ENVIRONMENTAL CONTAMINANTS IN BALD EAGLES NESTING IN HOOD CANAL, WASHINGTON, 1992-1997

FINAL DRAFT

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ABSTRACT

The number of bald eagles nesting along Hood Canal in Washington have increased from 2 known occupied territories in 1980 to 31 in 1999. Although the number of occupied bald eagle nests have increased, productivity remained significantly below the Washington State productivity average during most years and fluctuated dramatically. In 1992 a study was initiated to evaluate if contaminants were a possible cause of the lower productivity. From 1992 to 1993, fresh eggs were collected from eight Hood Canal bald eagle territories. Nests with the highest failure rate were selected for sampling. Only addled eggs were collected from 1994 to 1997, with a total of five additional eggs collected from Hood Canal nests. Five addled eggs from outside Hood Canal in Washington were also collected for comparison. Total PCB concentrations measured in the Hood Canal eggs exceeded threshold levels described for normal bald eagle reproduction. The geometric mean of total PCBs in the eggs from outside Hood Canal were close to the threshold level of 4.0 ppm. Only two eggs collected during the study (one from Hood Canal and one outside Hood Canal) exceeded the threshold limit for p,p'-DDE. Non-ortho and mono-ortho planar PCBs accounted for the majority of the dioxin-like toxicity found in the bald eagle eggs. PCB 126 contributed the highest dioxin-like toxicity with PCB 77 contributing the second highest level. Only the five eggs collected in 1992 were analyzed for metals, and concentrations of mercury, selenium and arsenic were found to be below levels of concern. Detectable levels of PCBs were found in five nestling and 6 adult bald eagle blood samples and detectable concentrations of p,p'-DDE were present in all the samples except for three of the nestling blood samples. Low concentrations of dioxin-like compounds were measured in a small number of fish and sediment samples from Hood Canal. At this time additional contaminant studies on the Hood Canal bald eagles are not planned; however, productivity monitoring needs to be continued. Productivity has been generally higher the past four years. If the productivity levels continue to dramatically fluctuate, or if they remain consistently lower than the statewide values, we recommend re-initiating a contaminants study.

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is currently listed as a federally threatened species in Washington. In July 1999, the U.S. Fish and Wildlife Service published a proposal to delist the bald eagle under the Endangered Species Act (USDI 1999). At the time the species was listed, environmental contaminants were cited as the primary reason for its decline. Beginning in the 1940s, dichloro diphenyl trichloroethane (DDT) and other organochlorine pesticides became widely used as insecticides. In the late 1960s and early 1970s, it was determined that dichlorophenyl-dichloroethylene (DDE), the principal breakdown product of DDT, accumulated in the fatty tissues of adult female bald eagles and resulted in thin shells and reproductive failure (Wiemeyer *et al.* 1972, 1984; Grier 1982). Due to the bioaccumulative and persistent nature of DDT and the adverse reproductive effects elicited by DDT, particularly on birds, its use was banned in the United States in 1972. Restrictions on organochlorine pesticides combined with concerted efforts to protect and manage habitat and to stop persecution have resulted in recovery of bald eagle populations throughout most of the contiguous United States (USDI 1999).

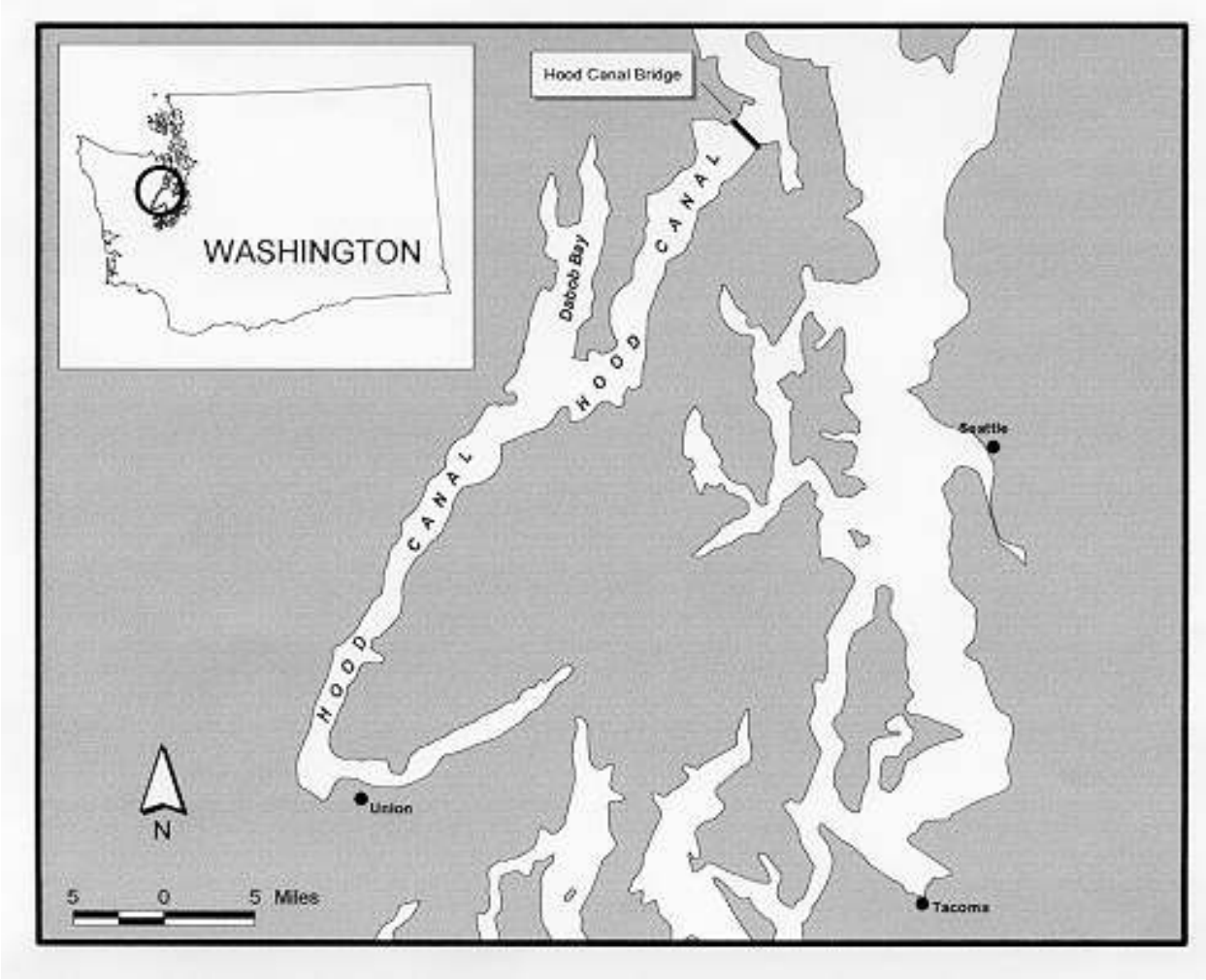
Bald eagles and other fish-eating birds continue to be exposed to persistent environmental chemicals that accumulate in their prey since they are predators at the top of the food chain. Productivity of bald eagles (Anthony *et al.* 1993, Buck *et al.* 1999) and double-crested cormorants (*Phalacrocorax auritus*, Buck and Sproul 1999) nesting near the Lower Columbia River continue to be impacted by dioxin-like compounds, including PCBs. Bald eagles (Colborn 1991, Kozie and Anderson 1991, Bowerman 1993, Best *et al.* 1994), Forster's terns (*Sterna forsteri*, Kubiak *et al.* 1989) and double-crested cormorants (Tillitt *et al.* 1992) from the Great Lakes also continue to be adversely impacted by organochlorine contaminants. Poor reproductive success of bald eagles in Maine may be linked to high PCB concentrations (Welsh 1994).

