ENVIRONMENTAL CONTAMINANTS IN BALD EAGLES NESTING ALONG THE LOWER COLUMBIA RIVER

INTERIM REPORT

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INTRODUCTION

The lower Columbia River, located along the border of Oregon and Washington (Figure 1), supports a variety of fish and wildlife resources. The area provides important foraging habitat for bald eagles (*Haliaeetus leucocephalus*), which are currently listed in Oregon and Washington as a threatened species under the Endangered Species Act of 1973, as amended. Forty-one nesting pairs of bald eagles and a wintering population of over 100 birds currently use the lower Columbia River (Garrett et al. 1988, Issacs and Anthony 1995). Bald eagle nesting territories and productivity of eagles along the lower Columbia River have been monitored since the early 1970s, and the number of occupied nesting territories has increased in the last six years. However, five-year productivity averages have been nearly 50 percent lower than five-year, state-wide averages for eagles nesting in either Oregon or Washington (Figure 2; Issacs and Anthony 1995; Washington Department of Fisheries and Wildlife, unpubl. annual census reports). While productivity levels of eagles along the lower Columbia River are low, levels of nesting populations in other areas of the two states are nearing some of the recovery guidelines required to remove the species from the endangered species list (U.S. Fish and Wildlife Service 1986).

The organochlorine pesticides DDT, dieldrin, and their metabolites have been implicated in nationwide population declines of bald eagles, either though direct mortality or from reductions in productivity (Prouty et al. 1977, Kaiser et al. 1980, Wiemeyer et al. 1993). During a study conducted along the lower Columbia River from 1985 to 1987, Anthony et al. (1993), found elevated concentrations of DDE and polychlorinated biphenyls (PCBs) in bald eagle eggs, in blood obtained from eight- to ten-week-old nestlings, and in eagle carcasses collected near the river. Eggshell thinning, commonly associated with DDE concentrations, was also observed in this study and was correlated with poor reproductive success (Anthony et al. 1993). Prey items of eagles (primarily fish) exhibited detectable concentrations of DDE, PCBs, and other organochlorines (Anthony et. al. 1993). In addition, evidence of DDE and PCBs in blood of eagle nestlings indicated that recent exposure was occurring after the nestlings fed on contaminated prey items taken from the river (Anthony et al. 1993).

The primary sources of organochlorine pesticides and PCB contaminants in Columbia River bald eagle tissues are largely unknown. The use of large quantities of DDT in orchard crops in the Columbia Basin prior to 1974 (Terriere et al. 1966, Blus et al. 1987) and use of PCBs in electrical transformers or as dust suppressants could have contributed to the organochlorine burdens found in biota associated with the river. DDT and its metabolites are very persistent and they have recently been detected in water in the Yakima River Basin, which drains into the Columbia River (Rinella et al. 1992). Dredging activities conducted in the river to maintain a navigation channel could be resuspending persistent compounds and increasing their bioavailability. In laboratory studies with fish, Seelye et al. (1982) demonstrated the potential for uptake of contaminants such as DDE and PCBs from dredging. Steidl et al. (1991) found organochlorine contaminants highest in eggs of ospreys (*Pandion haliatus*) nesting near Delaware Bay. The authors suspected high concentrations of contaminants detected in upper portions of the estuary, and resuspension of contaminants by dredging, were the probable source of contaminants in the osprey eggs.

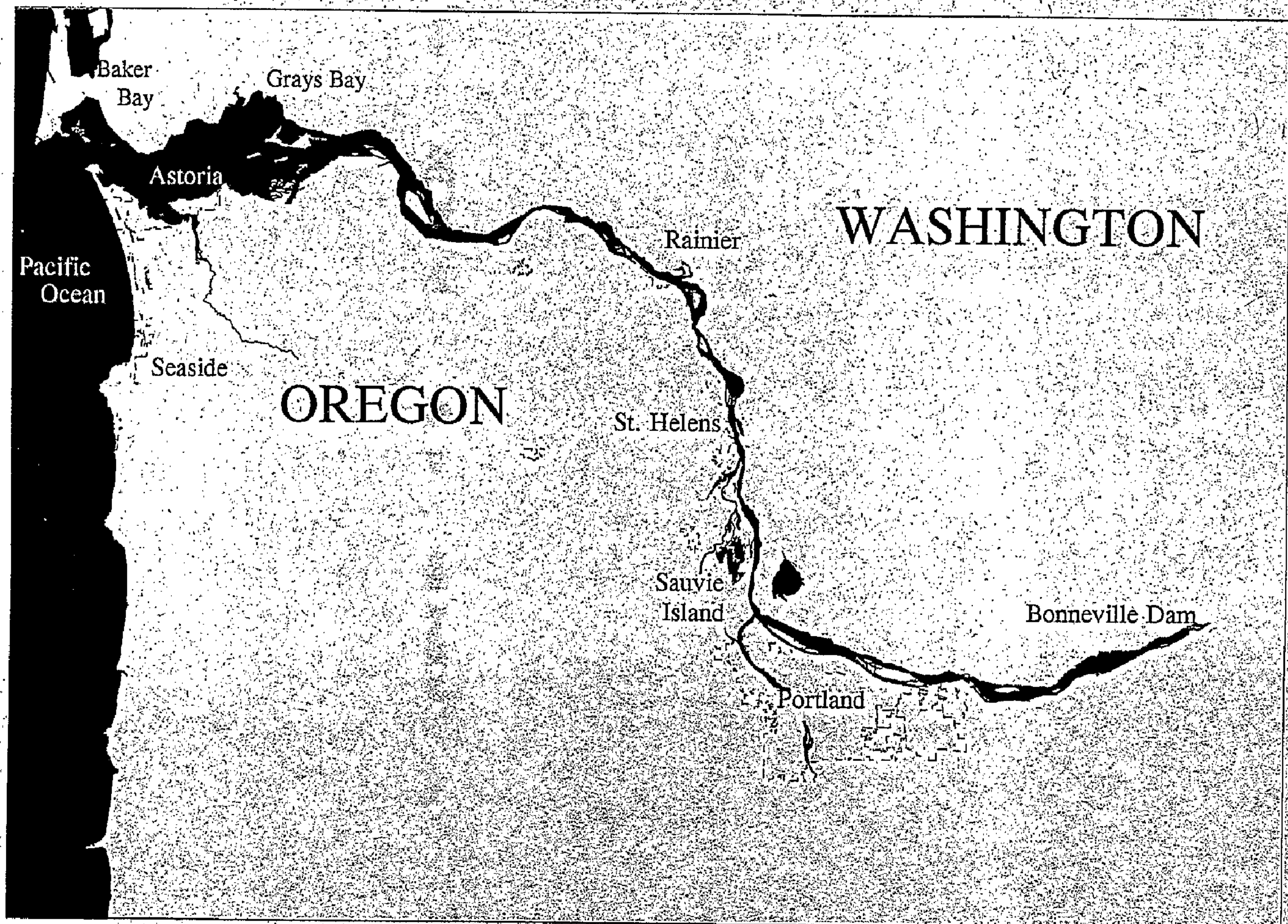

Figure 1. Bald eagle study area (mouth of the Columbia River to the Bonneville Dam).

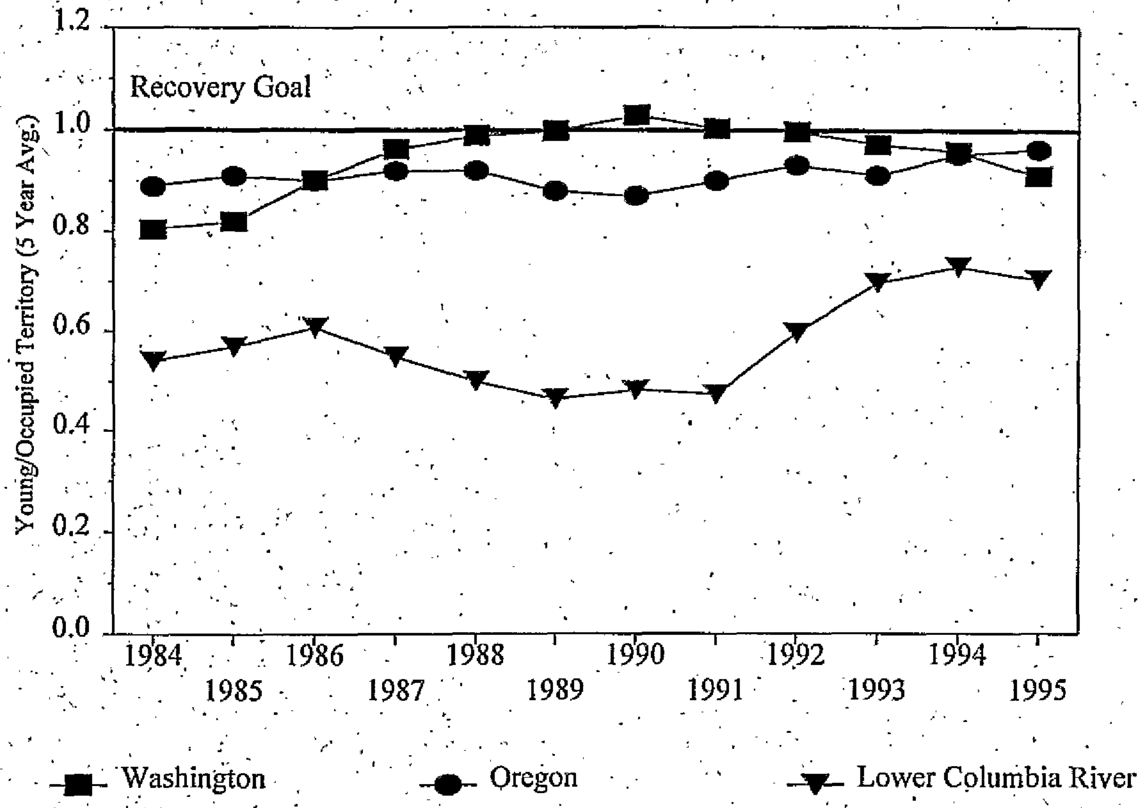

Figure 2. Five-year average productivity (young produced/occupied territory with known outcome) for bald eagles nesting in Washington, Oregon, and the lower Columbia River.[1] Statewide values for Washington from 1993 to 1995 are estimates. Statewide data include values from the lower Columbia River.

