

## ASSESSMENT OF BIOLOGICAL EFFECTS OF CHLORINATED HYDROCARBONS IN OSPREY CHICKS

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**Abstract**—Osprey (*Pandion haliaetus*) eggs were collected during 1995 and 1996 at seven sites along the Fraser and Columbia River systems of British Columbia, Canada, and Washington and Oregon, USA. Fifty-four eggs were placed into a laboratory incubator. Thirty-eight of the hatched chicks were sacrificed within 24 h. Hatching success did not differ among sites and therefore between treatment and reference areas. Residual yolk sacs of eggs collected downstream of the large bleached-kraft pulp mill at Castlegar contained greater mean concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 2,930 ng/kg lipid) compared with reference sites such as the Nechako River, an upper tributary of the Fraser system (33.7 ng/kg). Total polychlorinated biphenyls (PCBs) in yolk sacs were also higher at Castlegar and in samples from the Columbia River downstream of Portland, Oregon, compared with those from the Nechako River. Concentrations of measured chemicals, including TCDD toxic equivalents (TEQs), total PCBs, *p,p'*-dichlorodiphenylethylene (*p,p'*-DDE), and other organochlorines were not different in eggs that failed to hatch compared with calculated whole-egg values for hatched eggs. There were significant biochemical responses; a hepatic cytochrome P4501A (CYP1A) cross-reactive protein was detected in all samples tested and correlated positively with ethoxyresorufin *o*-deethylase (EROD) activity and yolk sac concentrations of TEQs and total PCBs. Tissue concentrations of vitamin A compounds varied among sites and correlated positively with yolk sac concentrations of TEQs and PCBs. Morphological, histological, and other physiological parameters, including chick growth, edema, deformities, and hepatic and renal porphyrin concentrations, neither varied among sites nor showed concentration-related effects.

**Keywords**—Osprey    Dioxin    Toxic equivalents    CYP1A    Vitamin A

## INTRODUCTION

The Columbia and Fraser Rivers (British Columbia, Canada, and Washington and Oregon, USA) drain much of the Pacific Northwest region of North America and receive effluent from numerous municipal and industrial sources. Ospreys are common breeders throughout the region and, as piscivorous top predators, they readily accumulate lipophilic chlorinated hydrocarbon and methyl-mercury contaminants. Thus, we selected the osprey as an indicator of contaminant effects in the aquatic ecosystems of the region [1]. In 1991, we began monitoring contaminant concentrations in osprey eggs at selected sites, particularly around bleached kraft pulp mills, known point sources of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Osprey eggs from nests downstream of pulp mill sites contained significantly higher concentrations of both 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and -dibenzofuran (TCDF) compared with those upstream [1]. At the same time, we found lower breeding success of ospreys nesting downstream of the pulp mill at Kamloops on the Thompson River. The compromised breeding success did not seem related to obvious factors such as reduced food availability. Although data were not available for ospreys, bald eagle (*Haliaeetus leucocephalus*) populations, another fish-eating, albeit resident, species, exhibited reduced reproductive

successes at nests along the lower Columbia River, which were related to chlorinated hydrocarbon exposure [2].

Various approaches have been used to study the impact of contaminants on reproduction of birds in the wild. Egg swap experiments attempt to address effects mediated both via direct effects on the embryo (egg intrinsic) and via adult behavior (egg extrinsic) [3]. Although logistically complex, egg exchanges have been successfully applied to ospreys because of the relative accessibility of nests and insensitivity to disturbance [4,5]. However, confounding influences from other variables present in the field, such as weather, predators, and food supply, still present difficulties in isolation of mechanisms. Recently, Woodford et al. [6] effectively combined a multiyear egg swap experiment with field observations of growth and behavior of ospreys breeding downstream of a pulp mill in Wisconsin, USA. They found no effects of contaminants on hatching or fledging rates but that chicks grew more slowly at the contaminated site.

In determining an experimental approach, we considered findings from laboratory studies that, regardless of significant interspecific variation in toxic responses, developing embryos were consistently more sensitive to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like chemicals and to *p,p'*-dichlorodiphenylethylene (DDE) than other life stages [7,8]. Thus, we opted for an artificial incubation experiment to investigate contaminant effects on ospreys. This technique, pioneered in studies of Great Lakes fish-eating birds [9], has since been applied to

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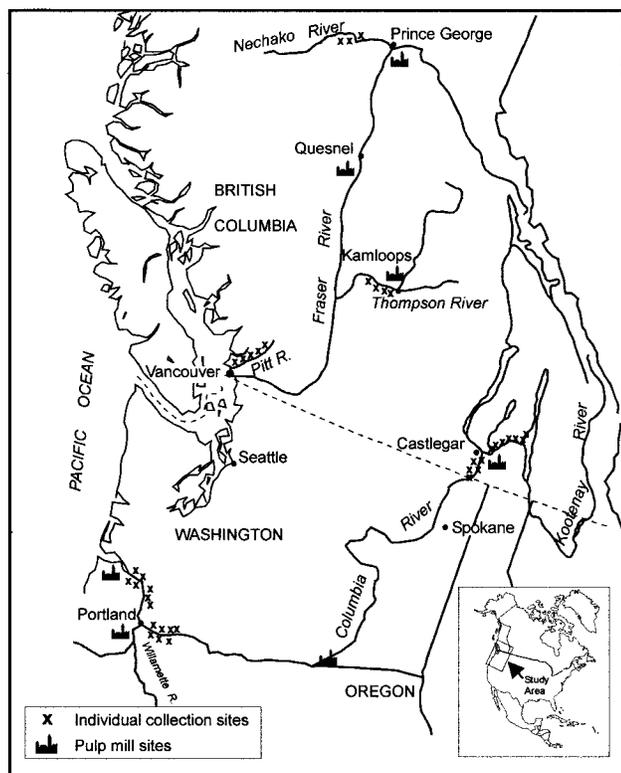


Fig. 1. Locations of osprey eggs collected for laboratory incubation. Locations are shown only for eggs that hatched successfully.

a variety of species and contaminated locations [e.g., 10–15]. It effectively examines the egg intrinsic mechanism and also permits determination of deformities, edema, morphology, and a variety of biochemical and physiological endpoints associated with contaminant exposure.

We hypothesized that ospreys breeding downstream of treatment sites, defined as bleached-kraft pulp mills and urban industrial centers, compared with reference sites, would exhibit symptoms of chlorinated hydrocarbon exposure such as reduced hatching success, smaller embryos, and altered biomarker responses. We also hypothesized that there would be correlations between those parameters and individual exposure to chlorinated hydrocarbon contaminants.

## MATERIALS AND METHODS

### Sample collection

We collected osprey eggs from 54 nests (Fig. 1). At each location, we attempted to collect eggs when birds had been incubating on average for one to two weeks. At two sites, the cities of Kamloops (referred to as the Thompson River) and Castlegar, nests were located downstream of single large bleached kraft pulp mill. Eggs also were collected from nests upstream of Castlegar (referred to as the upper Columbia), which was considered a reference area. We collected eggs from the United States side of the Columbia River around Portland, Oregon. The nests were divided into two groups, upstream of Portland (referred to as Portland nests) and downstream of the urban area (referred to as lower Columbia nests). The lower reaches of the Columbia River are subject to contaminant input from a large number of industrial, municipal, and agricultural point and nonpoint sources. Eggs were also collected from the Pitt and Nechako Rivers for use as references, being free of

any major industrial inputs. The Pitt River enters the lower Fraser River just above Greater Vancouver; the drainage area has areas of intensive agriculture but no industrial point sources. The Nechako River is a northwestern tributary of the Fraser River.

One egg was taken from each nest. Eggs were placed initially into a portable cooler. The temperature was maintained between 25 and 30°C using hot water bottles, replenished as required from thermos bottles. Within 2 h of collection, the eggs were transferred into a battery-powered incubator (Curfew RX 50, Curfew Incubators, Chelmsford, Essex, UK) kept at a temperature of 34°C. The eggs were rotated about hourly and turned on their long axis every 8 h.

Within a maximum of 24 h, eggs were brought to the laboratory at the Department of Animal Science, University of British Columbia (Vancouver, BC, Canada) and were weighed, measured, candled to determine fertility, and placed into a forced-air incubator (Curfew RX200, Curfew Incubators) at 37.5°C with a relative humidity of 59%. The eggs were rotated once per hour and turned twice per day in opposite directions on their long axes. At pipping, the eggs were placed into a hatcher.

Egg volume was determined using Hoyt's equation [16],

$$\text{volume} = 0.51 \text{ length} \times \text{width}^2 \quad (1)$$

### Sample preparation

Within 24 h of hatching, the birds were weighed, wing lengths were measured, blood samples were obtained by cardiac puncture using a heparinized tuberculin syringe, and the birds were sacrificed by decapitation. The yolk sac was removed, weighed, and frozen. The liver was removed, weighed, and samples taken, with 0.25 g taken from the tip of the left lobe for vitamin A analysis and 0.10 g taken from the tip of the right lobe for porphyrin analysis; these samples were then frozen. The remaining liver was stored in liquid nitrogen for later preparation of microsomes. Selected organs were removed, weighed, and measured, including the tibia, tarsus, heart, gall bladder, kidneys (left and right), bursa of Fabricius, thymus, thyroid, and adrenals (sum of both). Sex was determined by visual inspection of gonads.

Tissues fixed in 10% buffered formalin for histological examination were adrenals, bursa, gonads, thymus, and thyroids. At the University of Saskatchewan (Saskatoon, SK, Canada), tissues were processed routinely and embedded in paraffin blocks. Sections were cut at 6 µm and stained with hematoxylin and eosin and examined by light microscopy. Slides were then read without prior knowledge of their origin.