The number of occupied bald eagle territories in Washington increased from 105 in 1980 to 638 in 1998. The estimated number of young produced increased by over 700 percent between 1980 and 1998 (WDFW, unpublished data). The bald eagle recovery plan for the Pacific Region established recovery goals of an average reproductive rate of 1.0 fledged young per occupied breeding area and an average success rate for occupied breeding areas of not less than 65% over a 5 year period (USFWS 1986). These goals have been largely met in Washington (USDI 1999).

However, recovery objectives have not been met in all areas in Washington, including Hood Canal. Hood Canal is a deepwater fjord characterized by restricted circulation and extreme water depths in Western Washington (Figure 1). Although the number of occupied bald eagles nests in Hood Canal have been increasing, there was concern because their productivity remained significantly below the Washington State's productivity average in most years and fluctuated more dramatically than the statewide values (Figure 2). In 1992, a contaminants study was initiated to evaluate if contaminants were a possible cause of the lower productivity.

The primary study objectives were to: 1) evaluate productivity of the Hood Canal territories, 2) determine if elevated concentrations of dioxins, furans, polychlorinated biphenlys (PCBs), organochlorine pesticides, and metals were present in bald eagle eggs collected from nests near Hood Canal, and 3) evaluate whether the contaminant levels present could be significantly contributing to the reproductive failures seen in the bald eagles from Hood Canal. An additional objective was to evaluate if resident bottom fish could be a source of contaminants.

Figure 1: Hood Canal, Washington where bald eagle eggs were collected for the



contaminants study

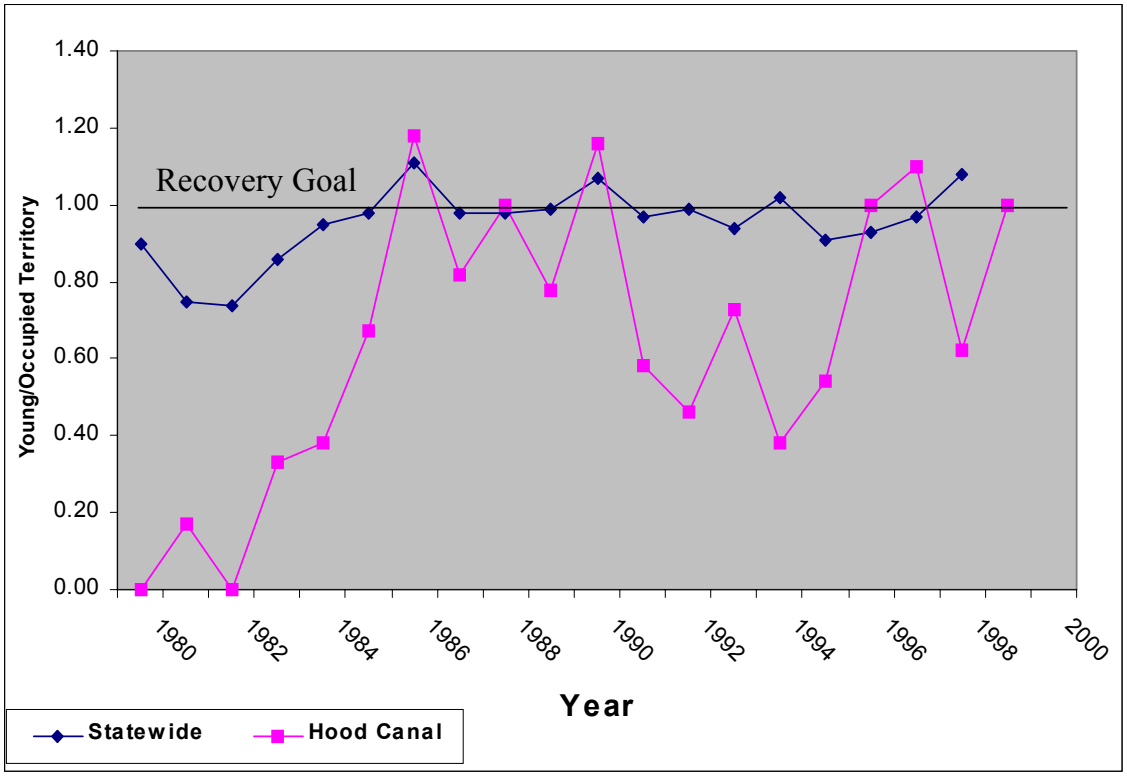


Figure 2: Annual bald eagle productivity (young/occupied territory with a known outcome) at the Hood Canal territory nests compared to the overall Washington State bald eagle productivity values.

