.06-1.25)	0.50A (0.29-0.91)
delta-BHC	NC <10	NC <5.0	NC <5.0	NC <2.00	NC <1.90				NC < 0.06	NC < 0.06
Hexachloro- benzene	14.1[h] (9.20-17.9)	6.94[h] (<9.08-8.77)	12.9[h] (9.08-25.6)	13.7 (7.03-42.0)	21.3 (11.4-50.5)		13.2 (<17-19.5)	25.6 (<17-47.3)	4.24[h] (2.24-7.54)	9.00[h] (5.32-20.4)
heptachlor epoxide	NC (<10-35.7)	6.94 (8.77 - <10)	8.91 (<10-17.0)	6.26 (<1.83-25.3)	NC (<1.90-13.0)		NC (6.7 - <17)	NC <17	5.30A (1.77-32.5)	8.82A (3.63-16.3)
heptachlor	NC <10	NC <5.0	NC <5.0	NC <2.00	NC <1.90				NC[g] < 0.13	NC[g] <0.13
mirex	NC[g] <10	NC <10	NC (8.52 -<10)	3.32 (1.59-7.26)	6.15 (3.43-13.0)		NC (2.0 - <17)	NC <17	0.75[h] A (0.40-1.64)	3.16[h] B (1.33-27.3)
toxaphene	NC <10	NC <5.0	NC <5.0				NC <86	NC <86		
trans-nonachlor	NC[g] (8.93 -<10)	NC <10	NC <10	1.57 (<2.00-2.92)	1.81 (<1.88-4.48)		NC (0.42 - <17)	NC <17	0.19[h] A (<0.14-2.10)	0.28[h] A (<0.14-0.91)
cis-nonachlor	NC[f] <10	8.74 (<10-17.5)	12.9 (<10-51.1)	17.0 (3.38-8.28)	9.85 (3.81-23.4)				3.42A (0.81-20.3)	6.51A (2.50-46.0)
Total PBrDE[i]									96.4A (32.2-161)	201B (112-580)

[a] Means with different capital letters between islands and within the reported year were significantly different ($P<0.05$).
[b] Calculated as the sum of individual PCB congeners. Concentrations not statistically compared to other islands due to different analytical techniques.
[c] The < sign denotes the concentration of the analyte in one or more samples was below the highest detection limit (indicated following the sign) reported for the group of samples.
[d] Only four egg samples were analyzed.
[e] NC=Not calculated. Geometric means were calculated only when chemicals were above detection limits in the majority of samples.
[f] A single value denotes that all samples were below the highest detection limit (reported following the < sign) for that group of samples.
[g] Only three egg samples were analyzed.
[h] Matrix spike recovery was poor for this analyte. Values reported are considered estimates.
[i] Polybrominated diphenyl ethers.

and p,p -DDE was detected in all samples except one egg collected in 1990. The maximum concentration of total PCBs (10.8 µg/g) was found in an egg from Rice Island in 1991, and the maximum concentrations for p,p -DDE (9.88 µg/g) were found in two eggs from Rice Island (one in 1991 and another in 1995; Table 2). Concentrations of p,p -DDT, p,p -DDD, dieldrin, HCB, heptachlor epoxide, and *cis*-nonachlor were consistently above detection limits during most years except 1994, when detection limits were elevated due to insufficient sample material (Table 2). Concentrations of o,p -DDE, delta-BHC, and toxaphene were rarely above detection limits in any sample. Total mercury was elevated in eggs collected in 1990 and 1991, but was not analyzed in other years. All compounds were less than one µg/g except for p,p -DDE, total PCBs, and total mercury (Table 2).

At the Hunters Island reference colony, all mean DDT metabolites and total-summed PCB (measured by summation of PCB congeners) concentrations were below one µg/g (Table 2). In 1993 samples, p,p -DDE concentrations were lower at the reference colony than at the East Sand Island (P=0.009) or Rice Island (P=0.005) colonies (Table 2). Likewise, p,p -DDT concentrations were lower at the reference colony than at the East Sand (P=0.001) or Rice (P=0.007) colonies (Table 2). The sum of individual PCB congeners from the reference colony was lower than the total PCBs detected in eggs from the Columbia River colonies, but values were not directly comparable due to the differences in analytical techniques for obtaining a total PCB value. Most PCB congeners evaluated in the Hunters Island samples were below detection.

Eggs from Rice Island exhibited higher total PCB concentrations than in eggs from East Sand Island in 1995 (P=0.026), 1994 (P=0.018), and 1991 (P=0.018; Table 2), but concentrations in 1993 samples were not different (P=0.610) between islands. Higher concentrations of p,p -DDE were observed in Rice Island eggs compared to East Sand Island eggs in 1995 (P=0.005) but not in 1994 (P=0.336), 1993 (P=0.999), or 1991 (P=0.052; Table 2). Other organochlorine pesticides with concentrations higher at Rice Island compared to East Sand Island in 1995 were p,p -DDT (P=0.042), cis-chlordane/octa-chlordane (P=0.010), alpha-BHC (P=0.016), HCB (P=0.006), and mirex (P=0.008). No other differences between islands were detected for any other organochlorine pesticides with concentrations allowing statistical comparisons (Table 2). Mean concentrations appeared to decline for most organochlorine pesticides from 1990 to 1995 (Table 2), but statistical comparisons were precluded due to differences in collection techniques and variation in detection limits among years.

Total mercury concentrations were not different (P=0.507) between Columbia River Islands in 1991 (Table 2). Mean total mercury concentrations in four eggs sampled in 1990 on Rice Island were nearly five times lower than concentrations found in 1991 eggs (Table 2).

Egg Measurements

Egg length was not different (P=0.910) in eggs between the two lower Columbia River islands for combined samples across years, although egg breadth and egg mass were greater (P=0.013 and 0.001, respectively) in samples from Rice Island (Table 3). Eggshells from both lower Columbia River islands exhibited mean thickness below the pre-1947 mean reported by Henny et al. (1982) and were below averages from Hunters Island eggshells reported by Kiff (1994) (Table 3). Eggshells were up to 14.5% and 31.3% thinner in eggs from East Sand and Rice Island,

Table 3. Arithmetic mean, standard deviation, and ranges (parentheses) for egg mass (g) and shell measurements (mm)of double-crested cormorant eggs from Rice and East Sand Islands in the lower Columbia River (collected in 1990, 1991, and from 1993 to 1995), and from Hunters Island (reference colony) located off the southern Oregon coast. Mean values for the Hunters Island colony were reported by Kiff (1994) from eggs collected in 1992.