### Chemical analysis

Osprey yolk sacs were homogenized and prepared for analysis at the National Wildlife Research Centre (Hull, PQ, Canada). Analysis of PCDDs, PCDFs, and non-ortho polychlorinated biphenyls (PCBs) were carried out on a VG AutoSpec double-focusing high resolution mass spectrometer (Micro-mass Canada, Pte-Claire, PQ, Canada) linked to a Hewlett-Packard 5890 Series II data system (Hewlett-Packard, Avondale, PA, USA) using <sup>13</sup>C-labeled internal standards after gel permeation/carbon chromatographic cleanup [17]. An in-house herring gull (*Larus argentatus*) egg pool from Lake Ontario was included with each batch of samples as a reference material for quality assurance. Concentrations were corrected for recoveries since they were calculated using an internal standard

quantification method. Percent recoveries were in the range of 70 to 100% for the major PCDD, PCDF, and non-*ortho* PCB congeners. Recoveries for CB-77 were lower, generally around 50%.

Organochlorine pesticides and other PCBs were determined at the National Wildlife Research Centre using a gas chromatograph/electron capture detector according to methods described previously [18] except that total PCB concentrations are reported as the sum of 42 congener peaks (CBs 31, 28, 52, 49, 44, 42, 64, 74, 70, 66, 60, 101, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 137, 138, 158, 129, 182, 183, 128, 185, 174, 171, 200, 172, 180, 170, 201, 203, 195, 194, 206). Organochlorine pesticides routinely analyzed for included *p,p'*-DDE, *p,p'*-DDT, *p,p'*-dichlorodiphenylethane (*p,p'*-DDD), dieldrin, heptachlor epoxide, *cis*-nonachlor, *trans*-nonachlor, oxychlorane, *cis*-chlordane, *trans*-chlordane, mirex, photomirex,  $\alpha$ -hexachlorocyclohexane,  $\beta$ -hexachlorocyclohexane,  $\gamma$ -hexachlorocyclohexane, tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, and tris(4-chlorophenyl)methanol. Analyses were carried out using a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 7673A auto injector. Aliquots of diluted herring gull reference material were analyzed with each batch of samples.

#### Biochemical assays

**Cytochrome P450-related activity.** The cytochrome P450-related activity was evaluated by measuring the 7-ethoxyresorufin *o*-deethylase (EROD) activity in liver microsomes. The EROD and protein assays were carried out simultaneously in 48-well plates following a modification of a previously described method [19]. The assay conditions were optimized for avian microsomes. The reaction mixture consisted of 110  $\mu$ l (sample wells) and 160  $\mu$ l (blank wells) of 0.05 M sodium phosphate buffer (pH 8.0) and, in each well, 25  $\mu$ l of microsomes (~50  $\mu$ g protein) and 50  $\mu$ l of 7-ethoxyresorufin prepared in buffer from a stock solution of 1,000  $\mu$ M in methanol. The final substrate concentration in each well was 2  $\mu$ M in methanol. The plate was preincubated at 37°C for 5 min, and the reaction was started by the addition of 50  $\mu$ l of a 2.4 mM solution of nicotinamide adenine dinucleotide phosphate (prepared immediately before use) to the sample wells (not to blank wells), for a final reaction volume of 235  $\mu$ l. The reaction was allowed to proceed for 10 min, after which 100  $\mu$ l of cold acetonitrile containing fluorecamine at a concentration of 600  $\mu$ g/ml was added. The plates were then scanned with a fluorescence plate reader (Cytofluor model 2350, Millipore, Bedford, MA, USA) for resorufin at 530 nm excitation (25 nm bandwidth) and 590 nm emission wavelengths (35 nm bandwidth) and for proteins at 400 nm excitation (35 nm bandwidth) and 460 nm emission wavelengths (40 nm bandwidth). Five concentrations of resorufin (25–350 nM) and bovine serum albumin (50–500  $\mu$ g/ml) standards were prepared on each plate. Fluorescence data were imported into QPRO (Borland International, Scotts Valley, CA, USA) for curve fitting and quantification.

**Immunoblotting.** Based on the original western blot method developed by Towbin et al. [20], hepatic microsomal proteins were separated on sodium dodecyl sulfate polyacrylamide gels (9% acrylamide) and electrophoretically transferred to Rad-free membranes (Schleicher and Schuell, Keene, NH, USA). Aroclor® 1254 (Monsanto, St. Louis, MO, USA)-induced rat liver microsomes (prepared from commercially available post-

mitochondrial supernatant; Molecular Toxicology, Annapolis, MD, USA) were used as standards. Immunodetection of CYP1A was performed using monoclonal antibody 1-12-3 prepared against scup cytochrome P4501A, which recognizes CYP1A in all taxonomic groups of vertebrates examined so far [21,22]. The secondary antibody was a goat antimouse immunoglobulin G linked to alkaline phosphatase. Immunoreactive proteins were detected by chemiluminescence (Rad-Free; Schleicher and Schuell), and the light intensities of the immunoreactive protein bands were quantified by video imaging densitometry (UVP Gel Documentation System 7500, San Gabriel, CA, USA).

**Liver and kidney vitamin A analysis.** Samples of liver or kidney were dehydrated to a pink powder by grinding approximately 300 mg of tissue with anhydrous sodium sulfate. The internal standard, retinyl acetate (40 ng/20 ml methanol), was added to an equivalent of 0.20 g of tissue, and the vitamin A compounds were extracted with 5 ml of a dichloromethane:methanol (1:1, v/v) solvent mixture in an amber vial. After centrifugation (10 min at 600 rpm at 10°C), 4 ml of the supernatant was evaporated under nitrogen to approximately 1.5 ml. After the addition of 200  $\mu$ l of dichloromethane, the volume was completed to 2 ml with methanol, then filtered through a 0.2- $\mu$ m Acrodisc LC13 polyvinylidene fluoride filter (Pall Canada, Mississauga, ON, Canada), and a 20- $\mu$ l aliquot was analyzed by nonaqueous reverse phase high-performance liquid chromatography (HPLC; Varian Star System, Varian, Mississauga, ON, Canada). Separation of retinol, retinyl acetate, and retinyl palmitate was achieved with a 15-cm-long, 5- $\mu$ m ODS Zorbax column (Chromatograph Specialities, Brockville, ON, Canada) with 100% methanol at 1 ml/min for 5.5 min followed by a linear gradient, which brought the mobile phase to 30% dichloromethane and 70% methanol within 0.5 min. This composition was held until the end of the run at a flow rate of 2.0 ml/min. With these conditions, retinol, retinyl acetate, and retinyl palmitate had retention times of 3.1, 4.2, and 9.7 min, respectively.

**Plasma vitamin A analysis.** The retinol-protein complex was dissociated by the addition of 200  $\mu$ l of acetonitrile to 100  $\mu$ l of plasma containing a known amount of the internal standard, retinyl acetate. The retinol was extracted twice using 4 ml and 1 ml of hexane. The organic and aqueous phases were separated by centrifugation (Sorvall model RT600B, DuPont Canada, Mississauga, ON, Canada), and the combined organic phases were evaporated to dryness under a stream of nitrogen. The residues were reconstituted in 0.5 ml of methanol and filtered through a 0.2- $\mu$ m Acrodisc LC13 PVDF filter, and a 50- $\mu$ l aliquot was analyzed by HPLC using the column described above for liver. With 100% methanol as the mobile phase and a flow rate of 1 ml/min, retinol and retinyl acetate had retention times of 3.3 and 4.5 min, respectively.

**Hepatic porphyrins.** Porphyrin levels in liver were determined using a previously described method [23]. Briefly, 100 mg of tissue were extracted with 1 M hydrochloric acid/acetonitrile mixture (1:1, v/v). The porphyrins were then concentrated on Sep-Pak Plus t C18 cartridges (Waters, Mississauga, ON, Canada) followed by separation and quantification by reverse phase HPLC.

#### Statistical analysis

Statistical analyses were carried out using JMP™ Statistical Package (SAS Institute, Cary, NC, USA). Data on chemical concentrations in yolk sacs are presented on a lipid weight

basis in accord with previous studies [10,14,24] in an attempt to normalize the data, given the greater than fivefold variability in lipid content among individual yolk sacs. Because there were no significant differences among locations for the yolk sac weights or percent lipid content of yolk sacs, we did not consider location a cofactor. Data were transformed to common logarithms, and geometric means and 95% confidence intervals were calculated with the data grouped by collection site. Contaminant levels were compared among locations with a one-way analysis of variance; significant differences were determined using Tukey's multiple comparison procedure.