Bald eagle eggs collected from the estuary in 1987 and 1991 also exhibited elevated concentrations of 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlordibenzofuran (TCDF; Anthony et al. 1993). The TCDD concentrations found in the five eggs analyzed were within a range of TCDD concentrations found in eggs of fish-eating birds exhibiting poor reproductive success in Michigan (Kubiak et al. 1989, Anthony et al. 1993). In addition, analysis of fish collected from the lower Columbia River revealed TCDD concentrations exceeding human health guidelines (U.S. Environmental Protection Agency 1986a; U.S. Fish and Wildlife Service, unpubl. data). High TCDD concentrations in the fish tissues led to the designation of the Columbia River as "Water Quality Limited" in 1990. The U.S. Environmental Protection Agency (EPA) restricted the concentration of allowable dioxin in the river through the establishment of a Total Maximum Daily Load (TMDL) for TCDD in order to protect aquatic resources.

The current investigation was designed to evaluate the reproductive success of bald eagles nesting along the lower Columbia River and to identify specific organochlorine residues in the eggs of these eagles. This interim report summarizes the results of productivity data gathered in 1994 and 1995, and reports the residue results from eagle eggs analyzed in 1994. Residue results for bald eagle eggs collected in 1995 were not available for inclusion in this report, but will be included in a final report scheduled for completion in the Fall of 1996. Information obtained from the study will be used to determine if organochlorine compounds continue to accumulate in eagle eggs and assess whether planar polychlorinated hydrocarbons (PCHs), which include dioxins, furans, and planar PCBs, are contributing to the reduced reproductive success observed in eagles nesting in this area. The results will assist resource management agencies in their assessment of water quality standards for the lower Columbia River and its tributaries and in the management of eagle populations in the area.

METHODS

Productivity Surveys and Site Selection

Productivity of bald eagle nest sites was determined in 1994 and 1995 by helicopter overflights and ground surveys, in coordination with annual state-wide surveys conducted by the Oregon Cooperative Wildlife Research Unit (OCWRU) and the Washington Department of Fisheries and Wildlife (WDFW). Overflights were conducted in April to determine site occupancy and nesting status, in May to confirm nesting status and to locate abandoned nests containing nonviable (addled) eggs, and in early July to count juveniles in nests and evaluate final productivity. The outcome of nests not monitored by helicopter surveys were determined by volunteers who monitor nests on an annual basis and report findings to OCWRU, WDFW, or the Ridgefield National Wildlife Refuge in Washington.

Selection of nesting sites for egg collection occurred immediately following the April overflight. Sites selected for sampling included nests where eggs were collected in previous contaminant investigations, nests exhibiting historically low productivity or repeated nesting failures, and nests in recently established territories.

Egg Collection and Processing

Bald eagle eggs were collected in April and May of 1994 and in April of 1995. Nest trees were climbed by professional tree climbers with experience handling eagle eggs. Only one fresh egg was collected from active nests, and all eggs were collected from abandoned nests. Each collected egg was wrapped in aluminum foil, placed in a well padded coffee can, and lowered to an observer or brought down by the tree climber in a backpack. The can containing the egg was placed in a cooler with Blue Ice® and transported to the U.S. Fish and Wildlife Service's Oregon State Office in Portland, where it was refrigerated at 3 °C until processing. In addition to eggs, one common carp (*Cyprinus carpio*) and one starry flounder (*Platichthys stellatus*) were collected from two different nest sites in 1994. The prey items were double bagged and frozen at -20 °C until shipment to the contract laboratory.

Eagle egg processing occurred within 24 hours of collection and included measuring length, breadth, whole egg mass, and volume by water displacement. Eggs were cut along the equator and the contents removed to determine embryo mass. Development stage was noted as early, mid, or late in comparison to developing pheasant (*Phasianus colchicus*) embryos (Fant 1957). Egg contents were stored in chemically-cleaned jars and frozen at -20°C until shipment to laboratories conducting residue and bioassay analyses.

Eggshell thickness (shell and membranes) was measured on shells from 25 bald eagle eggs collected intact and on eggshell fragments from four damaged eggs. Each eggshell half from the intact eggs was rinsed with water and air-dried for a minimum of 30 days after harvesting. Eggshell thickness was measured with a dial micrometer with rounded contacts at five sites along the equator on each eggshell half, resulting in 10 measurements per egg. The 10 measurements were averaged to determine the mean thickness of each shell. Thickness was measured on the four damaged eggs using eggshell fragments large enough to approximate the equator region of the egg. A minimum of five measurements were collected and averaged for the damaged eggs. For each eggshell with detached membranes, the estimated thickness (0.13 mm; Stan Wiemeyer, U.S. Fish and Wildlife Service, Reno, Nevada, pers. comm.) of bald eagle eggshell membranes was added to the mean thickness measurement. Eggshell thinning was determined as the percent difference between eggshell thickness of each egg and mean eggshell thickness (0.6088 mm) determined for bald eagle eggs collected in the Northwest prior to 1947, when DDT was not in widespread use (Dan Anderson, Univ. Calif., Davis, unpubl. data).

Residue Analysis

Eagle embryo samples and prey items were shipped overnight on dry ice to laboratories contracted through the National Biological Service, Patuxent Environmental Science Center for residue analysis. Embryos and prey items were chemically analyzed for PCHs at Midwest Science Center, Columbia, Missouri. The PCHs included eight polychlorinated dibenzo-*p*-dioxins (PCDDs), ten polychlorinated dibenzofurans (PCDFs), and non ortho- and mono ortho-substituted PCBs (planar PCBs). Nomenclature for the planar PCB congeners discussed in this report follow International Union of Pure and Applied Chemists (IUPAC) numbers (Ballschmiter and Zell 1980). Non-ortho PCB congners analyzed include PCB 77 (3,3′,4,4′-tetrachlorobiphenyl), PCB 81 (3,4,4′,5-tetrachlorobiphenyl), PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl), and PCB 169 (3,3′,4,4′,5,5′-hexachlorobiphenyl). Embryos and prey items also were chemically analyzed for organochlorine pesticides, total PCBs, and mercury at Hazleton Laboratories America, Inc., Madison, Wisconsin.

Egg and fish tissue samples were prepared and analyzed for PCHs according to Feltz et al. (1995). Approximately 25 g aliquots of egg or fish tissue were homogenized with anhydrous sodium sulfate for dehydration and extracted with methylene chloride in an extraction column. Extracts were concentrated by rotoevaporation and treated by a 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 by an automated C-18/PX-21 carbon column high performance liquid chromatography system, isolating four fractions: 1) bulk and di-ortho-PCB congeners; 2) mono-ortho-PCB congeners; 3) non-ortho-PCB congeners; and 4) PCDD/PCDFs. Following isolation, the PCDD/PCDF fraction was eluted through basic alumina for removal of potential co-contaminants (ethers and residual polychlorinated napthalenes and PCBs), and the alumina fractions were transferred under a steam of nitrogen.

Aliquants of purified sample extracts were analyzed by capillary gas chromatography/electron capture detection (CGC/ECD) to measure mono ortho-PCB residues (Schwartz and Stalling 1991). The PCDD/PCDF and non ortho-PCB fractions were determined by gas chromatography/high resolution mass spectrometry by monitoring five sequential windows of selected ions during the chromatographic separation (Kuehl et al. 1991).

Sample preparation, extraction, and cleanup of organochlorines pesticides and total PCBs followed methods outlined by the EPA (1986b). Samples were homogenized, ground and prepared with anhydrous sodium sulfate. Analytes were recovered by Soxhlet extraction using the solvent methylene chloride and concentrated in Kaderna-Danish apparatus. Sample cleanup occurred by gel-permeation chromatography. Additional cleanup and separation of PCBs from organochlorine pesticides was conducted using silica gel.

Sample preparation for mercury analysis included digestion with a sulfuric and nitric acid mixture and reduction of mercury using sodium borohydride (Monk 1961). Mercury was determined by cold vapor atomic absorption (Hatch and Ott 1968). The detection limit for this procedure was <0.01 μg/g.

Laboratory quality control samples for all residue analyses consisted of procedural blanks, duplicates, and spiked samples. Accuracy and precision as determined by spike sample recovery and duplicate sample analysis were within specified ranges for most organochlorine pesticides and total PCBs. Quality control samples for planar PCH analysis also included chicken egg (matrix) blanks, chicken egg spikes, and positive control Saginaw Bay carp samples processed and analyzed concurrently with actual samples (see Appendix 1). Recoveries of the ^{13}C-labeled PCHs concentrations were generally within range ($\pm$ 20%) of expected values. Analyte concentrations in control carp compared closely with previous QA/QC data.

H4IIE Rat Hepatoma Cell Bioassay

Embryo and prey item tissues were used in a H4IIE rat hepatoma cell bioassay to assess exposure to all planar halogenated compounds with the ability to biochemically elicit dioxin-like toxicity. The bioassay was conducted at the Midwest Science Center following the methods of Ribick et al. (1981) as modified by Tillitt et al. (1991a). The potency of extracts tested in the H4IIE bioassay is compared to the potency of TCDD. The results are expressed as TCDD-equivalents (TCDD-EQs) and are a reflection of the overall dioxin-like potency found in the sample, inclusive of TCDD and all other planar halogenated compounds in the samples (Tillitt et al. 1991a). The H4IIE bioassay is a screening tool and it is not completely understood if the potency of the planar compounds in the bioassay are directly related to the overall potency of these compounds to cause embryo lethality in bald eagles. The potency of planar compound mixtures in the H4IIE cells has been correlated to the hatching success in double-crested cormorants (*Phalacrocorax auritus*) from the Great Lakes (Tillitt et al. 1992).