Island	n	Whole egg	Length	Breadth	Shell thickness	%Diff[a]
East Sand	31	46.8A[b] ± 5.6 (23.1 - 57.0)	61.6A ± 4.2 (46.1 - 66.5)	38.3A ± 2.0 (30.8 - 40.6)	0.428A ± 0.040 (0.370 - 0.530)	-0.92 (-14.5 - +23.5)
Rice	37	51.0B ± 4.3 (39.9 - 60.0)	61.7A ± 2.3 (56.7 - 66.8)	39.3B ± 1.5 (34.7 - 43.4)	0.416A ± 0.042 (0.297 - 0.479)	-3.7 (-31.3 - +10.8)
Hunters (Kiff 1994)	15	NR[c]	NR	NR	0.433 ± 0.033 (NR)	+0.23 (NR)

[a] Percent difference from eggshell thickness (0.432 mm) measured on eggs collected in the Northwest prior to 1947 (Henny et al. 1982).
[b] Means different ($P<0.05$) from one another between islands (within a column) do not share a common letter.
[c] Not reported in referenced literature.

respectively, compared to pre-1947 eggs. However, eggshell thickness was not different between the two lower Columbia River islands (P=0.230; Table 3). Shell thickness and p,p -DDE concentrations in individual eggs were correlated (r= -0.47, P=0.001, n=46; Figure 2).

Dioxins, Furans, and Planar PCBs

Numerous dioxin congeners accumulated in eggs of lower Columbia River cormorants, and concentrations well above detection limits were noted for TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PnCDD), 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD), and octachlorodibenzo-*p*-dioxin (OCDD; Table 4). In contrast, only one furan, 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) was well above detection limits (Table 4). Furans near detection limits in the majority of 1994 and 1995 samples included TCDF, 1,2,3,4,7,8-hexachlorodibenzofuran, 1,2,3,6,7,8-hexachlorodibenzofuran, and 2,3,4,6,7,8-hexachlorodibenzofuran (Table 4). Concentrations of dioxins and furans were not detected in 1993 egg samples from the reference colony, although the detection limits for the non-tetra congeners were higher in 1993 than in other years (Table 4). Concentrations of TCDD, PnCDD, HxCDD, and OCDD in eggs from lower Columbia River samples were elevated well above the detection limits from the 1993 reference samples (Table 4). Maximum concentrations of TCDD, the most toxic dioxin congener, were found in eggs from both Rice (51 pg/g) and East Sand Islands (53 pg/g) in 1993.

Some dioxins and furans exhibited significantly higher concentrations at Rice Island compared to East Sand Island (Table 4). Rice Island eggs exhibited higher concentrations of TCDD (P=0.002), OCDD (P<0.001), and TCDF (P=0.015) in 1995, and PnCDD (P=0.047) and OCDD (P=0.016) in 1994 (Table 4). No other relationships between concentrations in East Sand and Rice Island eggs were significant, even though nearly all mean dioxin and furan values appeared higher in Rice Island eggs (Table 4). Dioxin and furan concentrations appeared to decline or be relatively stable from 1990 to 1995, although the relationships over time were not compared statistically due to differences in collection techniques and detection limits among years (Table 4).

Non-ortho-chlorinated PCBs accumulated in all eggs from the lower Columbia River islands in 1994 and 1995 (Table 5). The concentration pattern was the same for both islands in 1994 and at Rice Island in 1995, with mean concentrations of PCB 126>> 81>77>169. This pattern was similar in eggs from East Sand Island in 1995, except PCB 81 was higher than PCB 77. All non-ortho-chlorinated PCB congeners appeared higher in Rice Island eggs than in eggs from East Sand Island, but only PCB 81 was significantly higher (P=0.016; Table 5). PCB 126 concentrations were well above other non-ortho PCB congeners in eggs from both islands, and the two maximum concentrations of this congener were found in an egg from East Sand Island (1,790 pg/g) and an egg from Rice Island (1,770 pg/g).

The mono-ortho chlorinated PCB congeners were only determined in five 1994 eggs (two from East Sand and three from Rice Island); only eggs from Rice Island contained detectable concentrations of all mono-ortho PCBs (Table 6). The mono-ortho PCB congeners at both

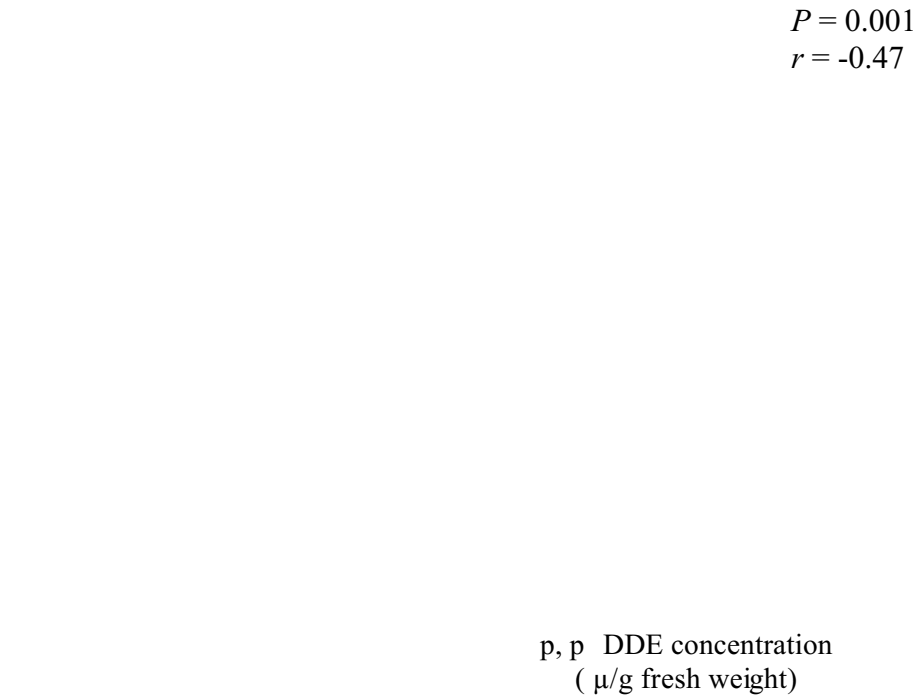

Figure 2. Relationship between eggshell thickness and DDE concentration in double-crested cormorants from the lower Columbia River, 1990 to 1995.

Table 4. Geometric mean and ranges (parenthesis) of dioxins and furans in double-crested cormorant eggs collected from East Sand and Rice Islands (Columbia River) and Hunters Island (south Oregon Coast).