We also compared chemical residue concentrations in yolk sacs from the hatched chicks with concentrations in whole eggs that did not hatch, detailed results of which were presented elsewhere [1,25]. Concentrations of four unhatched eggs were adjusted for moisture loss, as described previously [1,25]. The concentration of each chemical in the yolk sac was used to estimate the corresponding concentration that would have been present in a fresh egg. This was done by assuming that metabolic losses for compounds such as *p,p'*-DDE, total PCBs, and 2,3,7,8-TCDD in the developing embryo would have been negligible, and therefore the mass of a chemical in newly hatched chicks would approximate the mass of the chemical in the fresh egg. The formula used to calculate the concentrations of each compound [X] that would have been present in a fresh egg from the concentrations in each yolk sac (YS) was

$$\begin{aligned} \text{Mass of X} = & ([X] \text{ in yolk lipids} \times \text{mass of YS} \\ & \times \text{lipid fraction of YS}) \\ & + ([X] \text{ in yolk lipids} \\ & \times \text{yolk-free mass of hatchling} \\ & \times \text{lipid fraction of hatchling}) \quad (2) \end{aligned}$$

$$[X] \text{ in fresh egg} = \text{mass of X} / \text{mass of egg contents} \quad (3)$$

Individual data were available from each egg for all of the parameters in Equations 2 and 3 with the exception of the lipid fraction of hatchlings minus the yolk sacs. Therefore, we used an average value of 1.1% based on lipid determination of two osprey hatchlings. For this analysis, data are presented on a wet weight basis to facilitate comparison with published data and criteria. Results were transformed to common logarithms and geometric means, and 95% confidence intervals were calculated. A *t* test with adjustment for unequal variance was used to determine differences between log-transformed contaminant concentrations in hatched versus unhatched eggs. The relationship between hatching success, a binary dependent variable, and log-transformed contaminant concentrations in yolk sacs was also modeled using logistic regression analysis.

Concentration-effect relationships were determined using coefficients of determination ( $r^2$ ) using least-squares linear regression. Unless stated otherwise, a value of  $p < 0.05$  was considered statistically significant in all analyses.

The TCDD toxic equivalents were calculated using the toxic equivalency factors proposed by WHO (World Health Organization, Paris, France) [26].

## RESULTS

### Chemical contaminant levels

**PCDDs and PCDFs.** Concentrations of 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-pentachlorodibenzofuran (Pn-

CDF) were significantly higher in samples from Castlegar compared with other sites (Table 1). Concentrations of 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,4,6,7,8,9-heptachlorodibenzo-*p*-dioxin (HpCDD), 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD), 1,2,3,6,7,8-hexachlorodibenzofuran (HxCDF), and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were significantly elevated in yolk sacs from the Thompson River relative to most other locations.

Traces of 1,2,4,6,7,9/1,2,4,6,8,9-HxCDD (38.2–240 ng/kg) and 1,2,3,6,7,9/1,2,3,6,8,9-HxCDD (114–365 ng/kg) were found in samples with elevated 1,2,3,4,6,7,8,9-OCDD concentrations, including all four yolk sacs from the Thompson River near Kamloops and two samples from the Nechako River. A few samples had detectable concentrations of 1,2,4,6,8-PnCDF (2.38–77.6 ng/kg), particularly those collected on the Thompson River and the two from the Nechako. The 1,2,4,6,7,8-HxCDF congener was detected more frequently; concentrations ranged generally from 5 to 10 ng/kg but were higher in samples from the Thompson River (47.4–302 ng/kg), which also contained 1,2,4,6,8,9-HxCDF at slightly lower concentrations.

**PCBs.** Mean concentrations of total PCBs were two- to sixfold higher in the total of all the yolk sacs from sites on the Columbia compared with those on the Fraser River, and the differences were significant in most cases (Table 2). The pattern of individual congeners varied among sites. Whereas the relative proportion of more stable congeners such as CB-153 and CB-138 was spatially consistent, the relative amounts of less chlorinated compounds, such as CB-101 and CB-99, were greater in the samples from the lower Columbia compared with the Castlegar or upper Columbia reaches. The reverse was true for the higher chlorinated congeners, e.g., CB-170 and CB-180, which were higher relative to total PCBs in samples from Castlegar compared to the lower Columbia (Table 2). Within the Columbia basin, mean concentrations of the lower chlorinated non-*ortho* CB-81, CB-77, and CB-126 were highest in samples from the lower Columbia River below Portland (Table 3). However, CB-169 was highest in yolk sacs from the upper Columbia and Castlegar reaches.

**Toxic equivalents.** Toxic equivalents (TEQs) were highest in samples from Castlegar and the lower Columbia, where they were significantly higher than those from the Pitt River (Fig. 2). The lower Columbia was significantly different from the Nechako River. Non-*ortho* PCBs made the major contribution to TEQs at most sites, with the exception of Castlegar, where 2,3,7,8-TCDD was the major contributor at about 50%.

**Other organochlorines.** Mean concentrations of *p,p'*-DDE and *p,p'*-DDD were highest in eggs from the lower Columbia and Portland but were not significantly different from either the Castlegar area or the Nechako (Table 4). Highest mean concentrations of chlordane-related chemicals were found in yolk sacs collected along the Canadian side of the Columbia River. Other organochlorine pesticides did not vary significantly among sites, and concentrations ranged from nondetected to about 1 mg/kg lipid weight.

### Laboratory hatching success and condition of embryos

Of the total of 54 eggs collected for incubation, five were infertile. Of the 49 fertile eggs, 38 hatched, giving a successful hatching rate for artificial incubation of 78%. Of the 11 eggs that failed to hatch, three chicks died early in development, three died in middevelopment (one of which apparently dehydrated and one of which had a bacterial infection), and five

Table 1. Concentrations of selected PCDDs and PCDFs in egg yolk sacs during the 1995 and 1996 breeding seasons (ng/kg lipid weight)<sup>a,b</sup>

	Concentration (geometric mean, 95% confidence interval, range)						
	Nechako River (n = 3)	Pitt River (n = 5)	Thompson River (n = 4)	Upper Columbia (n = 7)	Castlegar (n = 5)	Portland (n = 7)	Lower Columbia (n = 7)
2,3,7,8-TCDD	33.7 A 2.90-393 (ND-84.0) 127	64.8 A 31.7-132 (25.7-122) 173	504 A 131-1,930 (190-1,360) 326	137 A 86.2-219 (73.5-343) 354	2,930 B 1,960-4,360 (1,940-4,600) 310	206 A 83.2-512 (54.5-843) 193	543 A 320-923 (235-1,200) 365
1,2,3,7,8-PnCDD	8.14-1,910 (46.2-404) 370	59.3-507 (39.7-329) 338	141-755 (190-668) 1,420	178-703 (99.6-1,130) 442	231-416 (257-456) 627	77.6-479 (68.1-735) 350	188-710 (119-977) 660
1,2,3,6,7,8-HxCDD	17.8-7,700 (181-1,520) 181 AB	93.5-1,220 (61.0-972) 53.1 A	680-2,960 (734-2,090) 960 B	196-994 (107-1,030) 149 A	332-1,190 (342-1,090) 247 A	163-753 (161-1,440) 60.8 A	311-1,400 (296-2,690) 138 A
1,2,3,7,8,9-HxCDD	5.64-5,790 (63.6-882) 2,540 A	11.0-256 (9.63-337) 413 A	530-1,740 (696-1,630) 13,400 B	56.2-397 (32.6-478) 1,280 A	95.4-638 (105-478) 1,690 A	21.9-169 (16.6-457) 990 A	43.5-436 (32.7-1,330) 1,210 A
1,2,3,4,6,7,8-HpCDD	71.4-90,400 (663-11,600)	61.1-2,790 (141-6,070)	7,500-24,100 (9,130-21,600)	353-4,630 (249-10,100)	583-4,890 (662-4,490)	262-3,740 (215-9,030)	333-4,410 (103-6,750)
1,2,3,4,6,7,8,9-OCDD	12,200 AB 745-201,000 (4,130-39,100) ND	645 A 82.2-5,060 (129-8,790) 6.98 A	40,400 B 17,700-92,200 (18,900-57,000) 161 A	3,280 AB 658-16,400 (297-79,600) 57.2 A	6,530 AB 1,870-22,800 (2,510-29,100) 1,200 B	1,900 AB 274-13,200 (112-54,300) 15.8 A	4,010 A 1,010-15,900 (326-20,400) 38.2 A
2,3,7,8-TCDF	ND	4.46-10.9 (ND-8.48) 40.9 A	57.9-447 (62.7-266) 74.1 A	34.8-94.1 (31.6-132) 99.1 A	547-2,650 (527-2,040) 348 B	7.91-31.7 (ND-47.4) 69.2 A	18.6-78.5 (ND-120) 116 A
2,3,4,7,8-PnCDF	17.8 A 0.17-1,860 (ND-111)	10.8-155 (6.13-78.0) 25.6 AB	39.3-140 (48.3-108) 203 B	55.2-178 (49.1-269) 35.8 A	249-486 (237-463) 41.1 AB	28.1-171 (22.6-300) 9.14 A	38.3-350 (ND-323) 29.8 A
1,2,3,6,7,8-HxCDF	0.04-3,980 (ND-127) 202 AB	2.29-286 (62.4-790) 34.7 A	53.7-769 (76.2-584) 1,650 B	15.6-82.0 (9.32-86.4) 63.0 A	23.3-72.6 (24.8-67.6) 125 A	2.96-28.2 (ND-93.9) 14.9 A	9.28-95.4 (ND-214) 49.0 A
1,2,3,4,6,7,8-HpCDF	0.30-135,000 (15.4-2,890)	2.48-485 (ND-1,330)	627-4,340 (957-3,750)	10.7-372 (ND-2,170)	23.7-665 (ND-1,230)	1.86-119 (ND-563)	11.5-210 (ND-336)

<sup>a</sup> Means that do not share the same letter (i.e., A or B) are significantly different ( $p < 0.05$ ).<sup>b</sup> PCDD = polychlorinated dibenzo-*p*-dioxin; PCDF = polychlorinated dibenzo-*p*-dibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PnCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzo-*p*-dibenzofuran; PnCDF = pentachlorodibenzo-*p*-dibenzofuran; HxCDF = hexachlorodibenzo-*p*-dibenzofuran; HpCDF = heptachlorodibenzo-*p*-dibenzofuran; ND = not detected.