Data Analyses
Contaminant residues were compared for bald eagle eggs collected in 1994 only. Analytical chemistry results have not been completed on eggs collected in 1995. Arithmetic means were calculated on organochlorine pesticide and mercury residues found in eggs collected in 1994 and compared to residue concentrations in bald eagle eggs in various locations in the United States (Wiemeyer et al. 1993) and from data collected previously from the lower Columbia River (Anthony et al. 1993).

Bald eagle breeding success (number of young produced/number of breeding attempts) for eagle pairs in the lower Columbia River was compared to percent eggshell thinning using simple linear regression. Eggshell measurements for shells of all fresh and addled eggs and all eggshell fragments collected in 1994 and 1995 were used in the analysis. Breeding information during years when fresh eggs were collected from a nest within a territory were excluded from the breeding success calculation. Breeding success will be compared to organochlorine pesticide concentrations in all eagles eggs collected from the lower Columbia River after analytical chemistry results are received on bald eagle eggs collected in 1995. All statistical tests were performed at the 0.05 level of significance.

The overall dioxin-like potency of PCHs in bald eagle egg tissues were summarized as TCDD toxic equivalents (TEQs). TEQs were determined by normalizing concentrations of individual PCHs relative to the potency of TCDD using toxic equivalency factors (TEFs; Ahlborg et al. 1992). The concentration of each PCH in an egg sample was multiplied by its corresponding TEF value. The values obtained for each PCH were then summed, which resulted in a single TEQ value of dioxin-like potency for a sample. TEQs for a set of samples can then be averaged and compared among populations.

Normalization of the concentrations of individual PCHs relative to the potency of TCDD is routinely accomplished with international TEFs (I-TEFs; Ahlborg et al. 1992). The I-TEFs are designed for use in risk assessment and based on mammalian toxicological endpoints. The only TEFs for avian risk assessment were derived from studies with chickens (C-TEFs; reviewed by Bosveld et al. 1995). TEQs were calculated in the present study using both I-TEFs and C-TEFs and are represented as I-TEQ for the mammalian based TEFs and C-TEQ for the avian based TEF values.

TEFs have been determined for many PCHs. However, some authors of previous studies have evaluated TEQs using TEFs determined for only PCDD and PCDF, while others have included TEF values for planar PCBs in the TEQ calculation. We determined both I-TEQ and C-TEQ values for bald eagle egg tissue using TEF values for PCDDs and PCDFs only, and also by calculating I-TEQ and C-TEQ values using TEFs determined for the PCDDs, PCDFs, and the planar PCBs. Thus, four separate TEQ values were used to assess dioxin-like potency in the bald eagle egg samples.

Residue values for each egg were adjusted for moisture and lipid loss using volume measurements. Volume was estimated using the mean of the whole egg volume and the egg content volume calculated from length and breadth measurements (Stickel et al. 1973). Contaminant concentrations were assigned a value of one-half the detection or quantification limit for egg samples that were below these limits for computational purposes. Concentrations of PCHs below detection or quantification limits were not used in the calculation of TEQs. All residues are reported as μg/g fresh weight for organochlorine pesticides and pg/g fresh weight for PCHs, unless otherwise noted.

RESULTS

Productivity

Bald eagles nesting along the lower Columbia River occupied 40 nesting territories in 1994 and 41 in 1995, and produced 0.70 and 0.54 young/occupied territory, respectively. Annual productivity was 23% lower in 1995 compared to 1994. Annual productivities were very similar on the Oregon and Washington sides of the river in 1994 and in 1995 (Table 1). However, annual productivities were lower by 18% in Oregon and 34% in Washington in 1995 compared to 1994 (Table 1). Productivity of the lower Columbia River eagles was very low in 1995 and at least ten unclimbed nest sites were abandoned at some point after initiation of incubation. Annual productivity of eagles nesting along the river was lower than statewide values by 23 to 28% in 1994 and 37 to 44% in 1995 (Table 1).

Table 1. Mean annual bald eagle productivity (young/occupied territory) within the lower Columbia River and statewide productivity in Oregon and Washington in 1994 and 1995. Statewide values include productivity values from the lower Columbia River.

	Lower Columbia River				Statewide		
Year	OR	WA	OR/WA	Total Occ. Terr.[a]	OR	WA	Total Occ. Terr.[b]
1994	0.71	0.76	0.70	40	0.97	0.91[c]	767
1995	0.58	0.50	0.54	41	0.96	0.86[c]	584

[a] Total occupied territories in the lower Columbia River.
[b] Total occupied territories in Washington and Oregon. Values for Washington are estimates and are not based on complete counts (Issacs and Anthony 1995, Washington Department of Fisheries and Wildlife, unpubl. annual census reports).
[c] Value may be underestimated due to incomplete survey.

Five-year average productivity for bald eagles nesting in the lower Columbia River was 0.73 young/occupied territory during the five-year period ending in 1994 and 0.70 young/occupied territory for the five-year period ending in 1995. The five-year average productivity values ending in 1993, 1994, and 1995 were higher than during any previous five-year time period (Figure 2). Statewide five-year average productivity in Oregon was 0.95 and 0.96 young/occupied territory for the periods ending in 1994 and 1995, respectively. Washington statewide five-year average productivities were 0.96 and 0.91 young/occupied territory for periods ending in 1994 and 1995, respectively. In Oregon, bald eagles occupied 230 territories in 1994 and 245 territories in 1995. In Washington, the number of territories was estimated at 537 in 1994 and 339 in 1995.

Egg Collection

Bald eagle eggs were collected from nest sites in 19 different territories over the two-year study. In 1994, fresh eggs were collected from nine territories, a broken egg from one territory, and five addled eggs from three territories. Seven of these territories were in Washington and five were in Oregon. Addled eggs from the same clutch (sister eggs) were collected from two territories. The broken egg was excluded from calculations of mean residue concentrations because fresh weight residue concentrations could not be determined.

In 1995, eight fresh and three addled eggs were collected from nests in 11 territories. Eggs were obtained from four territories in 1995 which were also sampled in 1994. Six eggs were collected from territories on the Oregon side of the river and five eggs were collected from the Washington side. Eggshell fragments were obtained from three of these territories and at one additional territory where no eggs were previously collected.

Organochlorine Pesticide and Mercury Residues

Residues of 12 organochlorine pesticides or metabolites and mercury were detected in one or more of the eagle eggs collected in 1994 (Table 2). Concentrations of most organochlorines, except p,p′-DDE and total PCBs, were <1 μg/g and considered, on an individual basis, to be below levels that would impair reproduction (Wiemeyer et al. 1993). Mean p,p′-DDE and total PCBs in the eggs were 6.84 μg/g and 6.15 μg/g, respectively (Table 2). Mercury was detected in all bald eagle eggs and averaged 0.221 μg/g (Table 2). Concentrations of p,p′-DDE and total PCBs were highest in one egg collected from a territory near the mouth of the river.

Organochlorine residues in the single broken egg collected from one territory were represented as lipid weight because insufficient volume remained in the egg to determine fresh weight. The lipid weight concentrations of p,p′-DDE, p,p′-DDD, and total PCBs in the broken egg were 152, 8.2, and 133 μg/g, respectively. These concentrations were very similar to lipid weight concentrations for p,p′-DDE ($\bar{x}$ = 154 μg/g), p,p′-DDD ($\bar{x}$ =9.06 μg/g), and total PCBs ($\bar{x}$ = 138 μg/g) in the 14 eggs collected in 1994. The shell from this egg was 14% thinner than shells from eggs collected prior to 1947. The contaminant concentrations in the egg, when compared on a lipid weight basis to other eggs collected in this study, were high enough to cause eggshell thinning and may have contributed to the egg breaking during incubation.

Table 2. Eggshell measurements and concentrations ($\mu g/g$ fresh weight) of select contaminants in 14 bald eagle eggs collected from nests in 11 territories along the lower Columbia River in 1994. Residue concentrations were averaged when multiple eggs were collected from the same nest.

Eggshell Parameter/ Contaminant	Mean ± Standard Deviation	Range
Eggshell Thickness (mm)	0.543 ± 0.040[a]	0.454-0.682
% Change[b]	-11 ± 6.6[a]	-25-+12
p,p′-DDT	0.03 ± 0.14	0.02-0.06
p,p′-DDE	6.84 ± 2.36	3.47-12.6
p,p′-DDD	0.41 ± 0.10	0.22-0.60
dieldrin	0.05 ± 0.02	0.02-0.07
hexachlorobenzene	0.02 ± 0.01	0.01-0.04
oxychlordane	0.02 ± 0.01	0.01-0.04
trans-nonachlor	0.17 ± 0.04	0.09-0.24
endrin	NC[c]	ND[d]-0.02
heptachlor epoxide	NC	ND-0.02
alpha chlordane	NC	ND-0.02
beta BHC	NC	ND-0.03
total PCBs	6.15 ± 2.28	3.43 -11.7
mercury	0.221± 0.43	0.167-0.292

[a] Combined sample size of 29 eggshells or fragments collected in 1994 and 1995.
[b] Change in mean eggshell thickness below value determined for bald eagle eggs collected prior to 1947.
[c] Not calculated. Only one sample was above detection limits.
[d] Not detected. Minimum level of detection at 0.01 $\mu g/g$ for organochlorine pesticides and mercury; 0.09 $\mu g/g$ for PCBs.

Eggshell Thickness and Breeding Success

With the exception of one egg, all bald eagle eggshells collected along the lower Columbia River in 1994 and 1995 exhibited some degree of eggshell thinning. Eggshells were up to 25% percent thinner ($\bar{x}$ = -11%) than the mean of eggs collected prior to the use of DDT (Table 2). Eggshell thinning >15-20% over a period of years is associated with poor reproductive success and a declining population (Anderson and Hickey 1972). Eggshells from five territories along the river were >15% thinner than the pre-DDT average, and breeding success at these territories ranged from 0 to 0.93 young/breeding attempt. One eggshell was 12% thicker than the pre-DDT average and was collected at the same territory where shells of normal thickness were collected in 1986 (Anthony et al. 1993).