Year	1990	1991		1993			1994		1995	
Island	Rice	East Sand	Rice	East Sand	Rice	Hunters	East Sand	Rice	East Sand	Rice
n	3	4	4	5	5	5	6	5	8	6
Chlorinated dibenzodioxins:										
2,3,7,8-Tetra	34 (28 - 44)	8.2A[a] (3.1-24)	12A (10-15)	13A (4.4-53)	21A (8.0 - 51)	NC[b] <2.0[c]	7.6A (2.8-16)	13A (6.6 - 25)	5.5A (4.3-7.9)	12B (5.3 - 23)
1,2,3,7,8-Penta				13A (<9.2 - 29)	15A (9.4 - 28)	NC <10	6.4A (2.8 - 13)	11B (8.9 - 13)	9.4A (5.5-18)	13A (6.6 - 19)
1,2,4,7,8-Penta							NC <0.2	NC <0.2	NC <0.1	NC <0.1
1,2,3,4,7,8-Hexa				NC <24	NC <10	NC <10	NC (<0.9 - 0.9)	0.9 (<1.3 - 2.2)	1.0A (<0.95 - 2.8)	1.3A (<0.4 - 3.7)
1,2,3,6,7,8-Hexa				15A (<9.2 - 27)	11A (<8.8 - 25)	NC <10	5.6A (2 - 10)	10A (7.7 - 12)	12A (6.6 - 27)	13A (5.2 - 32)
1,2,3,7,8,9-Hexa				NC <24	NC <10	NC <10	0.8A (0.1 - 2.0)	1.0A (<1.6 - 2.6)	1.9A (<1.7 - 4.2)	1.7A (<3.9 - 3.6)
1,2,3,4,6,7,8-Hepta				NC <24	NC <10	NC <10	1.4A (0.3 - 6.3)	3.6A (2.0 - 4.3)	3.3A (1.3 - 9.7)	4.4A (2.1 - 9.2)
Octa				NC <48 - 23	NC <20	NC <20	3.4A (1.8 - 9.4)	14B (3.4 - 36)	6.8A (2.8 - 39)	39B (31 - 57)
Chlorinated dibenzofurans:										
2,3,7,8-Tetra	NC (<3.8 -4.0)	0.47 (0.16-1.8)	0.65 (0.36-1.1)	NC <4.8	NC <2.0	NC <2.0	0.3A (0.04 - 2.0)	0.2A (0.2 - 0.3)	0.2A (<0.26 -0.8)	0.6B (<0.9 - 1.2)
1,2,3,7,8-Penta				NC <24	NC <10	NC <10	NC <0.3	NC (<0.2 - 0.2)	NC (<0.2-0.3)	NC (<0.4 - 0.3)
2,3,4,7,8-Penta				NC <24 - 20	NC (<10 - 15)	NC <10	3.0A (0.9 - 4.7)	5.7A (3.6 - 7.7)	4.2A (2.3-13)	6.5A (3.6 - 11)
1,2,3,4,7,8-Hexa				NC <24	NC <10	NC <10	0.5A (0.1- 2.2)	1.0A (<1.2 - 2.3)	0.7A (<0.6-3.1)	1.3A (<0.6 - 3.4)
1,2,3,6,7,8-Hexa				NC <24	NC <10	NC <10	0.4A (0.1 - 0.7)	0.7A (<0.68 - 1.0)	0.7A (<0.5 - 2.4)	1.1A (0.4 - 1.9)

Table 4. (Continued.)

Year	1990	1991		1993			1994		1995	
Island	Rice	East Sand	Rice	East Sand	Rice	Hunters	East Sand	Rice	East Sand	Rice
n	3	4	4	5	5	5	6	5	8	6
Chlorinated dibenzofurans:										
1,2,3,7,8,9-Hexa				NC	NC	NC	NC	<0.2	NC	NC
				<24	<10	<10	(<0.2 - 0.2)		(<0.1-0.2)	(<1.2 - 0.4)
2,3,4,6,7,8-Hexa				NC	NC	NC	0.3A	0.3A	0.6	NC
				<24	<10	<10	(0.13 - 0.5)	(<0.5 - 0.5)	(<1.1 - 1.8)	(<0.6 - 1.3)
1,2,3,4,6,7,8-Hepta				NC	NC	NC	NC	0.3	NC	<0.6
				<24	<10	<10	(<0.4 - 0.2)	(<0.4 - 0.5)	(<0.9 - 0.8)	
1,2,3,4,7,8,9-Hepta				NC	NC	NC	NC	NC	NC	NC
				<24	<10	<10	<0.2	<0.2	(<0.2-0.4)	(<0.4 - 0.4)
Octa				<48	<20	<20	NC	<0.7	NC	0.7
							(<0.7 - 0.3)		(<0.5-0.8)	(<0.8 - 1.4)

[a] Means with different capital letters between islands and within the reported year were significantly different ($P<0.05$).

[b] NC=Not calculated. Geometric means were calculated only if chemicals were above detection limits in the majority of samples.

[c] The < sign denotes the concentration of the analyte in one or more samples was below the highest detection limit (indicated following the sign) reported for the group of samples. A single value denotes that all samples were below the highest detection limit (reported following the < sign).

Table 5. Geometric mean (pg/g fresh weight) and ranges (parenthesis) of non-ortho-polychlorinated biphenyls (PCBs) in double-crested cormorant eggs collected from East Sand and Rice Islands in the lower Columbia River in 1994 and 1995.

Year	1994			1995		
Island n	East Sand 6	Rice 5	Probability value[a]	East Sand 8	Rice 6	Probability value
Chlorinated biphenyls:						
3,4,4 ,5-Tetra (81)[b]	87.5 (10.0-316)	127 (86.3 -331)	*P*=0.312	65.4 (6.6-191)	181 (75.3-436)	*P*=0.016
3,3 ,4,4 -Tetra (77)	61.3 (36.3-190)	79.4 (61.5-120)	*P*=0.205	72.3 (36.5-169)	125 (75.8-206)	*P*=0.081
3,3 ,4,4 ,5-Penta (126)	460 (105-950)	759 (490-1120)	*P*=0.245	493 (196-1790)	956 (545-1770)	*P*=0.096
3,3 ,4,4 ,5,5 -Hexa (169)	26.5 (6.51-51.7)	51.5 (38.8-74.3)	*P*=0.112	40.2 (19.7-120)	66.9 (33.8-106)	*P*=0.130

[a] Means between islands and within the reported year are significantly different if $P < 0.05$.
[b] International Union of Pure and Applied Chemists (IUPAC) number (Ballschmiter and Zell 1980).

islands followed the same concentration pattern, with mean concentrations of PCB 118>105>156>167>157>114>123>189. PCB 118 was the most elevated mono-ortho PCB congener detected, with concentrations up to 278,000 pg/g in a Rice Island egg. Mean values of mono-ortho PCB congeners appeared much higher in Rice Island eggs than East Sand Island eggs, although small sample size precluded statistical comparisons.

Toxic Equivalents

Mean TEQ concentrations appeared higher at Rice Island than East Sand Island in 1994 and 1995 egg samples, although none were significantly higher (Table 7). In 1995, sample sizes were somewhat greater than in 1994, and all probability values for between island comparisons were below 0.10 (Table 7). These low probability values were suggestive of statistical differences, and sample sizes may have been insufficient to explain the variability of values and the inability to detect differences for most TEQ values between the two islands.