Table 2. Concentrations of selected polychlorinated biphenyls (PCBs) in egg yolk sacs of ospreys collected during the 1995 and 1996 breeding seasons (mg/kg lipid wt)<sup>a</sup>

	Concentration (geometric mean, 95% confidence interval, range)						
	Nechako River (n = 3)	Pitt River (n = 5)	Thompson River (n = 4)	Upper Columbia (n = 7)	Castlegar (n = 5)	Portland (n = 7)	Lower Columbia (n = 7)
Total PCBs	24.3 AC 2.25–262 (8.09–46.5)	29.0 A 13.3–63.4 (9.87–49.5)	22.8 A 7.23–71.8 (9.79–50.9)	52.2 AC 33.0–82.4 (25.6–93.1)	138 B 109–174 (105–176)	109 BC 52.0–228 (36.8–426)	149 BC 109–204 (89.4–207)
CB-101	1.27 AB 0.05–32.1 (0.34–4.62)	1.50 A 0.56–4.06 (0.40–3.20)	1.61 AB 0.40–6.56 (0.69–5.06)	1.76 A 1.00–3.10 (0.88–3.71)	3.59 ABC 2.89–4.46 (2.92–4.59)	8.10 C 3.44–19.1 (1.90–34.5)	10.9 BC 7.41–16.1 (6.14–17.3)
CB-99	0.99 AB 0.05–18.1 (0.29–2.88)	1.16 A 0.43–3.14 (0.30–2.17)	0.98 AB 0.37–2.57 (0.47–1.62)	1.57 A 0.88–2.80 (0.67–3.43)	3.05 ABC 2.50–3.73 (2.66–3.90)	5.27 C 2.31–12.0 (1.34–22.6)	6.81 BC 4.41–10.5 (3.40–11.1)
CB-118	2.09 AB 0.11–39.4 (0.63–6.70)	2.77 A 1.19–6.46 (0.85–4.86)	1.87 A 0.66–5.29 (0.86–3.85)	2.97 A 1.59–5.54 (1.21–6.49)	6.67 AB 5.81–7.65 (5.56–7.37)	10.0 B 4.16–24.0 (2.18–46.4)	14.8 B 10.5–20.7 (8.72–21.4)
CB-153	3.68 0.35–39.0 (1.23–6.47)	4.35 2.17–8.71 (1.72–7.61)	3.39 1.38–8.29 (1.73–6.40)	8.89 5.73–13.8 (4.26–14.7)	19.7 15.4–25.2 (14.9–25.7)	15.2 7.22–32.0 (4.96–65.2)	21.5 16.3–28.5 (14.5–31.3)
CB-105	0.35 0.02–6.43 (0.10–1.05)	0.47 0.21–1.06 (0.15–0.76)	0.24 0.11–0.54 (0.13–0.47)	0.47 0.25–0.87 (0.20–1.09)	1.19 1.01–1.40 (1.03–1.45)	2.18 0.94–5.05 (0.55–9.55)	2.68 1.81–3.95 (1.46–4.43)
CB-138	3.66 0.28–48.0 (1.12–7.81)	4.59 1.98–10.7 (1.42–8.25)	3.48 1.27–9.56 (1.62–6.92)	7.23 4.57–11.5 (3.39–11.9)	17.8 15.1–21.0 (14.8–20.1)	16.0 7.58–33.9 (5.06–67.0)	21.6 15.7–29.8 (13.2–30.6)
CB-182	1.07 0.08–15.0 (0.36–3.01)	1.52 0.66–3.46 (0.49–2.59)	1.18 0.41–3.38 (0.52–2.10)	4.00 2.65–6.05 (1.92–6.14)	9.08 6.51–12.7 (5.92–11.4)	5.31 2.60–10.9 (1.87–20.3)	7.84 6.01–10.2 (4.91–10.8)
CB-180	2.03 0.18–22.3 (0.71–4.78)	2.87 1.38–6.01 (1.18–5.72)	1.76 0.72–4.35 (0.89–3.49)	6.33 4.08–9.81 (2.95–11.4)	23.3 15.8–34.3 (15.2–36.6)	8.04 3.83–16.9 (2.83–30.6)	12.2 9.54–15.7 (8.70–18.1)
CB-170	0.84 0.07–9.71 (0.28–1.76)	1.10 0.49–2.45 (0.39–2.25)	0.74 0.26–2.10 (0.35–1.64)	2.24 1.48–3.40 (1.20–3.69)	9.66 6.79–13.7 (6.48–14.3)	3.38 1.65–6.91 (1.25–13.0)	4.94 3.72–6.57 (3.02–6.52)

<sup>a</sup> Means that do not share the same letter (i.e., A, B, or C) are significantly different ( $p < 0.05$ ).

Table 3. Concentrations of non-ortho polychlorinated biphenyls in egg yolk sacs of ospreys collected during the 1995 and 1996 breeding seasons (ng/kg lipid wt)<sup>a,b</sup>

	Concentration (geometric mean, 95% confidence interval, range)						
	Nechako River (n = 3)	Pitt River (n = 5)	Thompson River (n = 4)	Upper Columbia (n = 7)	Castlegar (n = 5)	Portland (n = 7)	Lower Columbia (n = 7)
% Lipid (wet wt)	9.89 2.99–32.6 (6.55–16.8)	11.4 8.55–15.3 (9.44–17.1)	13.3 7.30–24.4 (8.00–19.6)	10.4 6.75–16.1 (4.22–16.9)	11.7 7.59–18.2 (8.48–17.9)	11.3 9.16–13.9 (7.94–15.5)	9.35 7.58–11.5 (6.08–11.7)
% Water (wet wt)	NA	NA	71.7 65.9–77.9 (67.6–76.1)	67.9 64.9–71.1 (63.9–73.7)	70.4 64.9–76.5 (64.8–75.5)	NA	68.9 <sup>c</sup> 66.4–71.4 (68.0–69.9)
CB-81	372 A 2.20–62,900 (35.2–1,660)	755 A 221–2,590 (132–1,430)	671 A 109–4,120 (250–2,300)	1,270 A 392–4,120 (179–7,360)	812 A 479–1,380 (525–1,640)	3,430 AB 1,550–7,590 (1,100–13,700)	8,310 B 5,110–13,500 (4,090–13,800)
CB-77	3,660 AB 113–118,000 (762–5,780)	2,560 A 830–7,910 (844–7,320)	4,040 AB 304–53,700 (852–32,600)	5,750 AB 2,010–16,500 (845–23,300)	6,070 AB 4,290–8,590 (3,920–8,500)	13,500 AB 8,040–22,700 (4,790–31,400)	18,000 B 13,300–24,400 (9,970–28,300)
CB-126	7,680 AB 435–136,000 (2,150–20,500)	8,630 A 3,550–21,000 (2,470–13,800)	7,880 A 1,870–33,300 (2,560–17,500)	14,200 AB 5,590–36,100 (2,880–60,100)	28,000 AB 23,400–33,500 (25,200–34,900)	24,400 AB 12,600–47,100 (11,500–100,000)	46,400 B 31,700–67,700 (23,000–67,800)
CB-169	2,040 AB 219–18,900 (730–3,870)	921 A 397–2,140 (312–1,690)	836 AC 325–2,150 (487–1,810)	3,920 BC 1,300–11,800 (538–14,100)	7,010 B 5,380–9,140 (5,590–9,750)	1,760 AB 886–3,510 (810–8,240)	2,310 AB 1,690–3,160 (1,270–3,350)
CB-189	37.2 0.60–2,290 (ND–167)	41.0 15.0–112 (13.1–119)	ND	57.6 12.1–275 (ND–555)	174 51.6–587 (66.2–763)	72.6 15.5–339 (ND–1,410)	124 36.4–424 (21.9–876)

<sup>a</sup> Means that do not share the same letter (i.e., A, B, or C) are significantly different ( $p < 0.05$ ).

<sup>b</sup> NA = not analyzed; ND = not detected.

<sup>c</sup> n = 3.

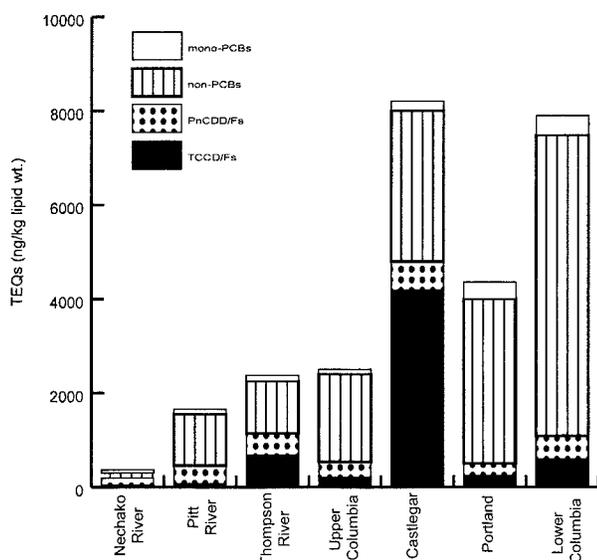


Fig. 2. The contribution of various chlorinated hydrocarbon groups to the sum of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs) in osprey yolk sacs from the Pacific Northwest, 1995 to 1996 (variances are in the tables). The TEQs were calculated using the toxic equivalents proposed by the World Health Organization [26].

died during late development or during pipping. Chilling during collection and/or less than ideal conditions during transport to the laboratory incubators should be considered as a factor in the case of chicks that were dead or died soon after placement in laboratory incubators.