Eggshell thickness was not correlated to breeding success for bald eagles along the lower Columbia River ($r = -0.06$, $n = 19$, $P = 0.79$). One territory was excluded from this analysis because an egg was collected in the only year (1995) the pair nested. Breeding success was quite variable and some nesting pairs with a history of relatively high breeding success also produced thin-shelled eggs.

Concentrations of PCDDs and PCDFs

Dioxin and furan residues were fairly consistent in the bald eagle eggs sampled from nests along the lower Columbia River. All egg samples contained PCDD and PCDF residues (Table 3), although the 1,2,4,7,8-PCDD and the 1,2,3,7,8,9-PCDF congeners were only present at or below the detection limits. TCDD and TCDF were the most elevated congeners in eggs. TCDD averaged 27.6 pg/g and ranged from 20 pg/g in an egg from the most upstream nest sampled to a high of 38 pg/g in an egg from a nest at the mouth of the river. TCDF averaged 21.1 pg/g and ranged from 6.90 to 40.0 pg/g. Other elevated congeners included 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HCDD, and 2,3,4,7,8-PCDF (Table 3).

The overall dioxin-like potency of the planar PCH congeners in eagle eggs was summarized using TEQs. As described in the Methods section of this report, TEQs were determined using I-TEFs (based on toxicity of planar PCHs to mammals; Table 3; Ahlborg et al. 1992) or C-TEFs (based on toxicity of planar PCHs to chickens; Table 3; Bosveld et al. 1995). The mean TEQ concentrations calculated using only I-TEFs for PCDD and PCDF was 40.2 pg/g (I-TEQ) and 68 pg/g (C-TEQ; Table 4), respectively. The majority of the dioxin-like potency in the I-TEQ value was due to TCDD (69%), followed by 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF, TCDF, and 1,2,3,6,7,8-HCDD, in decreasing order of importance (Table 3). The majority of the C-TEQ potency was due to TCDD (40%) followed by TCDF, 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF, and 1,2,3,6,7,8-HCDF. TCDF contributed more dioxin-like potency (28%) in the C-TEQ calculation than in the I-TEQ value, where the contribution was < 6%.

Concentrations of non ortho- and mono ortho-substituted PCBs

Planar PCB residues were elevated in all egg tissue from bald eagles. The most elevated non ortho-substituted PCBs were PCB 77 and 126, and the most elevated mono ortho-substituted PCBs were PCB 118 and 105 (Table 3). The average I-TEQ and C-TEQ calculated using TEFs established for PCDD, PCDFs, and the planar PCBs were 637 pg/g and 301 pg/g, respectively (Table 4). PCB 118 contributed the most dioxin-like toxicity (33%) of the average I-TEQ value, followed by PCB 126, PCB 105, PCB 156, and TCDD. PCB 126 contributed the most dioxin-like toxicity (54%) of the average C-TEQ value, followed by PCB 77, TCDD, TCDF, and 1,2,3,7,8-PCDD.

H4IIE Rat Hepatoma Cell Bioassay

Bald eagle eggs exhibited TCDD-EQ, as derived from the H4IIE bioassay, in the range of 16 to 278 pg/g, with a mean of 72 pg/g (see Appendix 3 for individual TCDD-EQ values). TCDD-EQ values for the two fish prey items ranged from 1 to 5 pg/g wet weight. These values for TCDD-EQ are comparable to less contaminated sites in the Great Lakes (Ankley et al. 1991, Tillitt et al. 1991b; 1992; Jones et al. 1993). However, the adverse effects these concentrations of TCDD-EQ may elicit on bald eagle embryos remains uncertain because the relative potency of planar

Table 3. Concentrations (pg/g fresh weight) of planar chlorinated hydrocarbons in 14 bald eagle eggs collected from nests in 11 territories along the lower Columbia River in 1994, and mammalian based toxic equivalency factors (I-TEFs) and avian based toxic equivalency factors (C-TEFs). Multiple eggs collected from the same nest were averaged to represent one egg from the nest.

Contaminant	Mean ± Standard Deviation	Range	I-TEF	C-TEF
Dibenzodioxins				
2,3,7,8-Tetrachloro	27.6 ± 5.87	20.0-38.0	1	1
1,2,3,7,8-Pentachloro	12.2 ± 3.47	8.70-21.0	0.5	1.2
1,2,4,7,8-Pentachloro	0.12 ± 0.03	ND[a]-0.30	NA[b]	NA
1,2,3,4,7,8-Hexachloro	1.03 ± 0.62	NQ[c]-2.40	0.1	0.05
1,2,3,6,7,8-Hexachloro	7.87 ± 2.78	4.40-13.0	0.1	0.01
1,2,3,7,8,9-Hexachloro	0.57 ± 0.53	NQ-2.0	0.1	0.1
1,2,3,4,6,7,8-Heptachloro	1.37 ± 0.78	NQ-3.10	0.01	0.001
Octachloro	10.3 ± 3.85	3.80-16.0	0.001	NA
Dibenzofurans				
2,3,7,8-Tetrachloro	21.1 ± 10.6	6.90-40.0	0.1	0.9
1,2,3,7,8-Pentachloro	1.13 ± 0.57	0.50-2.00	0.05	0.3
2,3,4,7,8-Pentachloro	5.85 ± 1.38	3.70-8.10	0.5	1.1
1,2,3,4,7,8-Hexachloro	0.72 ± 0.50	NQ-2.00	0.1	0.01
1,2,3,6,7,8-Hexachloro	1.00 ± 0.55	NQ-2.10	0.1	0.4
1,2,3,7,8,9-Hexachloro	0.10 ± 0.02	ND-0.150	0.1	NA
2,3,4,6,7,8-Hexachloro	2.05 ± 1.13	0.90-4.90	0.1	0.1
1,2,3,4,6,7,8-Heptachloro	2.70 ± 1.40	0.90-6.50	0.01	NA
1,2,3,4,7,8,9-Heptachloro	0.77 ± 0.67	NQ-2.10	0.01	NA
Octachloro	2.78 ± 1.17	1.63-5.90	0.001	NA
Non ortho-chlorinated Biphenyls				
3,4,4′,5-Tetra (81)[d]	358 ± 65.9	253-490	NA	NA
3,3′,4,4′-Tetra (77)	2675 ± 596	2040-3810	0.01	0.02
3,3′,4,4′,5-Penta (126)	1624 ± 450	1107-2620	0.1	0.1
3,3′,4,4′,5,5′-Hexa (169)	157 ± 72.2	84.5-325	0.05	0.001
Mono ortho-chlorinated Biphenyls				
PCB 123[d]	7,750 ± 3,450	ND-13,100	NA	NA
PCB 118	527,000 ± 136,000	345,000-859,000	0.0004	0.000004
PCB 114	12,000 ± 2,880	7,590-16,200	NA	NA
PCB 105	152,000 ± 38,600	96,100-230,000	0.001	0.
PCB 167	66,600 ± 28,700	30,900-136,000	NA	0.000003
PCB 156	75,600 ± 28,800	37,800-146,000	0.0004	0
PCB 157	20,400 ± 7,400	11,600-38,100	0.0003	0
PCB 189	7,780 ± 3,310	3,840-16,200	NA	NA

[a] Not detected. Detection limits ranged from 0.2 to 0.8 pg/g.
[b] Toxic Equivalency Factor not available.
[c] Not quantified. Quantification limits ranged from 0.2 to 0.8 pg/g.
[d] Number in parentheses is based on International Union of Pure and Applied Chemists congener number (Ballschmiter and Zell 1980).

Table 4. Mean total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs; pg/g fresh weight) in 14 bald eagle eggs collected from 11 nests along the lower Columbia River in 1994.

	Mean ± Standard Deviation	Range
I-TEQ (PCDD/F)[a]	40.2 ± 8.49	32.2-57.5
C-TEQ (PCDD/F)[a]	68.8 ± 16.9	46.3-102
I-TEQ (PCH)[b]	637 ± 159	439-1012
C-TEQ (PCH)[b]	301 ± 70.0	224-457
TCDD-EQ (H4IIE Bioassay)[c]	72 ± 78	16-278

[a] TEQ calculated using only TEF values for PCDD and PCDF (Ahlborg et al. 1992, Bosveld et al. 1995).
[b] TEQ calculated using TEF values for PCDD, PCDF, and planar PCBs (Ahlborg et al. 1992, Bosveld et al. 1995).
[c] TCDD-Equivalents determined by the H4IIE rat hepatoma bioassay (Tillitt et al. 1991a).

halogenated compounds to cause early life stage toxicity in bald eagles is unknown at this time. Further assessment of TCDD-EQs in eagle eggs will be provided following analysis of eggs collected in 1995.

Contaminants in Prey Items

Organochlorine pesticide, total PCB, and mercury residues found in two prey items (starry flounder and common carp) collected from two bald eagle nest sites were near or below detection limits. The carp sample contained 0.23 μg/g wet weight total PCBs and 0.12 μg/g wet weight p,p′-DDE, whereas the residues of these two contaminants in the starry flounder were below detection limits.

TCDD and TCDF were the only dioxin and furan congeners detected in the prey items, and the carp sample contained nearly double the values for these two congeners as did the flounder sample (see Appendix 1). The non-ortho PCBs 81, 77, and 126 were detected in the carp sample, and only PCB 77 was detected in the flounder (Appendix 1). The total for the three PCB congeners in the carp sample was 323 pg/g wet weight. The mono-ortho PCBs 105 and 118 were detected in the carp sample at 4 and 12.3 pg/g wet weight, respectively, and at 4 and 0.9 pg/g wet weight in the flounder, respectively. Other mono-ortho PCBs were below or near quantification limits.