The contributions of the PCDD and PCDF congeners toward the three mean TEQ values were nearly equal within each island and year (Figure 3). In contrast, the avian $TEQs_{nPCB}$ were higher than the mammalian- or H4IIE-based $TEQs_{nPCB}$ at each site (Figure 3). The higher avian-based TEQs reflects the greater sensitivity (higher TEF values) of birds than mammals to some dioxins, furans, and non-ortho-PCB congeners. In 1994 samples, the avian TEQs including both the non-ortho and mono-ortho PCBs ($TEQs_{nmPCB}$) contributions were nearly equal to the

Table 6. Geometric mean (pg/g fresh weight) and ranges (parenthesis) of mono-ortho-chlorinated polychlorinated biphenyls (PCBs) in double-crested cormorant eggs collected from East Sand and Rice Islands in the Columbia River in 1994.

Year	1994	
Island	East Sand	Rice
n	2	3
Mono-ortho-chlorinated PCBs:		
'2,3,3',4,4'-Penta (105)[a]	23,700 (6,330 - 88,600)	59,800 (41,200 - 78,500)
'2,3,4,4',5-Penta (114)	1,950 (530 - 7,160)	5,050 (3,810 - 6,980)
'2,3',4,4',5-Penta (118)	84,300 (25,800 - 276,000)	210,000 (154,000 - 278,000)
2',3,4,4',5-Penta (123)	NC[b] (<500 - 2,920)	2,850 (1,740 - 3,970)
'2,3,3',4,4',5-Hexa (156)	7,960 (2,520 - 25,200)	19,300 (14,700 - 27,500
'2,3,3',4,4',5-Hexa (157)	2,880 (918 - 9,000)	6,480 (4,660 - 9,560)
2,3',4,4',5,5'-Hexa (167)	6,117 (2,200 - 17,100)	14,500 (11,600 - 20,100)
'2,3,3',4,4',5,5'-Hepta (189)	NC (<500[c] - 2,330)	1,970 (1,540 - 2,700)

[a] International Union of Pure and Applied Chemists (IUPAC) number (Ballschmiter and Zell 1980).
[b] NC=not calculated due to insufficient sample size.
[c] Concentrations were below the detection limit reported following the < sign..

mammalian-based $TEQs_{nmPCB}$, whereas the avian- and mammalian-based $TEQs_{nmPCB}$ were at least 2.5-fold higher than corresponding H4IIE-TEQs (Figure 3).

The contribution patterns of five individual chlorinated groups to the three TEQ models were similar between islands in 1994 and 1995, although the mono-ortho PCBs were not analyzed in 1995 samples and could not be compared (Figure 3). The non-ortho PCBs contributed the majority (between 54 and 79 %) toward the overall avian- and mammalian-based TEQs, but contributed less than 44 % to the H4IIE-TEQs (Figure 3). In 1994 samples, the mono-ortho PCBs contributed a greater amount (up to 26% at Rice Island) to the total M-TEQs, and only a small amount (<8.0%) to the H4IIE- and avian-based TEQs. The H4IIE-TEQs were largely dominated by the dioxin and furan contributions.

The contribution of individual compounds to the overall dioxin-like toxicity exhibited a similar pattern in cormorant eggs from lower Columbia River both islands. The overall toxicity

Table 7. Geometric mean (pg/g fresh weight) and ranges (parenthesis) of Toxic Equivalents (TEQs) from avian- (A-TEQ), mammalian- (M-TEQ), and H4IIE bioassay-derived (H4IIE TEQs) endpoints in double-crested cormorant eggs collected from two islands along the lower Columbia River in 1994 and 1995. TEQs were calculated using Toxic Equivalent Factors (TEFs) reported for polychlorinated dioxins and furans, and included the non-ortho-chlorinated polychlorinated biphenyls (TEQ_{nPCBs}) or both non-ortho- and mono-ortho chlorinated polychlorinated biphenyls (TEQ_{nmPCBs}; van den Berg et al. 1998, Tillitt et al 1991; 1993).

Year	1994			1995		
Island n	East Sand 6	Rice 5	Probability value[a]	East Sand 8	Rice 6	Probability value
A-TEQ_{nPCBs}	77 (21-151)	134 (84-212)	*P*=0.151	80 (39-241)	153 (95-280)	*P*=0.066
A-TEQ_{nmPCBs}	81[b] (22-167)	145[c] (93-224)		NA	NA	
M-TEQ_{nPCBs}	62 (17-127)	113 (71-164)	*P*=0.123	69 (32-216)	126 (78-225)	*P*=0.087
M-TEQ_{nmPCBs}	79[b] (22-184)	156[c] (103-208)		NA[d]	NA	
H4IIE TEQ_{nPCBs}	26 (9.3-48)	42 (28-67)	*P*=0.140	27 (15-75)	49 (29-86)	*P*=0.062
H4IIE TEQ_{nmPCBs}	27[b] (10-51)	43[c] (31-65)		NA	NA	

[a] Means between islands and within the reported year were significantly different if $P < 0.05$. Means without probability values were not statistically compared.
[b] Only two egg samples analyzed.
[c] Only three egg samples analyzed.
[d] NA=Not analyzed. The mono-ortho PCBs were not analyzed in 1995 samples.

represented by the avian-based $TEQs_{nPCB}$ was dominated by PCB 126 (55 to 63%), followed by 1,2,3,7,8-PnCDD (8 to 12%), TCDD (7 to 12%), 2,3,4,7,8-PnCDF (4 to 5%), and the non-ortho PCB 77 (3 to 5%). In the 1994 eggs, the totaled mono-ortho PCBs contributed less than 6% total to the overall toxicity.

The toxicity of the mammalian-based TEQs was primarily due to the same dioxins, furans, and planar PCBs as the avian-based TEQs. The M-$TEQs_{nPCB}$ were dominated by PCB 126 (72 to 76%), followed by 1,2,3,7,8-PnCDD (10-14%), TCDD (8 to 12%), and 2,3,4,7,8-PnCDF (2 to 3%). In 1994 eggs, PCB 126 contributed the greatest (54 to 56%) to the overall M-$TEQs_{nmPCB}$, followed by PCB 118 (11 to14%), TCDD (9 to 10%), 1,2,3,7,8-PnCDD (8 to 10%), PCB 156 (5 to 6%), PCB 105 (3 to 4%), and 2,3,4,7,8-PnCDF (2 to 3%).