METHODS

Productivity Surveys

Productivity surveys have been conducted by Washington Department of Fish and Wildlife (WDFW) on Hood Canal since 1981. The Hood Canal bald eagle territories are defined as those south of the Hood Canal Floating bridge and associated with Hood Canal and Dabob Bay. Occupancy surveys of bald eagle territories are conducted using a fixed wing aircraft in early April. Territories are recorded as occupied if an adult eagle is seen in an incubation posture on the nest. Productivity surveys of the occupied nests are conducted by WDFW in early to mid-June using a helicopter. The number of young per nest are recorded and compared from year to year.

Site Selection for Contaminant Monitoring

Prior to the nesting season, bald eagle territories with a repeated history of failure were selected for the contaminant study. Five territories were selected in 1992 and four in 1993 for the collection of fresh eggs (Appendix A). Aerial surveys of the Hood Canal territories were conducted the last week in March to determine if bald eagles had initiated incubation. Beginning in 1994, only addled eggs were collected. Nests with addled eggs were identified during the mid-June productivity flights. Where available, addled eggs also were collected from nests outside the Hood Canal territories for comparative purposes. Stations for sediment and fish collections were selected based on the locations of the bald eagle nesting territories that were sampled.

Sample Collection and Processing

Eggs

In 1992, timing of the egg collection was targeted to be immediately after laying. One egg was collected from each selected nest between one to four days after the first evidence of laying or incubation. Nests were accessed by a professional tree climber. The eggs were wrapped in aluminum foil before being lowered by rope in a cushioned can to an observer on the ground. A minimal amount of time was spent in each territory during egg collection.

In 1993, only three of the four selected nests were included for egg collection because it was not possible to safely climb one nest tree. One egg was collected from each of the three nest trees. Only one of the eggs was collected one day after first evidence of incubation. The other eggs were collected after incubation had occurred for a longer period of time (7 days at one nest tree and 16 days at the other) in an attempt to decrease the chance of nest desertion.

Between 1994 and 1997, a total of 5 addled eggs were collected from Hood Canal nests. One nest sampled in 1996, Union, and one nest sampled in 1997, Toandos East, were the same nests where eggs were sampled in 1993 and 1994, respectively. Between 1992 and 1997, a total of five addled eggs were collected from territories outside Hood Canal. Four were from the outer coast and one from Skagit County.

The eggs were stored in a refrigerator (4^{o}C) until they were processed. Length, width and weight of the eggs were measured in the lab. Eggs were cut along the equator and contents placed in chemically-cleaned jars with teflon-lined lids and weighed. Eggshells were washed with water and air-dried for a minimum of 30 days. Using a dial micrometer, shell thickness (including membrane) was determined by averaging the measurements at five different locations on the approximate equator of the egg. Eggshell thinning was calculated as the percent difference between mean eggshell thickness of each egg and the mean eggshell thickness (0.6088 mm) of bald eagle eggs from the region prior to 1946 (Anderson and Hickey 1972).

Blood

Whole blood samples were collected from a total of four nestling bald eagles from different Hood Canal nests in 1995 and1996. Whole blood samples were also taken from five adult bald eagles in the Hood Canal area and one adult bald eagle from an Indian Island nesting territory (five miles north of Hood Canal) in 1995 and 1996. Three of the eagles captured in the Hood Canal area were transients and two were from Hood Canal nesting territories. The adult birds were captured as part of a satellite telemetry study (Watson 1998).

Approximately 6cc of blood was taken from each eagle using a 12-ml disposable syringe and a 21-gauge needle. The blood was immediately transferred to heparinized glass vacutainers and stored on ice until they could be frozen upright. Blood samples were stored for more than four months prior to residue analysis. A loss of DDE occurs within two weeks after freezing (Wiemeyer et al. 1984) so the reported blood DDE concentrations are minimum values.

Carcasses

Tissue samples were collected from three bald eagles in the study area in 1993 and 1994. It was not known if the birds were transients or resident birds. An immature eagle was found dead on Indian Island and one of the adult eagles was found dead near East Bremerton. The causes of death were unknown. The third eagle died of injuries following what appeared to be a territorial battle near Quilcene Bay.

Fish

In May 1994, composites of five starry flounder (*Platichthys stellatus*) were collected from five locations in the Hood Canal/Dabob area (Fig. 3). Starry flounder were selected to be sampled because bald eagles have been documented to feed on these fish in Hood Canal (Jim Watson, pers. comm 1993). One additional composite of five great sculpin (*Myoxocephalus polyacanthocephalus*) was collected at a shallower location at one of the five sites. A commercial trawler was used to collect the fish. Mixed age composites of 5 fish were collected at each sample location. Fish lengths and weights were measured, composites labeled, and samples frozen while still on the trawler.

Sediments

Sediment samples were collected using a ponar dredge at each site where fish were sampled (Figure 3). Samples were taken from a depth of approximately 10 to 18 meters. Each sample was obtained by mixing four subsamples representing approximately the top 10 centimeters of sediment at each site. The subsamples were homogenized in a decontaminated stainless steel bowl and placed in chemically clean jars. Sampling equipment was decontaminated between stations. One additional sediment sample was collected using a van Veen grab sample from a 192 meter deep site in Dabob Bay in April 1994. The top 2 centimeters of sediment were collected from several grab subsamples and homogenized in a decontaminated stainless steel bowl.