DISCUSSION

As a general trend, the mean p,p′-DDD, p,p′-DDE, total PCBs, and hexachlorobenzene values were lower than mean concentrations found in eggs collected along the lower Columbia River from 1985 to 1987 (Anthony et al. 1993). However, concentrations of total PCBs in Columbia River eggs were higher than either threshold values or no observable adverse effect concentrations estimated for bald eagles (Table 5; Wiemeyer et al. 1984, Kubiak and Best 1991 *as cited in* Giesy et al. 1995). The p,p′-DDE values in Columbia River eagle eggs also were

Table 5. Contaminant concentrations and eggshell measurements in bald eagle eggs from the lower Columbia River, and contaminant values associated with impacts in various avian species. All concentrations are based on fresh or wet weight egg values.

Eggshell parameter or Contaminant	Mean value for bald eagle eggs (Columbia River)	Value associated with impacts in various species[a]: Value	Species	Reference
% Eggshell Change[b]	11	>15-20	Bald eagle	Anderson and Hickey 1972
		10	Bald eagle	Wiemeyer et al. 1993
p,p'-DDE (μg/g)	6.84	3.6	Bald eagle	Wiemeyer et al. 1993
total PCBs (μg/g)	6.15	4.0[c]	Bald eagle	Wiemeyer et al. 1984
		6.0[d]	Bald eagle	Kubiak and Best 1991
mercury (μg/g)	0.221	>0.5	Bald eagle	Wiemeyer et al. 1993
2,3,7,8-TCDD (pg/g)	27.6	10	Chicken	Verrett 1970
		6-10[e]	Kestrel	Giesy et al. 1995
		7[f]	Bald eagle	Giesy et al. 1995
I-TEQ-PCDD/Fs (pg/g)	40.2	>20-50	Wood duck	White and Seginak 1994
PCB 77, 126, 169 (pg/g)	4456	5,500[g]	Forster's terns	Kubiak et al. 1989
		2,070[h]	Peregrine falcon	Jarman et al. 1993

[a] See text for explanation of impacts.
[b] Change in mean eggshell thickness below value determined for bald eagle eggs collected prior to 1947.
[c] Value an estimate of the no observable adverse effect concentration.
[d] Estimate of threshold concentration for healthy productivity (Kubiak and Best 1991 *as cited in* Giesy et al. 1995).
[e] Hazard assessment derived values (pg TCDD-equivalents/kg wet weight in eggs) represent the low observable adverse effect concentration estimated for American kestrels.
[f] Hazard assessment derived values (pg TCDD-equivalents/kg wet weight in eggs) estimate the lowest observable adverse effect concentration/no observable adverse effect concentration estimated for bald eagles.
[g] Median concentration in eggs of Forster's terns exhibiting reproductive problems in Green Bay, Wisconsin.
[h] Geometric mean concentration in eggs of peregrine falcons exhibiting reproductive problems in California.

nearly double the values typically associated with reduced productivity in bald eagles from other areas (Table 5; Wiemeyer et al. 1993), and shell thinning in eggs of some breeding pairs was above the values thought to be biologically significant in causing population declines (Table 5; Anderson and Hickey 1972, Wiemeyer et al. 1993). DDE has been linked to eggshell thinning in raptors and other birds (Bitman et al. 1969, Porter and Wiemeyer 1969), and Wiemeyer et al. (1993) found five-year production increased in bald eagle populations when shell thinning was less than 10%. Although eggshell thinning was not correlated to breeding success for eagles in the present study, p,p'-DDE concentrations and mean eggshell thinning (-11%) for eagles in this area are similar to values for bald eagles in other areas exhibiting impaired reproduction and reduced five-year productivity averages (Wiemeyer et al. 1993).

Mean mercury residues were similar to the mean concentration (0.20 $\mu g/g$) of mercury found in 13 bald eagle eggs collected along the river from 1985 to 1987 (Anthony et al. 1993). Anthony et al. (1993) also found lead and cadmium in eggs collected from 1985 to 1987, but concentrations were below levels thought to cause deleterious effects to the population. The highest concentration of mercury in eggs collected by Anthony et al. (1993) and during the present study did not exceed concentrations associated with adverse effects on bald eagle production (Table 5; Wiemeyer et al. 1984).

Five-year average productivity values for eagles nesting along the lower Columbia River from 1993 to 1995 were higher than any previous year, based on five-year productivity comparisons since 1984 (Issacs and Anthony 1995; Washington Department of Fisheries and Wildlife, unpubl. annual census reports). Using the equation $Y=1.081 - 0.709 \log_{10} X$ developed by Wiemeyer et al. (1984) to predict five-year average productivity (Y) with various levels of DDE contamination (X), we would predict a five-year average productivity for eagles along the river of 0.49 young/occupied territory based on mean DDE values of 6.84 $\mu g/g$ (Table 2). This productivity value is similar to historical five-year average productivity for these birds but lower than averages since 1993. Since 1990, 19 new territories were established along the river, and six of these territories were established during the past two years. The recent increase in five-year productivity averages reflects the higher breeding success observed from newly established pairs along the river. These pairs may not have accumulated DDE or other organochlorines to the extent of older pairs along the river. The high breeding success of the newly established pairs may have influenced the relationship between breeding success and percent eggshell thinning. Additional information will be available following analysis of eggs collected in 1995 regarding DDE concentrations in more recently established pairs along the river.

Currently, no studies have evaluated the dose-response relationship of TCDD in bald eagle eggs. In experimental studies, chicken embryos exhibited mortality, edema, and teratogenic effects with egg injections of TCDD of 10 pg/g (Table 5; Verrett 1970). Mortality and reduced egg production and hatchability were observed in adult female pheasants (*Phasianus colchicus*) injected with 1 ng/g TCDD for 10 weeks (Nosek et al. 1992). Field studies on avian species have revealed a variety of toxic responses to PCDDs and PCDFs. Great blue heron (*Ardea herodias*) reproduction was unsuccessful with mean TCDD concentrations in eggs of 252 pg/g, but reproduction was normal when mean concentrations in eggs were 92 pg/g (Elliot et al. 1988). Eggs collected from Forster's tern (*Sterna forsteri*) colonies having impaired reproduction exhibited a median concentration of 37.3 pg/g (whole egg, wet weight) for TCDD, although PCBs were thought to be primarily responsible for reproductive problems (Kubiak et al. 1989). Based on numerous field and laboratory studies, Giesy et al. (1995) estimated the lowest observable adverse effect level (LOAEL) or no observable adverse effect level (NOAEL) for TCDD-EQs in eggs ranging from 6 to 10 pg/g for American kestels (*Falco sparverius*) and at 7 pg/g for bald eagles (Table 5). Mean TCDD concentrations in the 14 bald eagle eggs in our study greatly exceeded the LOAEL/NOAEL in this hazard assessment, indicating dioxins are contributing to the reduced reproductive success observed in bald eagles nesting along the lower river.

The mean I-TEQ (PCDD/F) concentration in eggs of bald eagles along the study area were similar to I-TEQ concentrations in eggs of other avian species experiencing reproductive problems. In California, the geometric mean concentration (17 pg/g wet weight) found in eggs of peregrine falcons (*Falco peregrinus*) experiencing reproductive problems was lower than the I-TEQ in eggs from eagles along the lower Columbia River (Jarman et al. 1993). White and Seginak (1994) found impaired nesting and hatching success and reduced duckling production in wood ducks (*Aix sponsa*) when geometric mean TEQs were in the range of >20-50 pg/g. The wood ducks were nesting near a Superfund site highly contaminated with dioxins and furans. The authors also reported subcutaneous edema of the head and neck and lower bill deformities in some ducklings at the study site. TCDD contributed 70% of the TEQ values in the wood duck study (White and Seginak 1994) which was very similar to the TCDD contribution (69%) of the average I-TEQ found in eggs from bald eagles nesting along the lower Columbia River .

Kubiak et al. (1989) reported that increased incubation period, reduced hatchability, lower body weight, increased liver to body weight ratio, and occurrence of edema in Green Bay's Forster's terns appeared to be associated with contaminants, especially the non-ortho and mono-ortho PCBs. Jarman et al. (1993) suspected DDE and non-ortho PCBs were the most important compounds that may be adversely affecting reproduction in peregrine falcons in California. Total mean concentration (4456 pg/g, range = 3355-6255 pg/g) of three non-ortho PCBs (PCB 77, 126, and 169) in bald eagle eggs collected along the river was slightly lower than the median total (5,500 pg/g wet weight, range = 1,370-41,000 pg/g wet weight) of these three congeners found in Forster's terns in Green Bay (Kubiak et al. 1989), and more than double the geometric mean (2,070 pg/g wet weight, range = 260 to 6120 pg/g) found in peregrine falcon eggs in California (Table 5; Jarman et al. 1993). PCB congeners 126 and 105 accounted for more than 90% of the estimated TEQ in eggs from Green Bay (Kubiak et al. 1989). In California, PCB 126 accounted for 83% of the total TEQs in falcon eggs, although mono-ortho PCBs were not included in the analysis (Jarman et al. 1993). In eggs collected along the lower Columbia River, PCB 118, 126, and 105 accounted for the majority of the estimated TEQ average. Comparisons of TEQ concentrations and how they reflect toxicity in eagle embryos will be better discerned following analysis of eagle eggs collected in 1995.

Residues of organochlorine pesticides and total PCBs in prey item samples were similar to values obtained from various fish species collected from the lower Columbia River in 1986 (Anthony et al. 1993). TCDD and TCDF values in the prey items were within the range of concentrations observed in carp and other fish species from the lower Columbia River (U.S. Fish and Wildlife Service, unpubl. data). The preliminary estimated biomagnification factors (BMFs) from prey items to eagle egg, calculated as the ratio of the mean concentration of TCDD in the eggs of bald eagle to that of their prey item, was 54 (27/0.5) for TCDD and 57 (6.8/0.12) for p,p′-DDE. The BMF for TCDD was slightly greater, and for DDE somewhat lower, than the respective BMFs calculated from prey to herring gull egg at Lake Ontario (Braune and Norstrom 1988). Relationships between residues in eagle eggs and fish prey will be further examined after completion of the 1995 egg analyses.