The H4IIE-$TEQs_{nPCB}$ were most influenced by PCB 126 (37 to 45%), 1,2,3,7,8-PnCDD (17 to 31), TCDD (20 to 32%), and 2,3,4,7,8-PnCDF (15 to 22%). All other planar PCBs except 126

Figure 3. Geometric means of toxic equivalents (TEQs) from mammalian-(M-TEQ), avian-(A-TEQ), and H4IIE bioassay (H4IIE-TEQs)-based additive models in double-crested cormorant eggs collected from East Sand and Rice Islands along the lower Columbia River, 1994-95. TEQs were determined using toxic equivalent factors (TEFs) reported for polychlorinated dioxins and furans (PCDDs and PCDFs), non-ortho chlorinated PCBs (non-o-PCBs), and mono-ortho-chlorinated PCBs (mono-o-PCBs; van den Berg et al. 1998, Tillitt et al. 1991).

contributed less than five percent to the overall TEQ. In the 1994 eggs, mono-ortho PCBs contributed less than two percent to the overall H4IIE-$TEQs_{nmPCB}$.

Mean TCDD-EQs in cormorant egg tissue derived from the H4IIE-extract bioassay were not different (P=0.155) between Rice and East Sand Islands in 1993 samples, even though TCDD-EQs values appeared higher at Rice Island in both 1993 and 1994 (Table 8). Insufficient samples were available to compare differences between islands in 1994 or between 1993 and 1994. In 1993 samples, mean TCDD-EQs were as high as the TEQ_{nmPCB} values determined in eggs from 1994 and 1995, but mean TCDD-EQs in 1994 were much lower and more closely represented the TEQ values determined without including the planar PCBs.

Table 8. Geometric mean and ranges (parenthesis) of TCDD-Equivalents (TCDD-EQs) derived from the H4IIE bioassay conducted on extracts from double-crested cormorant eggs collected from two islands along the lower Columbia River in 1993 and 1994. Values derived from cormorant egg extracts are represented as pg TCDD-Equivalents per gram sample material (Tillitt et al. 1991).

Year	1993			1994	
Island	East Sand	Rice	Probability	East Sand	Rice
n	5	5	value	2	3
TCDD-EQs	73A[a] (16-179)	168A (66-305)	P=0.155	19 (12-31)	55 (38-115)

[a]Means with different capital letters between islands and within the reported year were significantly different (P<0.05). Means without capital letters were not statistically compared.

DISCUSSION

Organochlorine Pesticides, Total PCBs, and Mercury

Concentrations of total PCBs and p,p -DDE in lower Columbia River double-crested cormorant eggs exceeded reference samples from Hunters Island in 1993, and mean concentrations in Rice Island eggs during earlier years of the study approached estimated effect-threshold levels. Mean total PCBs in Columbia River eggs in 1990, 1991, and 1993 from Rice Island were near or above a low-observable adverse effect level (LOAEL) of 3.5 µg/g (based on egg lethality) estimated for double-crested cormorants in Lake Superior (Tillitt et al. 1992, Yamashita et al. 1993). Total PCBs in Rice Island eggs after 1993 were below this LOAEL. Eggs collected from Rice Island in all years except 1994 had p,p -DDE concentrations within the range (3 to 5 µg/g) of double-crested cormorants exhibiting 7 to 14% eggshell thinning from Lake Michigan in the late 1970's (Heinz et al. 1985), and were within concentrations implicated in reducing hatching success for cormorants in Green Bay Wisconsin (Custer et. al. 1999). Mean p,p -DDE concentrations in eggs from both Columbia River eggs were generally below values of 4.8 to 9.6 µg/g linked to population decreases (Price and Wesoloh 1986, Henny et al. 1989), although eggs collected from Rice Island in 1991 were within this range. All p,p -DDE concentrations in Columbia River eggs were below the concentration of 10 µg/g p,p -DDE considered by Pearce et al. (1979) to cause 20% eggshell thinning and complete reproductive failure in cormorants. All other

organochlorine pesticides were less than one µg/g and generally considered below levels associated with reproductive impacts in other fish-eating or predatory bird species (as reviewed in Wiemeyer et al. 1993 and Wiemeyer 1996).

Concentrations of p,p -DDE and total PCBs in 1990 to 1993 cormorant eggs were generally two to three times higher than means from great blue heron (*Ardea herodia*) colonies sampled in the lower Columbia River in 1994 to 1995, although samples from East Sand Island in 1994 and 1995 more closely resembled the heron egg values (Thomas and Anthony 1999). DDE concentrations in eggs of Caspian and Forster s (*Sterna forsteri*) terns nesting upriver from the cormorants at Crescent Island, Washington in the early 1990s were slightly lower or within the range of concentrations from the lower Columbia River cormorant colonies (Blus et al. 1998). However, total PCBs in the lower Columbia River cormorants were much higher than in terns from Crescent Island (Blus et al. 1998). Mean total PCB and DDE in eggs from Rice Island in 1991 were similar to concentrations found in eggs of bald eagles nesting in the same part of the estuary in 1994 to 1995 (U.S. Fish and Wildlife Service 1999). In contrast, cormorant egg concentrations from all other years at both islands were below the bald eagle values. Other detectable organochlorine pesticides in cormorant eggs were generally lower than concentrations in Columbia River bald eagle eggs, and within the range of concentrations in the great blue heron eggs (Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999).

The significant inverse correlation between p,p -DDE concentrations and shell thickness measurements indicates that p,p -DDE continues to have a small but measurable impact on Columbia River cormorants. An inverse correlation was also found between p,p -DDE and shell thickness for great blue herons along the Colombia River, but not for bald eagles (Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999). The mean percent difference (compared to pre-1947 eggs) was greatest at the Rice Island colony, and eggshells were up to 31% thinner than pre-1947 eggs. However, mean shell thickness for both islands were well below values associated with population impacts for cormorants in the Great Lakes (Pearce et al. 1979, Heinz et al. 1985), and below values (15 to 20% over a period of years) associated with declining populations (Anderson and Hickey 1972). Some individual eggs at the Columbia River colonies could exhibit increased moisture loss or mortality due to eggshell thinning, but the impact would not be expected to occur on the population level. It is unknown why egg mass and breadth would be greater at Rice Island than East Sand, although differences in these parameters have been reported between sampled colonies and museum reference specimens (Gress et al. 1973).

Mean mercury values in 1991 eggs were not different between Columbia River Islands, but were nearly five fold greater than mean concentrations in the eggs collected in 1990. The 1991 concentrations are within values (0.79 to 2.0 µg/g fresh weight) associated with impaired reproduction in various bird species (as reviewed by Eisler 1987) but lower than values found in eggs of common terns (*Sterna hirundo*) or herring gulls (*Larus argentatus*) where productivity was unrelated to mercury (Vermeer et al. 1973, Connors et al. 1975). Mercury in eggs from lower Columbia River bald eagles and great blue herons were two to three times below the 1991 cormorant values (Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999), yet mercury (=0.95 µg/g, range=0.62-2.1) in five 1991 Crescent Island Caspian tern eggs more closely resembled the cormorant values (Blus et al. 1998). Mercury could be negatively

influencing reproductive success for Columbia River cormorants, but only a few samples were analyzed in 1991 and concentrations in eggs could have dropped since this time. No egg samples were analyzed for mercury in cormorants after 1991, and additional cormorant egg samples would be necessary to assess any potential impacts and determine if mercury concentrations have declined.