Following sample processing in the laboratory or returning from the field where samples were collected, all of the samples (egg, fish, sediment, blood and tissue) were stored frozen at -20^{o} until
they were submitted for contaminant analyses. Samples were shipped overnight on dry ice to the U.S. Geological Survey, Biological Resources Division, Environmental Contaminant and Research Center (ECRC) in Columbia, Missouri for analyses.

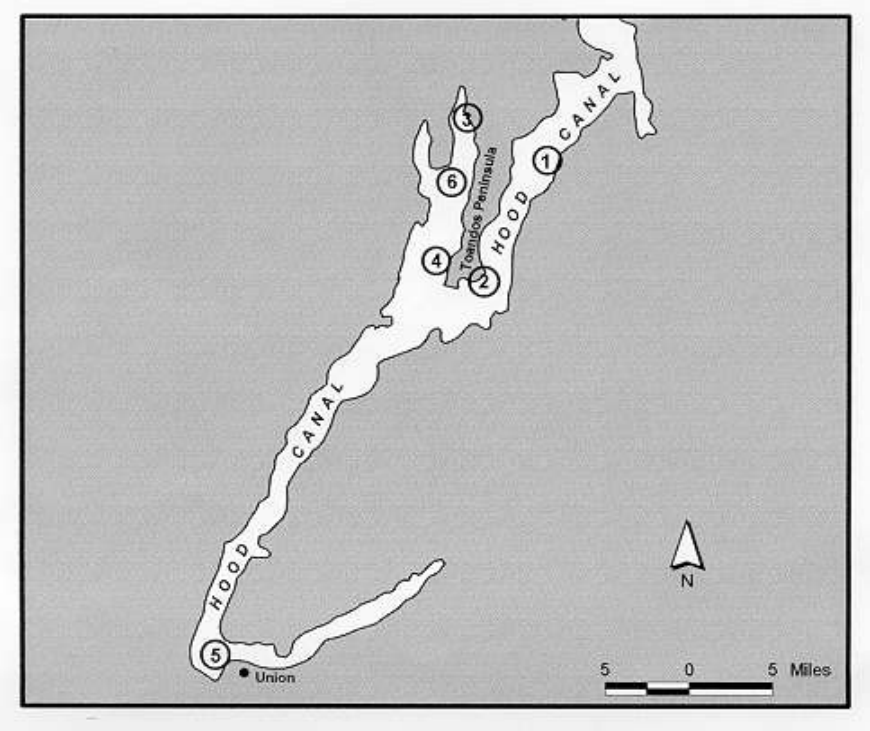


Figure 3: Sample locations for fish samples (Sites 1-5) and sediment samples (Sites 1-6) collected as part of the Hood Canal bald eagle contaminant study in 1994.

Residue Analysis

Samples were submitted to ECRC for homogenizing and chemical analyses. All of the bald eagle egg, fish and sediment samples were analyzed for dioxin-like compounds including seven polychlorinated dibenzo-*p*-dioxins (PCDDs), ten dibenzofurans (PCDFs) and four non- and eight mono-ortho-chloro substituted PCB congeners. Congener-specific analysis of PCB residues were conducted for the blood, tissue, egg, fish and sediment samples. The PCB congener identification numbers used in this paper follow that of the International Union of Pure Applied Chemistry (IUPAC). A screen for p,p'-DDE (a principle metabolite of DDT) was also conducted for all the samples. Prior to conducting the screen for p,p'-DDE and the congener-

specific PCB analyses, a scan was conducted for total PBS. The scan for total PCBs was not conducted on the last set of eggs analyzed so this concentration value is not available for all of the samples analyzed. Only the 1992 eggs were analyzed for metals (As, Se, and Hg) as well as a complete organochlorine pesticide scan.

All samples were thawed at room temperature. Sediment samples were air dryed. Egg samples were homogenized in a blender and tissue and sediment samples were ground. The samples were aliquoted for analysis (except the blood samples, in which the entire sample was used) and dehydrated with three-to-four times their weight of anhydrous sodium sulfate. The samples were extracted using methylene chloride as the solvent. The resultant extract was concentrated by rotoevaporation. The extracts were treated by a two stage reactive column clean-up; employing first a sulfuric acid silica gel/potassium silicate column and second, a column of sulfuric acid silica gel/potassium silicate/silica gel. The extracts were further purified with high performance gel permeation chromatography according to Meadows 1991. Extracts were analyzed for DDE and total PCBs at this point. The samples were then fractionated on an automated C-18/PX-21 carbon column system according to Feltz 1994, isolating four fractions: 1) bulk and di-*ortho*-PCB congeners, 2) mono-*ortho*-PCB congeners, 3) non-*ortho*-PCB congeners, and 4) PCDDs/PCDFs.

PCDD/PCDF fractions were eluted through basic alumina for removal of potential co-contaminants such as chlorinated diphenyl ethers and residual polychlorinated naphthalenes and PCBs. A total of 1 ng of the instrumental internal standard, ^{13}C-labeled 1,2,3,4-PCDD, was added to each semiconical autosampler vial prior to final transfer. The alumina fractions were transferred under a steam of nitrogen.

PCDD/PCDF fractions were determined by gas chromatography/high resolution mass spectrometry (GC/HRMS) by monitoring five sequential mass windows of selected ions during the chromatographic separation (Kuehl et al. 1991). The non-*ortho*-PCB congeners also were determined by GC/HRMS, by monitoring two sequential mass windows of selected ions during the chromatographic separation (Peterman 1994).

The bulk and di-*ortho*-PCB congeners and the mono-*ortho*-PCB congeners were determined by capillary gas chromatography/electron capture detection (CGC/ECD). Analysis of bulk and di-*ortho*-PCB congeners followed the methods of Schwartz and Stalling (1991) for congener-specific analysis. Analysis of mono-*ortho*-PCBs followed the methods of Smith et al. (1991) for samples collected in 1992 and Swartz et al. (1993) for and those collected in later years. Method limits of detection target concentrations were 1.0 pg/g for dioxins and furans and <1 ng/g for the PCB congeners.