For logistical and budgetary reasons, the study extended over two breeding seasons. Comparison between years is confounded by different locations. Overall, there was no difference between hatching success in 1995 (19/25, or 76% hatched) and 1996 (19/29, or 66% hatched). Hatching success did not vary significantly among locations (Table 5). Most birds hatched in good condition, although mild ascites and slight pericardial and/or cerebral edema were apparent in about 70% of the chicks. There was no association between presence of fluid build-up and concentrations of measured contaminants in yolk sacs. The slight fluid build-up could have been caused by less than optimum incubation conditions. One chick from the lower Columbia had a deformed toe.

The sex ratio overall was 21 males to 16 females and one unknown, for a determined ratio of 57% male to 43% female. The ratio varied among sites. For the Fraser basin sites, the ratio was 50:50 (6 males:6 females), which included 0 males:3 females at the Nechako, 3 males:1 female on the Thompson, and 3 males:2 females on the Pitt River. Overall in the Columbia basin, the ratio was 60% male to 40% female (15 males:10 females), with 3 males:2 females at Castlegar, 4 males:3 females on the upper Columbia, 4 males:2 females at Portland, and 4 males:3 females on the lower Columbia.

#### Morphological and histological measurements

None of the morphological parameters varied significantly among sites. Mean weights  $\pm$  standard deviation in grams ( $n = 38$ ) were, for yolk-free body weight,  $43.9 \pm 4.38$ ; for yolk sac,  $2.68 \pm 1.26$ ; for heart,  $0.335 \pm 0.042$ ; for gall bladder,  $0.196 \pm 0.048$ ; for liver,  $0.975 \pm 0.123$ ; for left kidney,  $0.330 \pm 0.045$ ; for right kidney,  $0.326 \pm 0.046$ ; for bursa,  $0.065 \pm 0.021$ ; for thymus,  $0.116 \pm 0.024$ ; for thyroid,  $0.014 \pm 0.006$ ;

and for adrenal,  $0.018 \pm 0.007$ . Mean egg volume was  $68 \pm 6$  ml.

Histological examination of tissues did not reveal any differences among groups. All of the bursa of Fabricius were growing with evident cell division but with few mature lymphocytes. Thymuses all exhibited thick cortices and abundant mature lymphocytes. There was no variation apparent from histological examination of tissues of ovaries and testes, which seemed uniformly normal, as did the adrenal and thyroid glands.

#### Biochemical measurements

Mean hepatic EROD activity in samples from the Thompson River was 3.1-fold higher than those from the Nechako River, and the difference was significant ( $F_{6,31} = 3.36$ ,  $p = 0.014$ ) if all values were included (Table 6). However, if a single individual measurement of 119 pmol/min/mg in a Thompson River chick, which lies outside two standard deviations of the mean at that site, was removed, the ratio was 2.4 and the difference was not significant ( $F_{6,30} = 2.47$ ,  $p = 0.053$ ). Hepatic CYP1A was 2.4-fold higher in samples from Castlegar compared with the upper Columbia, although the difference was not significant.

All of the retinoid compounds determined in kidney showed significant variability in mean concentration among sites, as did ester-2 in kidney (Table 6). The spatial pattern was complex, but generally chicks from the widely separated sites of the Nechako and Portland had the lowest concentrations of dehydro-retinol and the two esterified forms in kidney and plasma.

There were no significant differences among sites in porphyrin parameters in liver or kidney.

#### Relationships among biological and chemical parameters

Egg volume was positively related to mass of hatchlings ( $r^2 = 0.672$ ,  $F_{1,36} = 76.6$ ,  $p < 0.0001$ ) but not to mass of yolk sacs ( $r^2 = 0.069$ ,  $F_{1,36} = 3.71$ ,  $p = 0.061$ ; Fig. 3a and b). Smaller yolk sacs had significantly greater lipid content ( $r^2 = 0.275$ ,  $F_{1,37} = 15.0$ ,  $p = 0.0004$ ; Fig. 3f). Egg volume was negatively related to the lipid content of yolk sacs ( $r^2 = 0.379$ ,  $F_{1,36} = 23.6$ ,  $p < 0.0001$ ; Fig. 3c), as was hatching mass ( $r^2 = 0.501$ ,  $F_{1,36} = 38.1$ ,  $p < 0.0001$ ; Fig. 3d).

Data from the complete set of 38 osprey chicks were used to examine relationships between measured biological parameters and contaminant exposure. The gradient of exposure from highest to lowest individual values was 380-fold for 2,3,7,8-TCDD, 680-fold for 2,3,7,8-TCDF, 53-fold for total PCBs, 47-fold for CB-126, 46-fold for TEQs, and 24-fold for *p,p'*-DDE.

There were no significant relationships between egg volume or mass of hatchling and any of the chemical parameters. Hatching mass, if assessed minus the yolk sac, showed weak positive relationships with a number of PCB congeners, e.g., with lipid-normalized CB-118 ( $r^2 = 0.111$ ,  $F_{1,36} = 5.60$ ,  $p = 0.024$ ). There were some significant negative relationships between yolk sac weight and a few chemical parameters, e.g., log TEQs ( $r^2 = 0.129$ ,  $F_{1,37} = 6.47$ ,  $p = 0.015$ ).

Hepatic EROD activity regressed significantly with total PCBs and a variety of individual PCB congeners, although the best fit was with TEQs (Table 7 and Fig. 4). Hepatic CYP1A also exhibited a significant relationship with TEQ and total PCB concentrations in yolk sacs; however, the best fit was with *p,p'*-DDE. Catalytic (EROD) and immunoblot (CYP1A)

Table 4. Concentrations of selected organochlorines in egg yolk sacs of ospreys collected during the 1995 and 1996 breeding seasons (mg/kg lipid wt)<sup>a</sup>

	Concentration (geometric mean, 95% confidence interval, range)						
	Nechako River (n = 3)	Pitt River (n = 5)	Thompson River (n = 4)	Upper Columbia (n = 7)	Castlegar (n = 5)	Portland (n = 7)	Lower Columbia (n = 7)
HCB	0.15 0.01–4.02 (0.03–0.41)	0.08 0.05–0.15 (0.05–0.17)	0.18 0.06–0.56 (0.12–0.52)	0.19 0.11–0.35 (0.09–0.53)	0.15 0.13–0.19 (0.12–0.19)	0.26 0.11–0.62 (0.04–0.97)	0.26 0.20–0.34 (0.17–0.38)
<i>p,p'</i> -DDE	179 56.1–572 (133–307)	82.7 36.2–189 (41.0–237)	54.5 22.8–130 (24.6–85.7)	122 59.7–250 (51.7–402)	196 90.4–423 (99.7–496)	316 212–469 (185–588)	258 173–385 (145–389)
<i>p,p'</i> -DDD	6.81 0.64–73.0 (2.44–16.2)	4.42 1.40–14.0 (1.84–21.2)	3.38 0.73–15.7 (0.96–9.32)	4.28 0.81–22.5 (0.74–53.9)	6.27 0.91–43.4 (1.27–43.2)	18.3 9.84–34.0 (6.67–37.8)	16.8 6.85–41.1 (4.96–57.6)
<i>p,p'</i> -DDT	1.12 0.11–11.9 (0.48–3.12)	0.72 0.13–4.00 (0.12–2.24)	0.63 0.17–2.32 (0.28–1.58)	0.45 0.05–3.78 (ND–7.84)	1.41 0.52–3.82 (0.53–3.60)	1.55 0.51–4.76 (0.43–11.9)	1.61 0.38–6.76 (0.13–9.20)
Mirex	0.12 0.02–0.68 (0.06–0.25)	0.16 0.07–0.37 (0.06–0.26)	0.58 0.08–4.32 (0.14–2.87)	0.29 0.14–0.57 (0.10–0.86)	0.48 0.33–0.70 (0.34–0.77)	0.10 0.03–0.29 (ND–0.38)	0.12 0.04–0.37 (ND–0.43)
<i>p</i> -Mirex	0.08 0.001–6.15 (0.02–0.52)	0.38 0.21–0.70 (0.17–0.57)	0.14 0.05–0.34 (0.09–0.32)	0.11 0.03–0.40 (ND–0.41)	0.38 0.24–0.59 (0.23–0.62)	1.55 0.74–3.25 (0.52–6.71)	0.70 0.21–2.38 (0.12–2.77)
$\beta$ -HCH	0.15 0.03–0.72 (0.10–0.31)	0.03 0.004–0.32 (ND–0.16)	0.06 0.004–0.89 (ND–0.17)	0.06 0.01–0.49 (ND–0.79)	0.10 0.004–2.34 (ND–1.67)	0.14 0.07–0.28 (0.06–0.46)	0.05 0.004–0.67 (ND–0.92)
HE	0.22 0.01–7.76 (0.05–0.96)	0.10 0.07–0.13 (0.08–0.15)	0.31 0.09–1.06 (0.13–0.75)	0.50 0.31–0.79 (0.26–0.98)	0.47 0.24–0.94 (0.30–1.15)	0.65 0.36–1.16 (0.36–2.06)	0.58 0.48–0.70 (0.44–0.78)
Dieldrin	0.10 0.01–1.17 (0.04–0.28)	0.11 0.01–1.02 (ND–0.75)	0.16 0.02–1.34 (0.03–0.49)	0.11 0.04–0.26 (0.05–0.66)	0.11 0.07–0.17 (0.07–0.17)	0.29 0.16–0.53 (0.13–0.61)	0.16 0.09–0.27 (0.05–0.28)
TCPM	0.64 0.16–2.66 (0.35–1.11)	0.18 0.07–0.50 (0.09–0.67)	0.19 0.06–0.56 (0.08–0.37)	0.47 0.26–0.86 (0.19–1.02)	0.52 0.22–1.25 (0.28–1.72)	0.89 0.39–2.03 (0.22–3.99)	0.61 0.43–0.88 (0.29–0.91)
$\Sigma$ chlordanes	0.19 0.02–1.74 (0.08–0.44)	0.21 0.14–0.34 (0.12–0.31)	0.34 0.19–0.61 (0.24–0.54)	0.98 0.45–2.14 (0.27–3.12)	1.16 0.85–1.58 (0.81–1.52)	0.74 0.45–1.20 (0.46–2.10)	0.84 0.61–1.14 (0.50–1.36)

<sup>a</sup> HCB = hexachlorobenzene; DDE = dichlorodiphenylethylene; DDD = *p,p'*-dichlorodiphenylethane; HCH = hexachlorocyclohexane; HE = heptachlor epoxide; TCPM = tris(4-chlorophenyl)methanol;  $\Sigma$ chlordanes = sum of oxychlordanes, *trans*-chlordanes, *cis*-chlordanes, *trans*-nonachlor, and *cis*-nonachlor; ND = not detected.

methods of measuring activity of CYP1A-like proteins showed good coherence ( $r^2 = 0.460$ ,  $F_{1,12} = 11.2$ ,  $p = 0.007$ ).