Dioxin-like Compounds and Toxic Equivalents (TEQs)

The mean concentrations of TCDD, considered the most toxic dioxin congener, in Colombia River cormorant eggs was considerably below the median 50% lethal dose (LD50) of 4,000 pg/g determined from cormorant egg injections (Powell et al. 1998). However, sub-lethal impacts from TCDD may occur at concentrations well below the LD50, and a variety of responses to TCDD in other avian species have been observed in both laboratory and field studies (Kubiak et al. 1989, Nosek et al. 1992, Powell et al. 1996; 1997, Henshel et al. 1997, Henshel 1998). The maximum concentrations (up to 53 pg/g) of TCDD in eggs from Rice Island in 1990 and 1993, and in one egg from East Sand Island in 1993, were the highest recorded for any fish-eating species evaluated along the river (Blus et al. 1998, Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999, U.S. Fish and Wildlife Service unpubl. data). These maximum concentrations were above values found in eggs of Foster s terns exhibiting reproductive problems in the Great Lakes (Kubiak et al. 1989). In addition, concentrations in some cormorant eggs from Rice Island during all years exceeded the lowest response concentration (19 pg TCDD/g egg) or the probit-determined concentrations (34 pg TCDD/g egg) at which 50% of the evaluated populations responded (ED50) for brain asymmetry (Henshel 1998). Currently, brain asymmetry appears to be the most sensitive endpoint for measuring effects of TCDD to double-crested cormorants. TCDD, along with contributions from other dioxin-like compounds, could be eliciting brain asymmetry problems for some developing embryos in lower Columbia River colonies.

Mean concentrations of dioxin-like compounds (expressed as TEQs) in eggs of Columbia River cormorants approached or exceeded some no- or low-effect threshold values derived for cormorants or other avian species. Cormorant avian- and mammalian-based TEQ concentrations in eggs from both islands were higher than estimated egg values (>20 - 50 pg/g) associated with limiting reproduction in wood ducks (*Aix sponsa*), which appears to be one of the most sensitive species (White and Seginak 1994). Lower Columbia River cormorants also exceeded mean TEQs (41 pg/g) in eggs associated with lower reproductive rate in great cormorants (*Phalacrocorax carbo;* Peterson et al. 1993). Mean A-TEQs in eggs from Rice Island exceeded a suggested NOAEL for osprey embryo survival of at least 136 pg TEQ_{nPCBs} (Woodford et al. 1998). Henshel (1998) reported the lowest concentration of TEQs (based on H4IIE TEFs) eliciting a response for brain asymmetry measurements was 53 pg/g, and mean H4IIE TEQs in Rice Island eggs approached this value.

Tillitt et al. (1992) reported a strong correlation between the results of the H4IIE-extract bioassay and hatching success of double-crested cormorants in the Great Lakes. This correlation was stronger than the relationship identified between total PCB concentrations in eggs and egg mortality. In comparison to the egg concentration-response observed in the Great Lakes, our study results indicated that the mean TCDD-EQs would elicit 18 to 24% mortality in 1993 eggs

and 14 to 17% mortality in 1995 eggs, with the highest percent mortalities at Rice Island. In 1993 eggs, mean TCDD-EQs were similar to or higher than mean A-TEQs and M-TEQs (including planar PCBs) for eggs in 1994 and 1995. The similar mean concentrations suggests that the dioxin-like chemicals acted in an additive manner to elicit toxicity in the eggs. However, the 1994 H4IIE-extract values from both Islands were much lower than the calculated TEQ values, indicating chemical interactions may have lowered toxicity. Analytical chemistry results in 1993 were not sufficient to calculate TEQs using an additive model, and QA/QC problems affected bioassay results for some 1994 samples and all 1995 samples. Therefore, comparisons between TEQ and TCDD-EQs values could not be made during these years, and additional samples would be necessary to identify interactions and to determine if dioxin-like toxicity has decreased in eggs since 1993.

In cormorants from both lower Columbia River Islands, PCB 126 was the most elevated non-ortho-PCB and contributed the most dioxin-like toxicity toward all TEQ models. However, concentrations were well below the LD50 value of 177,000 pg/g for PCB 126 based on cormorant egg injection studies (Powell et al. 1998), and below values (44,000 pg/g) impacting hatching success of common tern (*Sterna hirundo*) eggs injected with PCB 126 (Hoffman et al. 1998). PCBs 77, 126, and 169 at a median concentration of 5,500 pg/g were considered the primary contaminants associated with increased incubation period, reduced hatchability, lower body weight, increased liver to body weight ratio, and edema in Green Bay s Forster s terns (Kubiak et al. 1989). Concentrations of these planar PCBs in lower Columbia River cormorants were below the Forster s tern values. Based on these studies, it appears unlikely that PCB 126 by itself is exhibiting an effect on lower Columbia River cormorants. However, PCB 126 is the primary contributor to the total dioxin-like activity in individual cormorant eggs for TEQ values that exceed threshold-effect levels, such as for brain asymmetry (Henshel 1998).

In addition to directly affecting cormorants, the dioxin-like compounds in cormorant eggs and nestlings from the lower Columbia River could impact mammalian and avian predators. Mink (*Mustela vision*) populations have declined along the lower Columbia River, and organochlorine contaminants and elevated TEQ values have been documented in Columbia River mink and river otter (*Lutra canadensis*; Henny *et al.* 1981, Henny et al. 1996, Elliott et al. 1999). Cormorant egg concentrations of M-TEQs and H4IIE-extract TCDD-EQs exceeded estimated threshold doses and low-effect level concentrations causing reproductive problems in mink (Tillitt et al. 1996). Bald eagles may be exposed to greater concentrations of dioxin-like compounds by consuming cormorant nestlings versus other prey from the river. Bald eagles are currently experiencing low productivity and have elevated dioxin-like compounds in eggs, especially eagles nesting within 10 miles of Rice Island (U.S. Fish and Wildlife Service 1999).