Aliquots of enriched sample extracts from the 1992 Hood Canal territory eggs were analyzed by CGC/ECD to measure residues of 25 organochlorine contaminants. The CGC/ECD analyses were similar to those described in Schmitt et al. (1990).

Only the 1992 Hood Canal territory eggs were analyzed for metals. Arsenic and selenium sample preparation included subjecting an aliquot of each to a combination nitric/hydrochloric acid wet digestion followed by dry ashing with magnesium nitrate. Prepared digestates were analyzed by hydride generation atomic absorption spectroscopy. For mercury, samples were digested by a microwave oven procedure employing both nitric acid and hydrogen peroxide. Digestates were analyzed for mercury using cold vapor atomic absorption spectroscopy.

Quality Assurance/Quality Control (QA/QC) procedures included analyses of spiked samples with appropriate standards, analysis of replicates, analysis of procedural and matrix blanks, and the demonstration of correct chemical identifications. Matrix samples (blanks and spikes) prepared from chicken eggs, clean bluegill tissue, and samples of ECRC's standard positive control tissue (common carp from Saginaw Bay, MI), and procedural blanks were processed concurrently with the actual samples as quality control.

Recoveries of the ^{13}C-labeled PCDFs and PCDDs were within the QC range of 25% to 125%, except OCDD in one of the egg samples and some of the flounder samples where recoveries were slightly below 25%. TCDD and TCDF recoveries in all the samples ranged from 40% to 100%. Recoveries of the ^{13}C-labeled non-*ortho* PCBs ranged from 42% to 95%. Recoveries of the congener-specific analysis of PCB residues averaged 63% to 104%. Recoveries of the organochlorine pesticide and total PCB analyses exceeded 80% for all compounds except: HCB (55%), PCA (69%), heptachlor (56%), delta-BHC (52%), oxychlordane (66%), trans-nonachlor (69%), p,p'-DDT (64%), and mirex (66%). Recoveries of the metals ranged from 96% to 98%.

The H4IIE Bioassay

The H4IIE rat hepatoma cell bioassay provided an estimate of the relative potency of complex planar hydrogenated hydrocarbons (PHHs) mixtures in extracts of the bald eagle egg, fish and sediment samples. The H4IIE rat hepatoma cell bioassay procedures were a modification of the standard methods reported by Tillitt et al. (1991). The modifications were to miniaturize and automate the procedures into a 96-well microtitre plate configuration. The samples were calibrated against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for the determination of TCDD-equivalents (TCDD-EQ) in the samples. The doses of each sample (g-equivalents /mg cellular protein) or TCDD standards (pg TCDD/mg cellular protein) were plotted against ethoxyresorufin *O*-deethylase (EROD) activity (pmol/min/mg cellular protein) to develop dose-response curves. The linear portions of these curves were used to compare the relative potencies of the samples with that of the standard, TCDD.

Data Analysis

All residues in eggs were corrected for moisture loss and are reported as fresh weight. Egg fresh weight corrections were determined using egg dimensions to calculate volume, assuming a density of 1 (Stickel *et al.* 1973). Egg volume was calculated using the formula: $(\text{width})^2$ (length) (0.51). Concentrations were adjusted by multiplying each analyte concentration, originally determined based on the eggs analytical wet weight, by the egg mass/egg volume. Tissue (fish and carcasses) concentrations are reported as wet weight. Sediment samples are reported in pg/g, dry weight and were not normalized to organic carbon because of the low total organic carbon (TOC) values, particularly at one site. Low TOC values may artificially increase organic contaminant concentrations above sediment quality standards if normalization is conducted (Michelsen 1992). The reported contaminant concentrations were not corrected for procedural internal standard losses. Contaminant concentrations below detection limits were assigned a value of one-half the detection limits for data analysis purposes.

The toxic equivalents (TEQs) were estimated using standard toxic equivalent factors (TEFs) for PCDDs, PCDFs, mono-*ortho* and non-*ortho* PCBs as suggested by the World Health Organization (Van den Berg et. al. 1998). The TEQs were estimated using both the mammalian-based and the avian-based TEF values, which are presented in Table 1.

Statistical analyses were not conducted on the sample results. The methods for egg collections were not consistent throughout the study. The first two years, fresh eggs were collected from the Hood Canal territories. Fresh eggs were never collected from outside the Hood Canal territories. In 1992, the eagle nests with the worst history for producing young in the Hood Canal area were selected for egg collections. In 1993, fresh eggs were collected from three additional nests, however; their history of failure was not as extreme as the nests selected for 1992. After 1993 in the Hood Canal area, and for all the eggs collected outside Hood Canal, egg availability and feasibility of climbing the nest trees were the only factors affecting egg collections. If addled eggs were observed during nest surveys, a climber was sent to collect the egg. In addition to differences in methodology for egg collections, the sample sizes were small and were spread out over a 6 year period.

Table 1: World Health Organization's toxic equivalency factors (TEFs) for the mammalian-based (M-TEF)and avian-based (A-TEF) endpoints used to calculate toxic equivalents (TEQs) for bald eagle eggs from Washington, 1992-1997 (Van den Berg et. al 1998) .