Concentrations of dehydro-retinol in liver displayed significant positive relationships with TEQs, CB-126, and total PCBs in yolk sacs (Table 7 and Fig. 5). Most other measured PCB compounds also regressed significantly with hepatic dehydro-retinol, e.g., CB-101 ( $r^2 = 0.4330$ ,  $p < 0.0001$ ), CB-99 ( $r^2 = 0.4100$ ,  $p = 0.0001$ ), CB-105 ( $r^2 = 0.4004$ ,  $p = 0.0001$ ), and CB-153 ( $r^2 = 0.3530$ ,  $p = 0.0004$ ). Similarly, hepatic levels of ester-2 also displayed a significant positive trend with TEQs and CB-126 but not with total PCBs or *p,p'*-DDE.

#### Comparison between hatched and unhatched eggs

There were no significant differences in concentrations of chemicals measured in unhatched eggs compared with esti-

mates of whole-egg concentrations calculated from yolk sacs (Table 8). The data for *p,p'*-DDE in unhatched eggs was particularly skewed; 8 of 13 values were less than 4 mg/kg, while the other 5 individual eggs contained concentrations of 5.2, 7.6, 8.8, 10, and 23 mg/kg. Among the hatched eggs, 14 of 38 had calculated whole-egg concentrations greater than 4 mg/kg; 10 of those were in the range of 4.0 to 6.0 mg/kg, while 4 eggs had individual concentrations of 7.0, 7.3, 7.8, and 9.0 mg/kg. Logistic regression of log-transformed data did not indicate that any of the contaminants were a significant factor in hatching success; DDE was the parameter closest to significance ( $\chi^2 = 1.05$ ,  $p = 0.307$ ).

#### DISCUSSION

We designed the present study to determine if ospreys breeding at industrial sites throughout the Fraser and Columbia

Table 5. Outcome of laboratory incubation of osprey eggs collected in 1995 and 1996 from the Pacific Northwest

	Nechako River	Pitt River	Thompson River	Upper Columbia	Castlegar	Portland	Lower Columbia
Treatment	Reference	Reference	Pulp mill	Reference	Pulp mill	Mixed exposure	Mixed exposure
No. collected	5	9	5	8	5	11	11
No. hatched	3	5	4	7	5	7	7
% Success	60	56	80	88	100	64	64

Table 6. Biochemical parameters measured in osprey chicks collected in 1995 and 1996 from the Pacific Northwest (arithmetic mean  $\pm$  standard error, wet wt)<sup>a</sup>

	Nechako River (n = 3)	Pitt River (n = 5)	Thompson River (n = 4)	Upper Columbia (n = 7)	Castlegar (n = 5)	Portland (n = 7)	Lower Columbia (n = 7)
CYP1A (pmol/g)	NA <sup>b</sup>	NA	3.17 $\pm$ 1.02	1.98 $\pm$ 0.77	4.75 $\pm$ 0.91	NA	4.60 <sup>c</sup> $\pm$ 1.17
EROD (pmol/min/mg p)	26.2 $\pm$ 10.4	34.3 $\pm$ 8.05	63.5 $\pm$ 12.7	35.1 $\pm$ 8.05	57.3 $\pm$ 9.00	46.9 $\pm$ 6.80	61.6 $\pm$ 8.05
Plasma dehydro-retinol ( $\mu$ g/L)	64.0 $\pm$ 14.1	75.4 $\pm$ 10.9	114 $\pm$ 12.2	117 $\pm$ 9.22	106 $\pm$ 10.9	86.1 $\pm$ 9.22	93.7 $\pm$ 9.22
Kidney dehydro-retinol ( $\mu$ g/g)	0.77 A $\pm$ 0.54	0.68 A $\pm$ 0.42	3.08 B $\pm$ 0.46	1.71 AB $\pm$ 0.35	2.03 AB $\pm$ 0.46	0.73 A $\pm$ 0.35	2.20 AB $\pm$ 0.35
Kidney ester 1 ( $\mu$ g/g)	3.17 BC $\pm$ 0.83	4.42 ABC $\pm$ 0.64	6.20 AC $\pm$ 0.72	3.99 BC $\pm$ 0.54	4.58 ABC $\pm$ 0.72	3.13 BC $\pm$ 0.54	6.61 A $\pm$ 0.54
Kidney ester-2 ( $\mu$ g/g)	1.77 A $\pm$ 0.36	2.42 AB $\pm$ 0.28	2.45 AB $\pm$ 0.31	1.99 A $\pm$ 0.24	2.15 AB $\pm$ 0.31	1.70 A $\pm$ 0.24	3.24 B $\pm$ 0.24
Liver dehydro-retinol ( $\mu$ g/g)	0.77 $\pm$ 0.18	0.60 $\pm$ 0.14	0.85 $\pm$ 0.23	0.70 $\pm$ 0.18	0.73 $\pm$ 0.16	1.04 $\pm$ 0.12	1.28 $\pm$ 0.14
Liver ester-2 ( $\mu$ g/g)	4.63 A $\pm$ 1.08	5.58 AB $\pm$ 0.84	5.05 AB $\pm$ 1.32	4.80 A $\pm$ 1.08	6.85 AB $\pm$ 0.94	5.79 A $\pm$ 0.71	9.36 B $\pm$ 0.84

<sup>a</sup> Means that do not share the same letter (i.e., A, B, or C) are significantly different ( $p < 0.05$ ).

<sup>b</sup> NA = not analyzed.

<sup>c</sup> n = 3.

River basins were affected by chlorinated hydrocarbon contaminants. Survival and health of osprey embryos were not significantly impacted by TCDD-like compounds, total PCBs, or organochlorine pesticides at measured concentrations. As has been shown in a number of other studies of fish-eating

birds, there were correlations between exposure to PCBs and hepatic concentrations of CYP1A and retinolic compounds.

#### Patterns and trends of contaminants in yolk sacs

Spatial patterns of 2,3,7,8-TCDD and 2,3,7,8-TCDF found in osprey eggs in this study are consistent with local pulp mill sources, as reported previously [1]. The highest concentrations were found in eggs taken in 1995 downstream of the pulp mill at Castlegar, which is consistent with a slower post-chlorine-substitution response curve at that site, compared, e.g., with ospreys monitored downstream of the pulp mill at Kamloops on the Thompson River.

We found the highest concentrations of OCDD and other higher chlorinated PCDDs and PCDFs in eggs from the Thompson River, which is also consistent with monitoring data [1]. As in the previous study, yolk sacs with higher concentrations of HpCDD and OCDD also contained 1,2,4,6,8,9-HxCDF and 1,2,4,6,8-PnCDF, which are considered to be indicative of pentachlorophenol sources. Noteworthy are the substantial concentrations of OCDD and HpCDD in yolk sacs of ospreys from the Nechako River, which are consistent with findings in eggs collected at that site in 1992 and which, together with the presence of 1,2,4,6,8,9-HxCDF and 1,2,4,6,8-PnCDF, are indicative of local chlorophenol sources, probably from sawmills in the Vanderhoof area [1].

The spatial pattern of total PCBs, particularly the higher concentrations in the Columbia compared with the Fraser basin, is consistent with the data from monitoring of whole eggs and is attributable to the greater development of hydroelectric generation and associated industries on the Columbia system [25]. Similarly, the data from yolk sac analysis confirms that eggs from the Canadian side of the Columbia River contained greater amounts of Aroclor 1260 type congeners compared with those from the lower Columbia, which contained more Aroclor 1254- and 1242-type peaks.