Bioaccumulation Patterns and Sources of Dioxin-like Compounds

The overall dioxin-like toxicity in eggs from both Columbia River islands, based on contribution of individual congeners to the mean TEQ concentration, was primarily due to four compounds in the general order PCB 126>>1,2,3,7,8-PnCDD TCDD>2,3,4,7,8-PnCDF. However, PCB 77 was of equal importance as 2,3,4,7,8-PnCDF in the avian-based model, and the mono-ortho PCBs contributed more in the mammalian-based model than the avian-based model. Columbia River cormorants accumulated similar dioxin and furan congeners as great blue herons and bald

eagles nesting along the lower Columbia River. Concentrations of TCDD, 1,2,3,7,8 -PnCDD, 1,2,3,6,7,8-HxCDD, OCDD, and 2,3,4,7,8-PnCDF were the most elevated dioxins and furans in all three species, although bald eagles accumulated TCDF to a much greater extent than the other two species (Thomas and Anthony 1999, U.S. Fish and Wildlife 1999). These congeners also accumulated in double-crested cormorants from the Great Lakes and Canada, and in great cormorants in the Netherlands (Norstrom 1988; Bellward et al. 1990; van den Berg et al. 1992, 1994). In the upper regions of the Columbia River at Crescent Island, TCDD accumulated in eggs of Forster s and Caspian terns sampled near a pulp and paper mill (Blus et al. 1998). In the tern study, TCDF accumulated to a much greater extent in the Caspian terns than in the Forster s terns or in lower Columbia River cormorants (Blus et al. 1998). In Canada, Elliott et al. (1996a) and Elliott and Norstrom (1998) attributed elevated concentrations of TCDD, 1,2,3,7,8-PnCDD, 1,2,3,6,7,8-HxCDD, and TCDF in bald eagle plasma and eggs to pulp mill sources. Other higher chlorinated PCDDs and PCDFs were largely attributed to chlorophenol-treated wood chips used in feedstocks by pulp and paper mills (Elliott et al. 1996a; 1996b; Elliott and Norstrom 1998). The accumulation pattern in eggs or lower Columbia River fish-eating birds suggests discharges from pulp and paper mills may be a primary source of dioxins and furans. However, releases from numerous wood treatment and municipal facilities along the lower Columbia and Willamette rivers (Rosetta and Borys 1995) could also contribute to the overall dioxin and furan loading in these birds.

Studies in Canada and the Great Lakes have documented the importance of PCB 126 and other planar PCBs in contributing to the total dioxin-like toxicity in double-crested cormorants and other fish-eating birds (Kubiak et al. 1989, Giesy et al. 1994, Sanderson et al. 1994). Similarly, PCB 126 contributed the most toward the dioxin-like toxicity in eggs of both great blue herons and bald eagles nesting along the lower Columbia River (Thomas and Anthony 1999, U.S Fish and Wildlife Service 1999). Concentrations (=340 pg/g, range =250-720) of PCB 126 in five Caspian tern eggs from Crescent Island were lower than in bald eagles or Rice Island cormorants, but within the range observed in herons (Blus et al. 1998, Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999). In Green Bay on Lake Michigan, high concentrations of PCBs in wildlife have been attributed to a primary source (Gilbertson et al. 1991). In contrast, PCBs are found at lower concentrations in wildlife tissues from the lower Columbia River than in the Green Bay area. PCBs enter the Columbia River from numerous sources, such as leaking electrical transformers, atmospheric deposition, use as dust suppressants, and from spills from electrical equipment at dam sites (Rosetta and Borys 1996).

The non- and mono-ortho PCBs were much higher in eggs of bald eagles than in eggs of double-crested cormorants or great blue herons nesting along the lower Columbia River (Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999). PCB 77 was the highest non-ortho PCB in the bald eagle eggs, whereas cormorants and herons each exhibited greater PCB 126 concentrations than PCB 77. The accumulation pattern of the mono-ortho PCBs was nearly identical in bald eagles and cormorants, with mean concentrations of PCBs 118>105>156>167>157>114. PCB 189 was higher in eagle eggs than PCB 123, while the reverse was true for cormorants. PCB 105 in eggs from two lower Columbia River great blue heron colonies in 1994 were similar to concentrations in double-crested cormorant eggs from

Rice Island (Thomas and Anthony 1999). Other mono-ortho PCBs in heron eggs were either not analyzed or co-eluted with other PCBs (Thomas and Anthony 1999).

PCBs 77 and 118 in lower Columbia River bald eagle eggs contributed much more toward the mean TEQ values than in cormorants. Other studies have documented that PCBs 105 and 118 were among the most important contributors to the total TEQ values in fish-eating birds (Norstrom 1988, Bellword et al. 1990, Sanderson et al. 1994, van den Berg, 1994). However, the TEFs used for PCBs 105 and 118 in these earlier studies were an order of magnitude higher than the WHO-TEFs, and therefore attributed a much higher sensitivity for PCBs 118 and 105. Therefore, these two congeners did not contribute much to the mean TEQ values in lower Columbia River cormorant eggs, even though their concentrations were relatively high and within the range found in other studies. In the bald eagle study, A-TEQs and M-TEQs in eggs collected in 1994 and 1995 were determined using the same WHO-based TEFs used in the cormorant study. The most important contributors towards the M-TEQ_{nmPCBs} in 1994 eagle eggs were PCBs 126 (48%), 118 (16%), 156 (11%), TCDD (8.3%), and PCB 105 (4.6%). The highest contributors toward the A-TEQ_{nmPCBs} in eagle eggs were PCBs 126 (37%), PCB 77 (31%), PCB 81 (8.3%), TCDD (6.4%), and TCDF (4.4%). In contrast, the total dioxin-like toxicities in cormorant eggs from both Columbia River islands were dominated primarily by PCB 126, 1,2,3,7,8-PnCDD, TCDD, and 2,3,4,7,8-PnCDF for all TEQ_{nmPCB} models. For cormorants, PCB 118 contributed up to 14% in the mammalian-based model, but PCBs 77 and 105 contributed less than five percent in either model.

Comparisons Between Columbia River Islands

Eggs from the Rice Island colony frequently exhibited significantly higher concentrations of organochlorine contaminants compared to eggs from the East Sand colony. Total PCBs were higher at Rice Island in 1991, 1994, and 1995, and DDE was higher in 1995. Concentrations of a number of other organochlorine pesticides were higher at Rice than East Sand during 1993 and 1995, when concentrations were well above detection limits. Of the dioxin-like compounds, only the 1995 concentrations of TCDD, TCDF and PCB 81 were higher in Rice Island eggs. Mean mono-ortho PCB concentrations appeared higher in Rice Island eggs, but sample size was insufficient to statistically compare means. Mean egg concentrations of detectable PCDDs, PCDFs, and PCBs 126, 105, and 118 from Rice Island exceeded values in cormorants from a relatively less contaminated colony in Saskatchewan, whereas concentrations of these congeners in East Sand Island eggs were similar to or less than eggs from the Saskatchewan colony (Sanderson et al. 1994). Avian- and mammalian-based TEQs in Rice Island eggs approached or exceeded concentrations (=139 pg/g, recalculated using WHO-TEFs) from a cormorant colony (Chisty Islet) in the Strait of Georgia receiving effluent from pulp and paper mills (Sanderson et al. 1994). In nearly all comparisons between islands and within a year, concentrations in Rice Island eggs appeared higher than in eggs from East Sand, and in no cases were East Sand Island eggs significantly greater than at Rice. In addition, the probability values were highly suggestive of statistical differences in 1995 for the non-ortho planar PCB and TEQ comparisons. The data indicate a consistent trend of higher contaminant burdens in eggs from the Rice Island colony versus the East Sand Island colony, although a greater sample size is needed to better represent the two cormorant colonies and address variability in analytical chemistry data.