	M-TEF	A-TEF
Chlorinated dibenzodioxins		

2,3,7,8-Tetra	1	1
1,2,3,7,8-Penta	1	1
1,2,3,4,7,8-Hexa	0.1	0.05
1,2,3,6,7,8-Hexa	0.1	0.01
1,2,3,7,8,9-Hexa	0.1	0.1
1,2,3,4,6,7,8-Hepta	0.01	<0.001
Octa	0.0001	0.0001
Chlorinated dibenzofurans		
2,3,7,8-Tetra	0.1	1
1,2,3,7,8-Penta	0.05	0.1
2,3,4,7,8-Penta	0.5	1
1,2,3,4,7,8-Hexa	0.1	0.1
1,2,3,6,7,8-Hexa	0.1	0.1
1,2,3,7,8,9-Hexa	0.1	0.1
2,3,4,6,7,8-Hexa	0.1	0.1
1,2,3,4,6,7,8-Hepta	0.01	0.01
1,2,3,4,7,8,9-Hepta	0.01	0.01
OCDF	0.0001	0.0001
Non ortho-chlorinated Biphenyls		
3,4,4',5-Tetra (81)	0.0001	0.1
3,3',4,4'-Tetra (77)	0.0001	0.05
3,3',4,4',5-Penta (126)	0.1	0.1
3,3',4,4',5,5'-Hexa (169)	0.01	0.001
Mono ortho-chlorinated Biphenyls		
2,3,3',4,4'-Penta (105)	0.0001	0.0001
2,3,4,4',5-Penta (114)	0.0005	0.0001
2,3',4,4',5-Penta (118)	0.0001	0.00001
2',3,4,4',5-Penta (123)	0.0001	0.00001
2,3,3',4,4',5-Hexa (156)	0.0005	0.0001
2,3,3',4,4',5'-Hexa (157)	0.0005	0.0001
2,3'4,4',5,5'-Hexa (167)	0.00001	0.00001
2,3,3',4,4',5,5'-Hexa (189)	0.0001	0.00001

RESULTS

Productivity

The number of known occupied territories has increased in the Hood Canal from 2 in 1980 to 31 in 1999 (Table 2). The number of young per occupied territory has reached the goal of 1.0 three out of the last four years. However, in 1994, 1995, and 1998, the number of young produced per

occupied territory was nearly half that of the other three years (Table 2). The percentage of the occupied territories that are successful has remained below the statewide levels in all but four years (Table 2, Figure 2). A relatively constant or increasing trend in the success rate of the Hood Canal territories has not been observed over the last 14 years.

Table 2: Comparisons of the estimated number of Washington statewide and Hood Canal bald eagle occupied territories, productivity and percent success.

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Statewide Data																				
No. occupied terr.	105	126	138	168	206	231	250	268	308	369	403	445	469	493	547	559	596	580	638	
Yng/occupied terr.	0.9	0.75	0.74	0.86	0.95	0.98	1.11	0.98	0.98	0.99	1.07	0.97	0.99	0.94	1.02	0.91	0.93	0.97	1.08	
% occ. terr. succ.	64	56	55	59	67	65	73	65	66	63	70	63	69	63	70	63	64	66	74	
Hood Canal Data																				
No. occupied terr.	2	5	4	6	8	9	11	11	16	18	23	26	25	24	21	21	29	27	32	31
Yng/occupied terr.	0	0.2	0	0.3	0.4	0.7	1.2	0.8	1	0.8	1.2	0.6	0.6*	0.9**	0.4	0.5	1.0	1.1	0.6	1.0
% occ. terr. succ.	0	20	0	17	25	44	82	55	59	50	76	38	41	50	27	38	75	72	50	58

*5 eggs removed and territories not included in the calculations
**3 eggs removed and territories not included in the calculations

In 1992, eagles from one of the five nests where fresh eggs were collected successfully fledged a young eagle. Three of the nests had two eggs in them prior to collection and two of the nests only had one egg. The adult eagles abandoned three of the nests shortly after the eggs were collected. A fourth nest failed even though the adults resumed incubation. At Squamish Harbor, a second egg was laid after the first egg was collected and one young successfully fledged. None of the nest sites where fresh eggs were collected in 1993 produced any live young.

Contaminants in Eggs

Eggshell thickness

Eggshell thickness of all the eggs collected for this study ranged from 13.1 percent thinner to 14.0 percent thicker than the mean eggshell thickness of bald eagle eggs collected prior to 1947 (Table 3). All bald eagle eggs collected from the Hood Canal territories exhibited eggshell thinning, except one, with a mean difference of 6.1 percent thinner. In contrast, all of the eggs collected from outside the Hood Canal territories, except one, were thicker than the pre-DDT average with a mean difference of 0.4 percent thicker (Table 3, Appendix A).

Organochlorines pesticides and total PCBs

All of the bald eagle eggs analyzed from both Hood Canal and outside Hood Canal contained PCBs and p,p'-DDE. Concentrations of total PCBs in the eggs from the Hood Canal territories derived from using a scan had a geometric mean of 13,270 ng/g (Table 3, Appendix A). In comparison, the geometric mean of total PCBs in the eggs was 6,255 ng/g by determining the concentrations by adding the mono-ortho-substituted PCBs and the congener specific PCBs. Concentrations of total PCBs in the eggs collected from outside the Hood Canal territories had a geometric mean of 4,142 ng/g using the scan and 2,806 ng/g determined by adding the mono-ortho-substituted PCBs and the congener specific PCBs (Table 3, Appendix A). Geometric means of p, p'-DDE concentrations were 1,921 ng/g and 1,943 ng/g for the eggs sampled from bald eagle nests from Hood Canal and outside Hood Canal, respectively (Table 3, Appendix A).

Individual egg sample concentrations of the congener specific PCBs are presented in Appendix B. The number of congener specific PCBs analyzed varied between a total of 199 and 123 congeners over the 6 years of the study.

Table 3: Eggshell measurements and concentrations (ng/g, fresh weight) of p,p'-DDE and total PCBs in bald eagle eggs collected from Hood Canal territories and outside Hood Canal in Washington, 1992 - 1997.

	Hood Canal			Outside Hood Canal		
	Mean[a]	**Range**	**n**	**Mean[a]**	**Range**	**n**
Eggshell Parameters						
Eggshell Thickness (mm)	0.572	0.513 - 0.694	12	0.607	0.529 - 0.658	5
Percent Change	-6.1	-15.7 - +14.0	12	+0.4	-13.1 - +8.1	5
Chemicals (ng/g fresh wt.)						
p,p'-DDE	1,921	936 - 4,334	13	1,943	367 - 5,683	5
Total PCBs (Scan)[b]	13,270	5,020 - 23,326	10	4,132	3,332 - 5,967	3
Total PCBs (Congeners)[c]	6,255	4,072 - 12,394	13	2,806	1,132 - 5,967	5

[a]Arithmetic mean for eggshell parameters and geometric mean for chemical concentrations.
[b]Total PCBs determined using a scan prior to determining PCB congeners.
[c]Total PCBs determined by adding the mono-ortho-chloro PCBs and the congener specific PCBs.