SUMMARY

The relationships between organochlorine compounds and reproduction of bald eagles nesting along the lower Columbia River have not yet been fully ascertained. Although eggshell thickness was not correlated to breeding success for bald eagles along the river, residue data show eagles continue to accumulate DDE and total PCBs at concentrations associated with limited productivity. Eagles are also bioaccumulating PCDD, PCDF, and PCB congeners. TCDD in lower Columbia River bald eagle eggs exceeded estimated LOAEL/NOAEL values, indicating dioxins and related compounds are contributing to the reduced reproductive success. Additional information provided from the 1995 field season will be useful in further elucidating relationships between toxins and reproductive effects. The final report in 1996 will provide a comparison to organochlorine concentrations over time and relate them to eggshell thinning and breeding success, as well as provide comparisons to previous studies conducted along the lower Columbia River.

PROJECT STATUS

Field work, productivity surveys, and egg collection have been completed for the second year of the bald eagle study. Productivity analyses and egg collection for 1995 has been summarized in the Methods section of this report. Bald eagle eggs obtained in 1995 were sent to the Midwest Science Center in August for residue analyses and for the H4IIE bioassay. Analytical chemistry results are expected to be received in the Spring of 1996. Dependent on completion of the residue analyses for the 1995 field season, we anticipate completion of the final report evaluating productivity, eggshell thickness, egg residue concentrations, and bioassay results in Fall 1996.

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Appendix 1. Concentrations (pg/g fresh weight) of 2,3,7,8-substituted polychlorinated dibenzo-p-doxins and dibenzofurans in bald eagle eggs and prey items from the lower Columbia River, and quality assurance samples.

	Bald Eagle Egg						
	271	606	300	137	137	610	308
Contaminant	CRRIBE08	CRFIBE06	CRCIBE09	CRWABE07	CRWABE07	CRTIBE02	CRWIBE14
DIOXINS							
2,3,7,8-Tetrachloro	20	24	26	31	30	24	23
1,2,3,7,8-Pentachloro	14	9.7	10	13	13	8.7	9.9
1,2,4,7,8-Pentachloro	0.2 NQ	0.2 ND	0.2 ND	0.2	0.2 ND	0.2 ND	0.2 NQ
1,2,3,4,7,8-Hexachloro	2.4	0.9	0.8 NQ	0.9	0.9	0.7 NQ	0.8
1,2,3,6,7,8-Hexachloro	13	6.9	5.5	11	12	4.4	6.0
1,2,3,7,8,9-Hexachloro	2	0.5 NQ	0.5 NQ	0.9	0.9	0.4 NQ	0.3
1,2,3,4,6,7,8-Heptachloro	3.1	2 NQ	0.9	2	2	2	0.8
Octachloro	16	3.8	15	9.8	8.9	11	5.8
FURANS							
2,3,7,8-Tetrachloro	16	6.9	17	13	13	9.3	22
1,2,3,7,8-Pentachloro	2	0.5	0.9	0.8	0.7	0.7	0.8
2,3,4,7,8-Pentachloro	5.8	3.7	5.2	6.5	6.2	4.2	5.5
1,2,3,4,7,8-Hexachloro	0.8	0.4 NQ	0.5	0.6	0.7	0.6 NQ	0.6 NQ
1,2,3,6,7,8-Hexachloro	0.8	0.6 NQ	0.8	0.9	0.9	0.9	0.7
1,2,3,7,8,9-Hexachloro	0.2 ND	0.2 ND	0.2 ND	0.2	0.2 ND	0.2 ND	0.2 ND
2,3,4,6,7,8-Hexachloro	1	1	2.1	2	2.1	2	0.8
1,2,3,4,6,7,8-Heptachloro	2	2	2.7	2	2.5	2.4	2
1,2,3,4,7,8,9-Heptachloro	0.4	0.4 NQ	0.7	0.4	0.7	0.5 NQ	0.5 NQ
Octachloro	2.7	2	2.7	2	2.1	3.0	2

ND Not Detected at Specified Detection Limit
NQ Not Quantitated at Specified Average Concentration due to Inaccurate Ion Ratio

	Bald Eagle Egg						
	308	248	130	565			304
Contaminant	CRWIBE15	CRJCBE03	CRBSBE11	CRGPBE04	CRBEGP12	CRBEGP13	CRCPBE10
DIOXINS							
2,3,7,8-Tetrachloro	21	30	37	22	21	28	28
1,2,3,7,8-Pentachloro	9.4	11	14	9.9	9.5	11	13
1,2,4,7,8-Pentachloro	0.2 ND	0.2 ND	0.2 ND	0.2 ND	0.2 ND	0.3	0.5 NQ
1,2,3,4,7,8-Hexachloro	0.9	0.9	2	0.7	0.9	0.9	0.9
1,2,3,6,7,8-Hexachloro	6.0	6.1	8.4	5.7	6.2	7.7	7.2
1,2,3,7,8,9-Hexachloro	0.3 NQ	0.6	0.6	0.4 NQ	0.4	0.5 NQ	0.5 NQ
1,2,3,4,6,7,8-Heptachloro	0.8	0.9	2	0.7 NQ	0.8	0.9	0.9
Octachloro	7.6	11	11	3.3	5.5	7.1	14
FURANS							
2,3,7,8-Tetrachloro	21	40	16	31	28	35	31
1,2,3,7,8-Pentachloro	0.9	2	2	0.9	0.9	0.9	0.9
2,3,4,7,8-Pentachloro	4.9	6.1	7.1	4.7	4.6	5.7	7.6
1,2,3,4,7,8-Hexachloro	0.5 NQ	0.9	2	0.6	0.5	0.5	0.9
1,2,3,6,7,8-Hexachloro	0.8	0.9	2.1	0.6	0.5	0.7	0.9
1,2,3,7,8,9-Hexachloro	0.2 ND	0.2 NQ	0.2 ND	0.2 ND	0.2 ND	0.2 ND	0.2 ND
2,3,4,6,7,8-Hexachloro	2	2.9	4.9	0.9	0.9	0.9	2
1,2,3,4,6,7,8-Heptachloro	2.2	3.3	6.5	0.9	0.9	0.9	2.7
1,2,3,4,7,8,9-Heptachloro	0.9 NQ	0.9	2.1	0.3 NQ	0.4	0.5	0.8 NQ
Octachloro	2.3	3.6	5.9	0.9	2	2	2.3

ND Not Detected at Specified Detection Limit

NQ Not Quantitated at Specified Average Concentration due to Inaccurate Ion Ratio

Contaminant		Bald Eagle Egg		Prey Items		
		304	145	Common Carp		Starry Flounder
		CRCPBE10	CRFCBE05	CRRICC01	CRRICC01	CRMSSF01
DIOXINS						
	2,3,7,8-Tetrachloro	29	38	0.7	0.6	0.3
	1,2,3,7,8-Pentachloro	13	21	0.2 ND	0.2 ND	0.2 ND
	1,2,4,7,8-Pentachloro	0.2 ND	0.2 ND	0.2 ND	0.2 ND	0.2 ND
	1,2,3,4,7,8-Hexachloro	0.9	0.9	0.2 ND	0.2 ND	0.2 ND
	1,2,3,6,7,8-Hexachloro	7.2	11	0.2 ND	0.2 ND	0.2 ND
	1,2,3,7,8,9-Hexachloro	0.8 NQ	0.6	0.2 ND	0.2 ND	0.2 ND
	1,2,3,4,6,7,8-Heptachloro	0.9	0.8	0.2 ND	0.8 NQ	0.2 ND
	Octachloro	13	11	1 NQ	1 NQ	1 NQ
FURANS						
	2,3,7,8-Tetrachloro	30	31	5.0	4.8	2.4
	1,2,3,7,8-Pentachloro	0.9	0.9	0.2 ND	0.2 ND	0.2 ND
	2,3,4,7,8-Pentachloro	7.6	8.1	0.2 NQ	0.2 ND	0.2 ND
	1,2,3,4,7,8-Hexachloro	0.8	0.9	0.2 ND	0.2 ND	0.2 ND
	1,2,3,6,7,8-Hexachloro	0.9	2	0.2 ND	0.2 ND	0.2 ND
	1,2,3,7,8,9-Hexachloro	0.2 ND	0.2 NQ	0.2 ND	0.2 ND	0.2 ND
	2,3,4,6,7,8-Hexachloro	2.6	2	0.2 ND	0.2 ND	0.2 ND
	1,2,3,4,6,7,8-Heptachloro	2.5	2.9	0.2 ND	0.2 ND	0.2 ND
	1,2,3,4,7,8,9-Heptachloro	0.9	2	0.2 ND	0.2 ND	0.2 ND
	Octachloro	2.3	2.5	0.7 NQ	0.2 ND	1 NQ

ND Not Detected at Specified Detection Limit

NQ Not Quantitated at Specified Average Concentration due to Inaccurate Ion Ratio

Contaminant		Quality Assurance Samples: Procedural Blank 1	Procedural Blank 2	Chicken Egg Blank 1	Chicken Egg Blank 2
DIOXINS					
	2,3,7,8-Tetrachloro	2 ND	2 ND	0.1 ND	0.1 ND
	1,2,3,7,8-Pentachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,4,7,8-Pentachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,4,7,8-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,6,7,8-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,7,8,9-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,4,6,7,8-Heptachloro	5.4	2 ND	0.3	0.7
	Octachloro	83	16	6.9	8.0
FURANS					
	2,3,7,8-Tetrachloro	2.7	2 NQ	0.1	0.1 ND
	1,2,3,7,8-Pentachloro	2 ND	2 ND	0.2 ND	0.2 ND
	2,3,4,7,8-Pentachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,4,7,8-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,6,7,8-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,7,8,9-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	2,3,4,6,7,8-Hexachloro	2 ND	2 ND	0.2 ND	0.2 ND
	1,2,3,4,6,7,8-Heptachloro	6.5	2 ND	0.2 ND	0.2 ND
	1,2,3,4,7,8,9-Heptachloro	2 ND	2 ND	0.2 ND	0.2 ND
	Octachloro	30	27	0.7 NQ	0.6 NQ