Double-crested cormorants are primarily exposed to bioaccumulative contaminants through ingestion of contaminated prey. Bioaccumulation of organochlorine compounds has been documented throughout the Columbia River food web in invertebrates, fish, eggs of fish-eating birds, and in mammals (Henny *et al.* 1981, 1984, 1996; Schmitt *et al.* 1985; Anthony et al. 1993; Elliott et al. 1999; Thomas and Anthony 1999; U.S. Fish and Wildlife Service 1999; U.S. Fish and Wildlife Service unpubl. data). Rice Island is located at RM 22 above the mouth of the estuary, and depositional areas for contaminants entering the estuary could occur within the feeding area of cormorants nesting on this island. In contrast, cormorants from East Sand Island at RM six may feed more in the open ocean and contact less contaminated prey. In eggs of lower Columbia River bald eagles, organochlorine compounds were highest from nests in the lower estuary below RM 60, especially where tidal flats were more abundant (U.S. Fish and Wildlife 1999). Bald eagle productivity was lowest between the RMs 13 and 31, which encompasses Rice Island (U.S. Fish and Wildlife 1999). Cormorants nesting in this area on Rice Island exhibit the greatest potential for contaminant impacts, although the reproductive success of the birds has not been directly assessed.

CONCLUSIONS

In summary, concentrations of p,p -DDE, total PCBs, mercury, and some dioxin-like compounds (TEQs) accumulated in eggs of double-crested cormorants from Rice and East Sand Islands, whereas 1993 samples from a reference colony were significantly lower or below detection limits. Nearly all contaminant concentrations in Rice Island eggs either appeared higher, or were significantly higher, than values in eggs from East Sand Island. Concentrations of these contaminants, particularly in eggs from the Rice Island colony, approached or exceeded some effect threshold levels during some years of the study. Eggshell thickness in lower Columbia River cormorants was inversely correlated to p,p -DDE concentrations, and eggshell thinning of some eggs in both colonies was documented. Eggs collected in 1991 from both islands exhibited mercury concentrations within a range associated with reproductive impacts for some avian species. Maximum concentrations of TCDD or TEQs in eggs from Rice Island also exceeded concentrations associated with reproductive impacts in birds from the Great Lakes, and concentrations affecting brain asymmetry measurements. Non-ortho PCB 126 was found to contribute the most dioxin-like toxicity toward the mean TEQ concentrations in all additive models, which coincides with studies of other piscivorus birds along the lower Columbia River. The H4IIE bioassay conducted on cormorant egg extracts revealed that up to 18 and 24% mortality could occur in East Sand and Rice Island eggs, respectively. Results indicate that lower Columbia River cormorants, especially birds nesting on Rice Island, are exposed to concentrations of contaminants that decrease shell thickness, adversely impact developing embryos, or elicit egg mortality in some individuals. However, contaminant concentrations since 1994 appear to be well below concentrations impacting double-crested cormorants at the population level, as compared to other field studies from the Great Lakes region.

RECOMMENDATIONS

Contaminants enter the Columbia River from numerous point and non-point sources. Persistent contaminants such DDT and PCBs were banned in the 1970s but still are present in the environment, and TCDD and dioxin-like compounds are currently released from industrial, municipal, and combustion processes. These chemicals have been detected at various locations in water, sediment, and biota along the Columbia River and in major tributaries such as the Willamette River (Fuhrer 1989, Rinella et al. 1992, Thomas and Anthony 1999, U.S. Fish and Wildlife Service 1999). Biomagnification of these contaminants has resulted in harmful concentrations in some fish-eating birds, particularly bald eagles. Additional prevention techniques such as reducing run-off from agricultural fields and minimizing disturbance to contaminated sediments are needed to limit the entry of these persistent contaminants into the Columbia River system.

The contaminants present in Columbia River double-crested cormorants are a result of industrial, municipal, and land use practices along the Columbia River. Actions taken by refuge personnel to prevent contamination of cormorants are limited. However, we recommend the following management actions be taken in order to help minimize contaminant uptake in cormorants and monitor exposure:

1) Ensure that adequate buffers exist on any land managed by the refuge that supports agriculture or pasture, or was formerly used for these purposes. Vegetative buffers should be present between agricultural land and the Columbia River or its tributaries to prevent erosion of soil associated with DDT or its metabolites from entering the waterways.

2) Continue population monitoring or aerial nest counts. Cormorants are an excellent indicator species for the region, and population estimates are crucial to determine the health of the species and as an indicator for other fish-eating species. In addition, dioxin-like chemicals may be influencing egg mortality at nest sites, and hatching or nesting success should be monitored if possible. Previous attempts to monitor hatching success were unsuccessful due to excessive predation by gulls during site visits, so monitoring should only be conducted if predation can be avoided.

3) In coordination with the Service s Environmental Contaminants Program, continue monitoring contaminants in cormorant eggs on a periodic basis (e.g., every five years) to more closely examine trends over time and to better evaluate the risks to mammalian and avian predators from consuming cormorant eggs or young. Results of the present study indicate contaminants are near effect-threshold concentrations, and a relatively small increase in mean egg burdens could impact the cormorant population. Contaminants released during large dredging projects, such as the proposed channel deepening of the lower Willamette and Columbia rivers, could result in increased egg burdens and further impact reproduction in the cormorants. In contrast, DDE and total PCB concentrations have declined in eggs of lower Columbia River bald eagles between the mid-1980s and 1995. This trend could be occurring in other fish-eating species as well, but the decline will be undetected unless monitored. In addition, the pulp and paper industry recently

changed from using elemental chlorine to chlorine dioxide for bleaching paper at pulp mills along the Columbia River. A similar process change to chlorine dioxide in Canada led to a corresponding decrease in the contaminant burdens in great blue heron eggs (Whitehead et al. 1992; Elliott et al. 1996b).

4) In coordination with the Service s Environmental Contaminants Program and the Oregon Department of Environmental Quality, collect and chemically analyze prey items collected from cormorant nest sites or regurgitated from cormorant nestlings. This information will allow determination of species-specific biomagnification factors (BMFs) for cormorants, and can be used to develop target tissue concentrations in fish that will be protective of cormorants and other fish-eating birds along the river. Prey items should be collected over one or two breeding seasons, identified to species, and individual or composite samples should be chemically analyzed for organochlorine pesticides, total PCBs, and dioxin-like compounds. The H4IIE-extract or other suitable bioassay could be used to address dioxin-like compounds and establish BMFs in cormorant eggs or fish prey to minimize analytical chemistry costs, similar to studies conducted in Great Lakes (Jones et al. 1994).

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