Concentrations of organochlorine pesticides in the five eggs collected from Hood Canal territories in 1992 are presented in Table 4 and Appendix C. Concentrations of p,p'-DDE were higher than any of the other organochlorine pesticides. The following organochlorines were below detectable limits: pentachloroanisole, alpha-BHC, Lindane (gamma-BHC), heptachlor, delta-BHC, Dacthal, o,p'-DDE, o,p'-DDD, Endrin.

Dioxins, Furans and Planar PCBs

All of the eggs contained concentrations of dioxins, furans, and planar PCBs. The most elevated dioxins were 1,2,3,7,8-PentaCDD, 2,3,7,8-TetraCDD, and 1,2,3,6,7,8-HexaCDD (Table 5, Appendix D) in the eggs sampled from Hood Canal and outside Hood Canal. The concentrations in the samples from outside Hood Canal were present at lower levels. Similar patterns were observed with the furans. The most elevated furans were 2,3,7,8-TetraCDF and 2,3,4,7,8-PentaCDF, with the highest concentrations present in the eggs from Hood Canal (Table 5, Appendix E).

All of the eggs also contained dioxin-like planar PCBs. Of the non ortho-substituted PCBs, congeners 126 and 77 had the most elevated levels. Congeners 118 and 105 had the highest levels of the mono-ortho-substituted PCBs (Table 5, Appendices F and G).

Table 4: Concentrations of organochlorine pesticides (ng/g, fresh weight) in five bald eagle eggs collected from different Hood Canal territories in 1992.

Organochlorine Pesticides	**Geometric mean**	**Range**

hexachlorobenzene	9	6 - 20
beta-BHC	33	13 - 51
oxychlordane	49	32-63
heptachlor epoxide	22	14-27
trans-chlordane	3	ND[a] - 5
cis-chlordane	6	ND - 16
dieldrin	23	ND - 44
p,p'-DDE	2,691	1,766 - 3,270
trans-nonaachlor	191	124 - 313
cis-nonaachlor	77	55 - 118
o,p'-DDT	18	8 - 27
p,p'-DDD	147	102 - 221
p,p'-DDT	8	ND - 14
mirex	20	12 - 33
toxaphene	441	281 - 783

[a] Not detected

The geometric means for the avian-based TEQs (A-TEQs) were higher in the eggs collected in the Hood Canal area than in the eggs collected from outside Hood Canal (Table 6). Similar results were noted for the mammalian-based TEQs M-TEQ geometric means (Table 6). The geometric mean for the total sum of the dioxin-like toxicity for the M-TEQs for the Hood Canal samples was higher than the sum of the A-TEQs. The geometric means of the sums of the dioxin-like activity in the samples from outside Hood Canal were similar for both the M-TEQs and the A-TEQs. The A-TEQ and M-TEQ values for individual egg samples are presented in Appendix H.
The non-ortho-PCBs contributed the most dioxin-like activity to both the A-TEQ and the M-TEQ va;ues (Figure 4). PCB 126 comprised the greatest percentage of the total dioxin-like toxicity for the both TEQ values, with geometric means that ranged from 45.1% to 49.3% (Table 7). PCB 77 contributed a large percentage of the total dioxin-like activity for the A-TEQ values, while PCB 118 contributed a large percentage of the total dioxin-like activity for the M-TEQ.values.

TCDD-EQs as derived from the H4IIE extract bioassay were detected in all eggs collected in 1992 to 1994 (Appendix H). The overall mean for the samples from Hood Canal was 52.3 pg/g, with a range of 6 ppt to 228 ppt. The values were the highest for the 1992 eggs with a range of 95 pg/g to 228 pg/g. The H4IIE extract bioassay was conducted on only one sample from outside the Hood Canal territories and resulted in a concentration of 5.4 pg/g.

Table 5: Concentrations (pg/g, fresh weight) of PCDDs, PCDFs, and planar PCBs in bald eagle eggs collected from territories in Hood Canal and outside Hood Canal in Washington, 1992 - 1997.