ND Not Detected at Specified Detection Limit
NQ Not Quantitated at Specified Average Concentration due to Inaccurate Ion Ratio

Contaminant		Quality Assurance Samples Chicken Egg Spike 1	Chicken Egg Spike 2	SAGINAW CARP #1	SAGINAW CARP #2
DIOXINS					
	2,3,7,8-Tetrachloro	9.3	9.4	21	21
	1,2,3,7,8-Pentachloro	9.5	9.1	11	11
	1,2,4,7,8-Pentachloro	10	9.8	0.2 ND	0.2 ND
	1,2,3,4,7,8-Hexachloro	9.5	9.7	4.4	4.5
	1,2,3,6,7,8-Hexachloro	9.2	9.4	13	15
	1,2,3,7,8,9-Hexachloro	8.4	9.6	2	2
	1,2,3,4,6,7,8-Heptachloro	9.8	9.9	17	19
	Octachloro	61	57	13	13
FURANS					
	2,3,7,8-Tetrachloro	8.4	9.3	32	33
	1,2,3,7,8-Pentachloro	9.2	9.5	12	12
	2,3,4,7,8-Pentachloro	10	9.8	34	32
	1,2,3,4,7,8-Hexachloro	10	10	10	9.9
	1,2,3,6,7,8-Hexachloro	9.6	9.6	6.0	7.0
	1,2,3,7,8,9-Hexachloro	9.8	10	0.2 ND	0.2 ND
	2,3,4,6,7,8-Hexachloro	10	14	5.6	6.5
	1,2,3,4,6,7,8-Heptachloro	9.8	12	12	12
	1,2,3,4,7,8,9-Heptachloro	8.5	8.9	0.2 ND	0.6 NQ
	Octachloro	47	47	2.1	1

ND Not Detected at Specified Detection Limit
NQ Not Quantitated at Specified Average Concentration due to Inaccurate Ion Ratio

Appendix 2. Concentrations (pg/g fresh weight) of non-ortho-chloro-substituted polychlorinated biphenyls in bald eagle eggs and prey items from the lower Columbia River.

		Non-o-Polychlorinated Biphenyls			
		Tetra:	Tetra:	Penta:	Hexa:
Submitter Number:	Sample Description: (nest/prey, Fresh wgt factor: Contents/Vol.)	3,4,4',5-TCB Congener 81	3,3',4,4'-TCB Congener 77	3,3',4,4',5-PeCB Congener 126	3,3',4,4',5,5'-HxCB Congener 169
Eagle Eggs					
CRRIBE08	271, 25 g, (0.82)	445	3,810	1,430	100
CRCIBE09	300, 25 g, (0.91)	330	2,290	1,510	115
CRWABE07	137, 25 g, (0.89) - Replicate 1	375	2,060	1,670	165
CRWABE07	137, 25 g, (0.89)- Replicate 2	365	2,220	1,880	160
CRTIBE02	610, 25 g, (0.93)	355	2,120	1,220	110
CRWIBE14	308, 25 g, (0.82)	300	2,380	1,380	120
CRWIBE15	308, 25 g, (0.85)	310	2,320	1,410	110
CRJCBE03	248, 25 g, (0.93)	380	3,090	1,620	135
CRBSBE11	130, 25 g, (0.90)	490	3,210	2,170	170
CRGPE04	565, 25 g, (0.90)	235	2,600	920	235
CRBEGP12	565, 25 g, (0.86)	230	2,510	1,090	230
CRBEGP13	565, 25 g, (0.87)	295	3,180	1,310	290
CRCPBE10	304, 25 g, (0.95)	300	2,230	1,790	155
CRCPBE10	304, 25 g, (0.95)	315	2,370	1,780	160
CRFCBE05	145, 25 g, (0.90)	370	3,310	2,620	325
Prey Items					
CRRICCC01	Common Carp, 25 g - Replicate 1	16	270	26	2 ND
CRRICCC01	Common Carp 25 g - Replicate 2	19	290	25	2 ND
CRMSSF01	Starry Flounder, 25 g	1 ND	32	7.6 NQ	2 ND

a Procedural Internal Standards / native analytes were not detected in sample extract.
b Procedural Internal Standard recoveries below QC limit (<25%).
c Total pg in blank sample
NQ Not Quantitated at Specified Concentration due to Incomplete Ion Cluster or Inaccurate Ion Ratio
ND Not Detected at Specified Detection Limit

Appendix 3. H4IIE bioassay-derived TCDD-EQ (pg/g fresh weight) in bald eagle eggs and prey items from the lower Columbia River.

Sample No.	Nesting Territory		TCDD-EQ (pg/g)	Standard Deviation
Bald Eagle Egg				
CRMFBE01	784		114[a]	12
CRTIBE02	610		39	3.3
CRTIBE02	610		80	5.2
CRTIBE02	610		75	7.8
		Bioassay Mean	65	
CRTIBE02	610		54	6.4
		Sample Mean	60	
CRJCBE03	248		20	2.7
CRGPBE04	565		8	0.8
CRFCBE05	145		18	2.0
CRFIBE06	606		143	13
CRWABE07	137		100	7.6
CRRIBE08	271		16	1.7
CRCIBE09	300		54	4.4
CRCIBE09	300		42	5.1
CRCIBE09	300		57	5.0
CRCIBE09	300		42	4.6
		Bioassay Mean	47	
		Sample Mean	51	
CRCPBE10	304		278	20
CRBSBE11	130		16	1.4
CRBEGP12	565		3	0.5
CRBEGP13	565		58	6.4
CRWIBE14	308		39	3.1
CRWIBE15	308		15	1.5
Prey Items				
CRRICC01	Common carp		5	0.6
CRRICC01	Common carp		1	0.5
		Sample Mean	3	
CRMSSF01	Starry flounder		1	0.1

[a]Egg concentration given as wet weight because egg volume could not be estimated.

Attachment

Response to Peer Review Comments on the draft interim report *Environmental Contaminants in Bald Ealges Nesting Along the Lower Columbia River*

Comments from Steven L. Stockton, U.S. Army Corps of Engineers, Oct. 4, 1995:

Comment 1) Page 3, Paragraph 1; The two sentences, "Various parts of the Columbia River are frequently dredged, and these activities can resuspend and increase bioavailability of contaminants such as PCBs and DDE (Seelye et al. 1982). Steidl et al. (1991) suspected elevated contaminants in osprey (*Pandion haliatus*) eggs in Delaware Bay were due to dredging activities." are misleading, in error, and dated.... and should be deleted.

Response 1) The sentences were modified to better represent the intentions of the original authors and to remove any implication that the studies were conducted in the lower Columbia River.

Comment 2) As presented, the Seelye reference insinuates that frequent dredging of the Columbia River dredged material resuspended and increased the bioavailability of PCBs and DDE. In actuality, the Seelye study conducted in 1977-1978 on Saginaw Bay... was a laboratory study to gather information to recommend how bioavailability tests should be designed to evaluate sediment quality. ...Contaminated sediments were introduced to these tanks every 5 to 10 minutes for the duration of the 10-day exposure. This is neither the typical material dredged, dredging cycle, nor contamination levels found in the "frequently dredged Columbia River sediments." Seelye did not work with the Columbia River sediment nor did he extrapolate the relationship of the laboratory exposure to the actual Saginaw Bay field exposure around a dredging operation. The same caution is prudent, therefore, not to extrapolate his laboratory work to the Columbia River in the present study.

Response 2) The two sentences were modified to remove any implications that Seelye et al. (1982) conducted their study in the Columbia River or that the authors supported the notion that Columbia River dredged material resuspended and increased the bioavailability of PCBs and DDE. However, Seelye et al. (1992) did suggest that based on their results dredging could cause uptake of contaminants by fish. Specifically, the authors stated "These results demonstrated not only the potential for uptake of contaminants by fish as a result of dredging but also the potential utility of fish bioassays in evaluating proposed dredging operations." The sentences in the final interim report have been modified to indicate that dredging has the potential to increase bioavailability of contaminants.

Comment 3) Seelye conducted 3 experiments. Total DDT levels in the test sediments are shown as 20 ppb for all 3 experimental exposures. No significant differences in DDE accumulation were noted in experiment 1 between controls and exposed hatchery or lake fish. Seelye did report that in experiment 3, using aerated sediments, concentrations of DDE were significantly higher in the exposed than in control fish. What is not mentioned in the text, but is displayed in the tables, is that the preexposed hatchery raised fish had significantly higher DDE levels than either the control or the exposed fish. This would be an indication of depuration of DDE during the 10 day duration of the test. Looking back at experiment 2, DDE concentrations in the exposed fish were less than the control and similar to preexposure fish. Therefore, Seelye does not show increased bioavailability of DDE even during prolonged laboratory exposure as stated. Extrapolation to dredging activities on the Columbia River and DDE uptake by fish hence to eagles based upon Seelye et al. 1982, is poor science if not bogus.

Response 3) Seelye et al. (1982) indicated in experiment 3 that concentrations of PCB, DDE, and some metals were significantly higher in fish exposed to aerated dredged sediments than control fish. The preexposure, hatchery-raised fish did not have significantly higher DDE levels than the control fish (P=0.700; Table VII, Seelye et al. 1982) and differences were not tested between the preexposure and the exposed fish. The authors did provide a plausible explanation for the tendency towards higher mean values in the preexposure fish compared to the control and exposed fish. The authors stated "We believe these increases were due to a loss of weight by the control fish that resulted from our withholding of feed from both groups (controls and exposed) during the experiment." Therefore, the results indicate that fish did have enhanced uptake of DDE when exposed to aerated sediments (experiment 3), even though the fish exposed to nonaerated sediments did not exhibit higher concentrations of DDE than controls (experiment 2). The results of Seelye et al. (1982) reveal the potential for increased bioavailability of DDE in fish exposed to dredged sediments under the specified conditions. The sentences in the final interim report on bald eagles have been revised to indicate that dredging has the potential to increase uptake of some contaminants.