As in the egg monitoring study, we found higher concentrations of chlordane-related compounds in yolk sacs from the Canadian side of the Columbia River. It is possible those os-

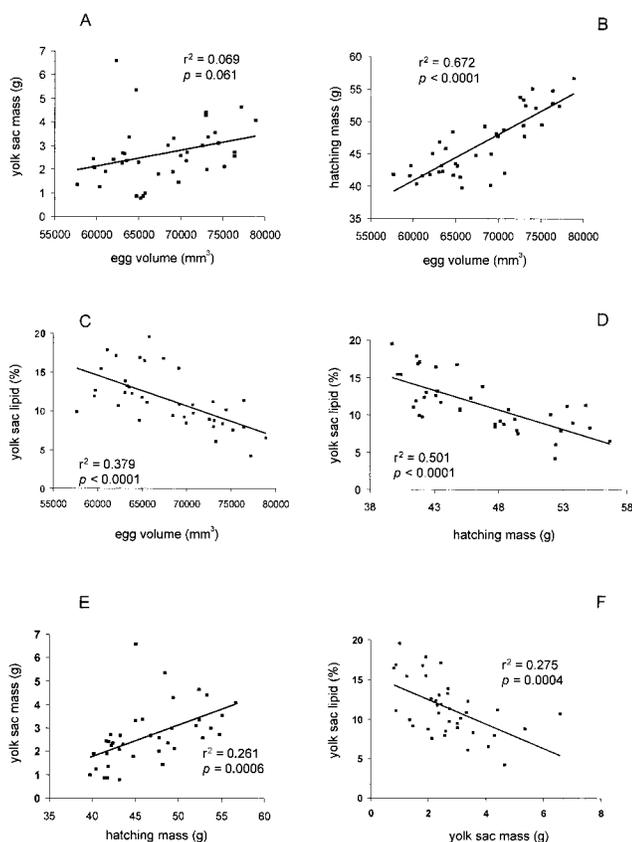


Fig. 3. Relationships between egg volume and yolk sac mass (A), hatching mass (B), and lipid content of yolk sac (C); between hatching mass and lipid content of yolk sac (D) and yolk sac mass (E); and between yolk sac mass and lipid content (F) of osprey chick samples collected in the Pacific Northwest, 1995 to 1996.

Table 7. Concentration–effect relationships between biochemical measurements and chlorinated hydrocarbon concentration in yolk sacs of osprey chicks collected in 1995 and 1996 from the Pacific Northwest<sup>a</sup>

Parameter	n	TEQs		PCBs		CB-126		TCDD		p,p'-DDE	
		r <sup>2</sup>	P	r <sup>2</sup>	P	r <sup>2</sup>	P	r <sup>2</sup>	P	r <sup>2</sup>	P
CYP1A	18	0.252	0.017	0.227	0.023	0.139	0.065	0.086	0.119	0.375	0.003
EROD	32	0.395	<0.0001	0.367	0.0002	0.144	0.018	0.081	0.063	0.052	0.115
Liver dehydro-retinol	28	0.219	0.006	0.385	0.0002	0.282	0.002	0.036	0.860	0.126	0.033
Liver ester-2	28	0.169	0.015	0.093	0.059	0.118	0.038	0.039	0.157	0.001	0.334

<sup>a</sup> TEQs = toxic equivalents; PCBs = polychlorinated biphenyls; TCDD = tetrachlorodibenzo-*p*-dioxins; DDE = dichlorodiphenylethylene.

preys were exposed to elevated chlordane while wintering around the Gulf of Mexico [25].

#### Laboratory hatching success

The overall laboratory hatching success (hatch of all eggs set, including infertiles) of 70% was similar to the field value of 73% reported for reference nests by Woodford et al. [6] and was greater than the 43% reported by Wiemeyer et al. [4] for unmanipulated eggs. Thus, it appears that collection, transport, and artificial incubation did not have an undue effect on overall hatching rate of osprey eggs.

Laboratory studies have found 2,3,7,8-TCDD and related iso-stereomers to be lethal at very low concentrations when injected into chicken eggs but to be less toxic to other avian species [7]. Most research on birds, primarily with fish-eating species, has not shown TCDD or related compounds to cause embryo lethality at concentrations encountered in the field. Studies of Forster's terns (*Sterna forsteri*) from the early 1980s

in Lake Michigan reported a total mean TEQ of 2,175 ng/kg (wet wt, whole egg), derived from TCDD and from PCBs using older toxic equivalency factors; hatching success was reduced by 50% in tern eggs collected and incubated in the laboratory at that time [11,27]. Herring gull eggs collected from colonies in Lake Ontario during the 1970s had TEQ concentrations in the thousands of parts per trillion [28], within the range of potential lethality, although those gull eggs also contained high concentrations of an array of other potent embryotoxins [29]. An initial report [30] that TEQs in the range of 200 to 300 ng/kg (H4IIE-derived TEQs) were associated with embryo mortality in double-crested cormorants (*Phalacrocorax auritus*) has not been substantiated by subsequent experiments [31]. In great blue heron chicks (*Ardea herodias*), mean TEQs of 472 ng/kg (recalculated using the latest toxic equivalency factors [26,32]) affected some biochemical and morphological variables but did not reduce survival of embryos [13,33].

In raptor species, reported no-observable-adverse-effect levels are at least 136 ng/kg TEQs for embryo survival in ospreys [6] and 210 ng/kg (Ahlborg et al. toxic equivalency factors [34]) in bald eagles [14]. In the present study, mean TEQs calculated on a whole-egg basis were 82 ng/kg for the entire 38 hatched eggs; concentrations were higher, 126 ng/kg, in five eggs from Castlegar that hatched. Those values were not different from the mean value of 77 ng/kg in the 13 unhatched eggs or even from the 6 unhatched eggs from the lower Columbia River of 134 ng/kg (range 45–450 ng/kg) TEQs. There is no indication, therefore, that 2,3,7,8-TCDD TEQs, at the concentrations encountered here, had a significant effect on hatching success of osprey chicks.

Polychlorinated biphenyl mixtures and individual non-*ortho* PCBs show a similar pattern of avian interspecific toxicity as TCDD, with chickens being particularly sensitive and other species less so [7,35]. We found no differences in concentrations of total PCBs or any individual congeners between hatched and unhatched eggs. Previous studies have failed to

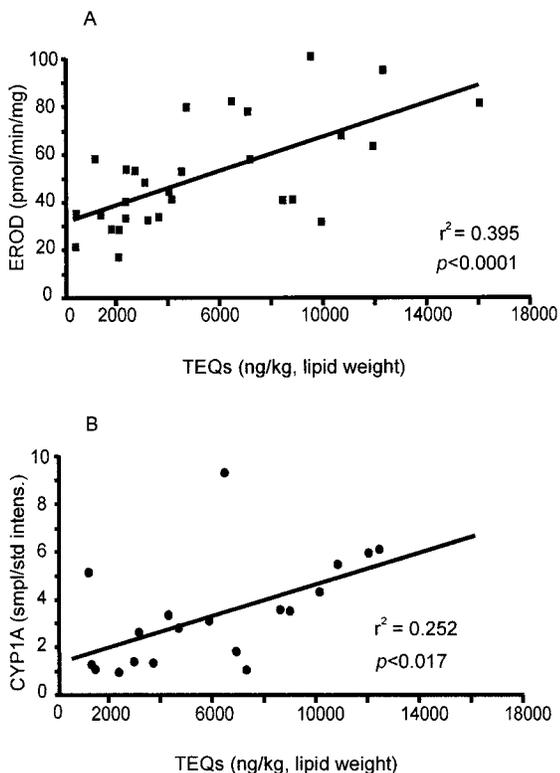


Fig. 4. Exposure–response relationships between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs) and hepatic ethoxyresorufin-*O*-deethylase (EROD) activity (A) or CYP1A concentrations (B) in osprey chicks from the Pacific Northwest, 1995 to 1996.

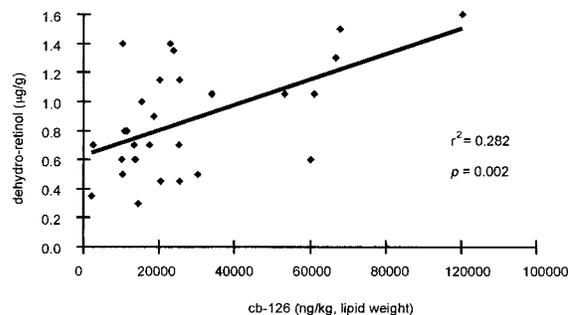


Fig. 5. Relationship between dehydro-retinol in liver and CB-126 concentration in yolk sacs of osprey chicks collected from the Pacific Northwest, 1995 to 1996.

Table 8. Comparison of concentrations of chemical contaminants measured in unhatched eggs and concentrations in whole eggs estimated from yolk sac values for hatched eggs for ospreys from the Pacific Northwest, 1995 to 1996<sup>a</sup>

	Concentration (geometric means, range; wet wt)	
	Unhatched eggs (n=13)	Hatched eggs (n=38)
<i>p,p'</i> -DDE (mg/kg)	3.33 (0.81–22.9)	2.62 (0.40–9.22)
PCBs (mg/kg)	1.08 (0.15–10.0)	0.83 (0.14–6.67)
CB-126 (ng/kg)	2.93 (27.3–2,760)	210 (37.0–1,570)
2,3,7,8-TCDD (ng/kg)	2.21 (0.17–25.5)	4.34 (0.20–59.4)
TEQs (ng/kg)	45.8 (8.63–450)	61.7 (6.03–254)

<sup>a</sup> DDE = dichlorodiphenylethylene; PCBs = polychlorinated biphenyls; TCDD = tetrachlorodibenzo-*p*-dioxins; TEQs = toxic equivalents.

find any effect of PCBs on osprey reproduction [36], even at mean concentrations of 25 mg/kg in eggs [37].