Comment 4) PCBs (1248) concentrations were 1350 ppb, 1190 ppb, and 950 ppb (dry weight) in the 3 experimental exposure sediments. These values are 2 orders of magnitude above the 3 hits in the 77 sediment samples collected and analyzed (532 separate analyses) during the Bi-State study of Columbia River sediments (85 ppb, 11 ppb, and 7.3 ppb). In experiment, 1 hatchery fish showed PCB uptake while lake fish showed a decrease, though not significant, from the control. Experiment 2 and 3 both show significant uptake after 10 days exposure in the tanks. In these experiments the contaminant, and exposure pathway are known, this is not true for the field. First, the contaminant must be present. Frequently dredged sediments in the Columbia River are clean sands low in fines and organic material and do not contain detectable levels of PCBs. Secondly, exposure to fish by actual dredging operations would be measured in minutes if not seconds as they instinctively avoid both the dredging and disposal operations. Again extrapolation of Seelye et al. 1982 to Columbia River dredging is inappropriate due to significant difference in contamination levels and exposure pathways.

Response 4) The objectives of the Seelye et al. (1982) study were to determine accumulation in contaminant concentrations in fish exposed to dredged sediments, and the results indicate that enhanced uptake of PCBs and other compounds can occur. Data collected by the U.S. Fish and Wildlife Service (Service) clearly show that bioaccumulation of DDE, PCBs, and dioxins is occurring in fish, bald eagles, and other birds associated with the Columbia River from the Bonneville dam to the mouth. This data indicates contaminants are present in the Columbia River at concentrations high enough to bioaccumulate in fish and cause reproductive impacts to bald eagles. Exposure pathways of the contaminants do need to be further examined during Columbia River dredging operations, although this does not preclude the results reported by Seelye et al. (1982) that dredging has the potential to increase bioavailability.

Fish may avoid conditions created by dredging operations (e.g., laboratory studies indicating smelt could avoid areas high in suspended solids; Wildish and Power 1985; Bull. Environ. Contam. Toxicol. 34:770-774). However, fish could still be exposed to organochlorines attached to suspended sediment even if the suspended sediment particles were present at low concentrations, or during the "learning process" that may occur before the fish exhibit the avoidance behavior. Therefore, the scenario in the interim report indicating the potential exists for fish to bioaccumulate contaminants during or after dredging appears plausible.

Comment 5) Steidl et al. (1991) is only partially quoted and is, therefore, misleading as presented. Steidl includes non dredged sediments in the upper reaches of Delaware Bay as possible sources of contaminant found in Delaware Bay osprey eggs. While Steidl does state "we believe the release and resuspension of contaminants through dredging," is also a likely source, the study was neither designed to collect nor presents any data or argument to substantiate the reason for this belief. The present Columbia River study is also lacking in data to establish contaminant pathways from dredging activities to eagle eggs. By solely listing dredging activities as the source of elevated contaminants in Delaware Bay osprey eggs, and therefore by reference Columbia River eagle eggs, without the first data point to establish any link is irresponsible. Further, it ignores other likely pathways which need to be assessed if proper management of eagle populations in the lower Columbia River is to be achieved.

Response 5) The sentences referring to Steidl et al. (1991) in the final interim bald eagle report have been revised to include the high organochlorine concentrations in sediment in the upper estuary as a possible cause of high concentrations observed in osprey eggs. In the Columbia River, the source of organochlorines is largely unknown, but the data show that fish are accumulating organochlorines and that bald eagles have organochlorine residues higher than elsewhere in the Northwest. One plausible hypothesis to explain this phenomena is that dredging operations may be resuspending and increasing the availability of contaminants. Data from Seelye et al. (1982) and observations made by Steidl et al. (1991) support this hypothesis and therefore are included in the text of the final interim bald eagle report. At this time, data are insufficient to determine whether dredging activities in the Columbia River are or are not contributing to the increased organochlorine burdens in the bald eagles. The dredging issue is only proposed as a possible hypothesis, and the previous studies indicate that the hypothesis should be considered in management decisions.

Comment 6) Lastly, continued references to dredging activities on the lower Columbia River and the health of eagle populations in the area shows a lack of knowledge by those writing the reports of dredging operations, material dredged, and the laws and regulations which need to be followed to dredge. The USACE, Portland District has both operational and regulatory authorities governing dredging in the lower Columbia River. We, therefore, have institutional and historical expertise and information available to any interested party regarding dredging and dredged material disposal in the lower Columbia River.

Response 6) The Service recognizes that the U.S. Army Corp of Engineers (COE) has operational and regulatory authorities concerning dredging operations. Under section 7(a)(1) of the Endangered Species Act of 1973, as amended, both the U.S. Fish and Wildlife Service and the COE, as Federal agencies, are obligated to "utilize their authorities in furtherance of the purposes of this Act by carrying out programs for conservation of endangered species and threatened species listed pursuant to section 4 of this act." We agree that insufficient information is available to adequately address the impacts of dredging activities on fish and wildlife communities associated with the Columbia River, and are committed to using the best available data to determine why reproductive impacts are occurring in the eagles. Consequently, the Service would appreciate the opportunity to consider data obtained by the COE during dredging operations to assist in the Columbia River bald eagle assessment.

Comments from Richard Olsen, Argonne National Laboratory, December 31, 1995

Comment 2) As with specific species studies, this study should eventually attempt to relate contaminant levels in eggs and eagles to contaminants in prey and also to the spatial distribution of contaminants in non-biotic parts of the ecosystem. The general pathway for transport of contaminants to eagles in the LCR is known, but the microhabitat distribution of contaminants within the foraging range of the eagles appears not be known. The substantial variability of analytical results presented emphasizes this factor. This is an important component that must be determined. Also, non-contaminant effects must also be partitioned out (e.g., habitat and human disturbance factors).

Response 2) Contaminant relations in eagle eggs and prey: The U.S. Fish and Wildlife Service collected numerous invertebrates, fish, and eggs of fish-eating birds from the Columbia River and is currently reviewing the data. This information will be used to assess contaminant relations in eagles and prey species and determine biomagnification factors in future reports. Microhabitat contaminant distribution: The objectives of the present study did not include an examination of microhabitat contamination within the foraging range of individual eagles primarily due to insufficient funding. However, eagles have been used as an indicator species in numerous studies and information is available to assess reproductive impacts based on contaminant levels in eggs. This assessment can be conducted regardless of contaminant variability in eggs, although future investigations should focus on microhabitat sampling if funding permits. Disturbance: Habitat and human disturbance factors for bald eagles nesting along the river have been assessed in previous investigations. This issue will be considered during the final phase of the project.

Comment 3) I am a little concerned with a few aspects of the study design. The egg analyses do not include heavy metals which may also be a factor. Unless this potential factor can be ruled on the basis of past studies, it should be included in this study. I am also concerned about the lack of specific reference sites in the design. The analytical results for this study are compared with state-wide results for analyses during the same year. This may be acceptable if it can be stated that the state-wide analyses do not also include eggs from contaminated or otherwise impacted sites. It would be far better to have included noncontaminated reference sites from within the region or even better within the LCR in the current study. One final concern I have is the statistical analysis. The study includes relatively few data points which appear to represent nests selected in the LCR where nonviable eggs existed or where previous nesting success was evident. The site selection component of the sampling design may therefore bias the results for statistical analyses and that in combination with the fact that the population may not be normally distributed due to the number of data points makes use of linear regression somewhat suspect. There is also some indication that state-wide values include the RZ10 values; is may also be a problem and I suggest a data correction be made.

Response 3) Trace elements: The trace elements lead, cadmium, and mercury were analyzed in Columbia River bald eagle eggs in previous studies, and mercury was analyzed in eggs from the present study (see Garrett et al. 1988, Anthony et al. 1993, and this study). The trace elements were found to be below levels thought to cause deleterious effects to the population. The dioxin and furan analysis requires substantial quantities of egg tissue and therefore only mercury was examined in the present study. The trace element information was added to the "Discussion" section in the final interim report. Reference sites/statewide comparisons: Contaminant residues and eggshell thickness are only compared to breeding success of eagles in the lower Columbia River and are not compared on a statewide basis in the present study. In this analysis, the annual breeding success of nest sites that were climbed for fresh egg collection were excluded, as described in the Methods section. The five-year productivity values of nests in the lower Columbia River were compared to statewide productivity values. This was a graphical comparison and was not based on statistics, and using the Columbia River data in Figure 2 had virtually no effect on the statewide five-year productivity values. Furthermore, excluding the lower Columbia River values from the complete statewide determination would misrepresent the state values. The figure demonstrates that the Columbia River bald eagle productivity (five-year averages) is much lower than statewide values *even when* Columbia River productivity values are included. Site selection/statistical analysis: Eggs were collected from sites with repeated poor productivity, a history of contaminants in eggs, and from newly established territories. True random sampling was impossible in this area because some nests sites were inaccessible, some nests were in snags which were un-climbable, and there was no way to predict early in the season which nesting pairs would begin incubation. Therefore, linear regression evaluated the relationship between breeding success of only the lower Columbia River eagles and the corresponding eggshell thickness values for the birds (nesting success information was available for all nest sites along the river). Although the sample size was small, it represented nearly half of the eagles nesting along the river. Sampling nearly 50% of a population is generally better than most field studies achieve, especially when working with a listed species. Therefore, the results from the regression are probably representative of the eagles nesting along the river. Statewide values including RZ10 values: See discussion above under "Reference sites/statewide comparisons."