Critical concentrations of DDE in osprey eggs have been estimated to be 4 mg/kg, a value associated with 0.8 young fledged per active nest, a rate needed to maintain osprey population stability [36]. Reduced hatching success has been determined to occur within the range of 5 to 10 mg/kg DDE in egg [37]. In the present study, a substantial fraction of both hatched (34%) and unhatched (38%) eggs contained greater than 4 mg/kg DDE. However, a larger proportion of unhatched eggs (4/13) than hatched eggs (4/38) had greater than 6 mg/kg DDE, and concentrations greater than 10 mg/kg were found only among unhatched eggs. Based on these data, we would speculate that the critical concentration of DDE affecting hatching success of ospreys is toward the higher end of the 5 to 10 mg/kg range. Mean concentrations of other organochlorine pesticides, including dieldrin and oxychlorane, were lower in unhatched eggs than those associated with embryotoxicity in laboratory and field studies with raptors and other wild birds [38].

We reported previously that 23% of osprey eggs collected from 1991 to 1997 across the same areas studied here had greater than 4 mg/kg DDE [25] and that there was a significant negative correlation between DDE and shell thickness. Outside of specific hot spots such as the lower Columbia River, instances of elevated DDE in ospreys were thought to have originated from exposure on the wintering grounds in Mexico. A number of other studies conducted during the 1990s have found continuing effects of DDE, e.g., on reproduction of double-crested cormorants [31] and on shell thickness of great blue herons [15].

#### Morphological and histological parameters

We found no evidence in these osprey chicks of any exposure-related pattern of edema, and only one mild deformity was noted. Among studied species, the chicken embryo is most sensitive to 2,3,7,8-TCDD, not only exhibiting lethality as discussed above but also a suite of other effects, particularly cardiac malformations, at 9 ng/kg egg concentration [7]. Some of the endpoints observed in chickens (e.g., edema, reduced hatching weight, and deformities) have been reported in fish-

eating birds, particularly from the Great Lakes [3], and in great blue heron chicks from near a pulp mill site in British Columbia [13]. Although derived from various toxic-equivalent schemes, TEQ concentrations in those cases were generally greater than 500 ng/kg, much higher than we measured in osprey chicks.

There were weak negative relationships between yolk sac mass and both TEQs and TCDD, which appear to be caused by the higher lipid concentrations in smaller yolk sacs. Similar negative relationships between yolk sac mass and contaminant concentrations were reported previously in bald eagles and cormorants from British Columbia [13]. A study of cormorants (*Phalacrocorax carbo*) in the Netherlands [10] found significant negative relationships between yolk sac mass and mono-ortho PCBs, although there was no significant relationship between mass and lipid content of yolk sacs in those cormorant chicks.

Lipid content and mass of yolk sacs in these ospreys also correlated with egg volume and hatchling mass. Murvoll et al. [39] reported that lipid content of yolk sac regressed significantly with egg volume and hatchling weight and therefore PCBs and body weight in Norwegian shags (*Phalacrocorax aristotelis*). They argued that their results and a number of the previously reported relationships between embryonic body weight and contaminants were the result of covariation between body weight and lipid content rather than being cause and effect. Recently, Custer et al. [31] also found a significant negative correlation of PCBs with egg volume and embryo weight in cormorants, although they did not report yolk sac size or lipid content. It is not clear why we found only weak positive relationships for a few contaminants, e.g., TEQs and some PCB congeners, with egg volume and yolk-free body weight. However, the studies of Custer et al. [31] and Murvoll et al. [39] focused on single colonies, whereas we collected from seven widely separated sites.

#### Biochemical responses

Our results indicate that ospreys have a CYP1A-like protein that is inducible by iso-stereomers of TCDD. We interpret the significant relationship between DDE and CYP1A as a result of correlation between DDE and the typical CYP1A inducers. Induction of EROD activity and/or immunoblotting measurement of CYP1A has been shown in a wide variety of wild birds [e.g., 10,13,40]. In ospreys, induction was measurable statistically over a 10-fold range of exposure, 1,400 to 14,000 ng/kg TEQs. We found that absolute EROD activity was about 10-fold higher in ospreys than reported for bald eagles [14]. Notwithstanding different methods of preparing microsomes between the osprey and eagle studies, we observed previously that this indication of higher P450 activity in ospreys was consistent with the findings of a much higher ratio of oxychlorane to *trans*-nonachlor in ospreys, an indication of increased capacity to metabolize xenobiotics [1].

Mean EROD activity at the Nechako River site was 26.2 pmol/min/mg, virtually at the *y*-intercept (32 pmol/min/mg) when EROD is regressed against TEQs. Mean TEQs at the Nechako River were 37 ng/kg calculated on a wet weight whole-egg basis; we suggest this value as a no-effects or even a no-response concentration for TEQs in osprey chicks. Induction, indicated by an approximate twofold increase in either CYP1A protein concentration or EROD activity, occurred at calculated whole-egg TEQs of about 130 ng/kg.

Previous investigations of avian species breeding near

bleached-kraft pulp mills found that regression of CYP1A type endpoints against 2,3,7,8-TCDD or 2,3,7,8-TCDF produced the best coefficients of determination ( $r^2$ ) [13,14]. However, in the present data set on ospreys, the best fits are with TEQs and total PCBs and with non-*ortho* PCBs, while there were no significant relationships with 2,3,7,8-TCDD or -TCDF. Such findings are in accord with most published studies of avian species [e.g., 10,12,40]. We conclude that, when the present study was undertaken in 1995 and 1996, as a result of chlorine-substitution programs at the targeted pulp mills, concentrations of 2,3,7,8-TCDD and -TCDF had decreased to the point where they were relatively inconsequential relative to the PCB congeners [1]. Similarly, a study conducted in 1983 on herring gulls in Lake Ontario found a significant positive relationship between hepatic aryl-hydrocarbon hydroxylase activity and 2,3,7,8-TCDD but not with PCBs [41]. Another study in 1997 failed to find any relationship between hepatic EROD activity and any of a wide range of chlorinated hydrocarbons in herring gull chicks [42]. However, mean concentrations of 2,3,7,8-TCDD had decreased by greater than 50% in herring gull eggs collected between 1982 and 1991 at a Lake Ontario monitoring colony [43].

The effects of vitamin A deficiency on development have been investigated in chickens [44]. In mammals, PCBs are reported to depress circulating retinol in plasma. The mechanism is thought to be mediated via binding of hydroxylated PCB metabolites to the protein transthyretin, which impairs formation of a retinol-binding protein-transthyretin plasma transport complex. Unbound plasma retinol is then cleared by glomerular filtration [45]. In wild birds, there are reports of associations between PCBs and plasma retinol concentrations, although the regressions are generally of a positive form. Murvoll et al. [39] recently reported a positive relationship between plasma retinol and total PCBs in shags. Murk et al. [46] reported a positive relationship between plasma retinol and hepatic EROD in common tern chicks. In osprey chicks, we found no differences among sites or significant relationships between concentrations of retinolic compounds in plasma and PCBs or other contaminants in yolk sacs. There were significant intersite differences in renal retinol-related parameters but no relationships with any of the contaminants measured in yolk sacs. For hepatic dehydro-retinol, there were significant positive correlations with total PCBs, a number of PCB congeners, including CB-126, and TEQs, and for retinyl-ester 2 with TEQs. A similar relationship between hepatic retinyl-palmitate and CB-126 was reported in bald eagle chicks [14]. Bishop et al. [47] found a negative relationship between liver retinol and EROD activity in tree swallow (*Tachycineta bicolor*) chicks. In the original studies of Spear and colleagues [48], an increase in the molar ratio of retinol to retinyl palmitate in herring gull yolk sacs correlated positively with egg concentrations of 2,3,7,8-TCDD and TEQs. Further study of the nature and consequences of vitamin A to contaminant exposure in birds is perhaps warranted.

#### SUMMARY AND CONCLUSIONS

Concentrations of PCDDs, PCDFs, and PCBs were higher in yolk sacs of osprey chicks collected downstream of pulp mill sites and industrial centers, such as Portland, compared with reference sites. However, there were no significant differences in hatching success among reference and treatment sites. There were also no differences in most morphological, histological, and physiological parameters among locations.

Contaminant concentrations based on estimated whole-egg values for hatched eggs compared to measured values for non-hatched eggs revealed no differences for TEQs, *p,p'*-DDE, PCBs, or other chlorinated hydrocarbon compounds. Our results suggest that the critical concentration of DDE affecting hatching of ospreys is toward the higher end of the range of 5 to 10 mg/kg. Our data support the value of 136 ng/kg TEQs (wet wt) in whole eggs suggested by Woodford et al. [6] for a no-observable-adverse-effect level for hatching in ospreys as a conservative estimate based on available data. Significant correlations between measurements of hepatic CYP1A and TEQs and various PCB compounds show a biochemical response to exposure in osprey chicks. Our data indicate a no-response level of 37 ng/kg TEQs and a lowest observed effect level of 130 ng/kg TEQs for CYP1A in osprey chicks. There were also significant positive correlations between dehydro-retinol and an unidentified retinolic ester in liver and yolk sac concentrations of TEQs, PCBs, and some non-*ortho* PCB compounds.

We conclude, therefore, that iso-stereomers of TCDD, primarily CB-126, induced hepatic CYP1A in osprey chicks at 130 ng/kg (whole egg, wet wt, lowest contaminant effect/response level); that PCBs correlated with hepatic dehydro-retinol concentrations, consistent with a number of field reports, but not with proposed mechanisms from laboratory studies; and that *p,p'*-DDE probably affected survival of individual osprey embryos.

The food chains of the Fraser and Columbia River systems continue to be contaminated with a wide array of persistent chlorinated hydrocarbon contaminants, particularly in the lower reaches. Osprey populations breeding on these river systems exhibit physiological responses to TCDD-like compounds and effects of *p,p'*-DDE on survival of individual eggs. However, average contaminant concentrations in both systems are less than those required to have a significant impact on hatching success of ospreys.

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