	Hood Canal	**Outside Hood Canal**

Chemical	n	Geo Mean	Range	n	Geo Mean	Range
Chlorinated dibenzodioxins						
2,3,7,8-Tetra	13	9.0	2.7 - 20.7	5	4.7	2.3 - 14.4
1,2,3,7,8-Penta	13	13.8	4.1 - 32.0	5	5.0	2.8 - 7.3
1,2,3,4,7,8-Hexa	13	1.2	0.4 - 2.3	5	0.4	0.2 NQ[a] - 0.4
1,2,3,6,7,8-Hexa	13	11.4	4.7 - 27.3	5	5.6	2.6 - 9.6
1,2,3,7,8,9-Hexa	13	0.6	0.2 NQ - 0.9	5	0.1	< 0.1 - 0.6
1,2,3,4,6,7,8-Hepta	13	0.9	0.4 - 16.7	5	0.3	< 0.1 - 1.3
OCDD	13	6.0	2.5 - 56.5	5	3.6	1.1 - 8.7
Chlorinated dibenzofurans						
2,3,7,8-Tetra	13	11.2	3.5 - 33.8	5	5.2	3.2 - 9.5
1,2,3,7,8-Penta	13	1.0	0.4 - 2.3	5	0.5	0.3 NQ - 1.1 NQ
2,3,4,7,8-Penta	13	7.9	2.0 - 18.8	5	2.7	1.5 - 6.1
1,2,3,4,7,8-Hexa	13	0.6	0.1 - 1.1	5	0.3	0.1 NQ - 0.3
1,2,3,6,7,8-Hexa	13	0.8	0.2 NQ - 3.0	5	0.5	0.2 - 0.6
1,2,3,7,8,9-Hexa	13	0.1	<0.1 - 0.3	5	0.1	<0.1 - 0.2 NQ
2,3,4,6,7,8-Hexa	13	0.8	<0.1 - 5.3	5	0.4	<0.1 - 2.9
1,2,3,4,6,7,8-Hepta	13	1.1	0.2 NQ - 6.8	5	0.6	0.3 - 1.7
1,2,3,4,7,8,9-Hepta	13	0.6	0.1 - 3.2	5	0.3	0.2 - 0.7 NQ
OCDF	13	2.3	0.5 - 10.0	5	3.1	2.3 - 5.5
Non ortho-chlorinated biphenyls						
3,4,4',5-Tetra (81)	13	114	54 - 222	5	53	35 - 63
3,3',4,4'-Tetra (77)	13	990	367 - 1,880	5	440	220 - 818
3,3',4,4',5-Penta (126)	13	1,250	442 - 2,820	5	476	328 - 782
3,3',4,4',5,5'-Hexa (169)	13	243	96 - 1,537	5	113	58 - 169
Mono ortho-chlorinated biphenyls						
2,3,3',4,4'-Penta (105)	13	136,000	49,900 - 277,200	5	45,000	28,600 - 68,700
2,3,4,4',5-Penta (114)	13	3 ,800	<100 - 24,100	5	900	<100 - 18,200
2,3',4,4',5-Penta (118)	13	442,300	157,700 - 1,012,000	5	127,300	99,200 - 158,100
2',3,4,4',5-Penta (123)	4	5,000	3,200 - 6,400	1	1,200	1,200
2,3,3',4,4',5-Hexa (156)	13	76,100	40,700 - 144,800	5	24,500	10,900 - 57,500
2,3,3',4,4',5'-Hexa (157)	7	16,200	14,400 - 24,000	3	8,000	2,900 - 13,400
2,3'4,4',5,5'-Hexa (167)	13	43,500	14,100 -119,700	5	17,700	9,200 - 31,900
2,3,3',4,4',5,5'-Hepta (189)	13	6,200	2,000 - 15,300	5	2,900	1,400 - 6,700

[a] Non Quantifiable
[b] Not Detected

Table 6: Geometric means (pg/g, fresh weight) of the toxic equivalents (TEQs) from the avian-based (A-TEQ) and mammalian-based (M-TEQ) endpoints in bald eagle eggs collected from territories in Hood Canal and outside Hood Canal in Washington,1992 - 1997. The total is the sum of the

geometric mean of the dioxin-like toxicity of PCDDs, PCDFs, non ortho-PCBs, and the mono-ortho-PCBs for each sample.

TEQ	Locations	n	PCDD/PCDF	non ortho-PCBs	mono-ortho-PCBs	Total PCBs
A-TEQ	Hood Canal	13	42.1 (13.0-106.3)	188.0 (72.5 - 392.5)	26.4 (13.0 - 55.2)	258.0 (105.8 - 549.3)
A-TEQ	Outside HC[a]	5	18.8 (10.3 -29.2)	76.5 (61.6 - 125.4)	9.3 (5.6 - 14.6)	106.4 (80.7 - 166.5)
M-TEQ	Hood Canal	13	30.4 (8.9 - 86.9)	128.2 (45.2 - 287.3)	120.3 (36.6 - 212.3)	315.1 (97.9 - 554.4)
M-TEQ	Outside HC l	5	15.2 (7.0 - 27.1)	48.8 (33.4 - 80.0)	34.1 (22.6 - 59.7)	102.3 (80.6 - 145.7)

[a]Bald eagle territories outside Hood Canal

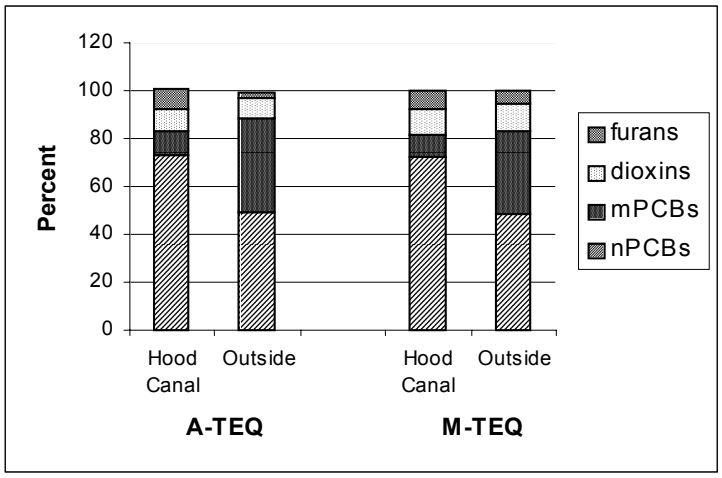


Figure 4: Average percent composition of compounds with dioxin-like activity in bald eagle eggs collected from territories in Hood Canal and outside Hood Canal in Washington, 1992-1997 for both avian- and mammalian-based TEQ values.

Table 7: The average percentages of the primary congeners that comprised the total dioxin-like toxicity for the avian-(A-TEQ) and the mammalian-(M-TEQ) based endpoints in bald eagle eggs collected from Hood Canal and outside Hood Canal in Washington, 1992 - 1997.

	Hood Canal (n=13)		Outside Hood Canal (n=5)	
Congener	**A-TEQ**	**M-TEQ**	**A-TEQ**	**M-TEQ**
Non ortho-chlorinated Biphenyls				
PCB 126	49.3	48.3	45.1	47.3
PCB 77	19.2	0.1	22.0	0.05
PCB 81	4.3	0.003	5.0	0.006
PCB 169	0.1	1.2	0.1	1.1
Mono ortho-chlorinated Biphenyls				
PCB 105	5.2	5.3	4.3	4.5
PCB 114	0.4	1.8	0.4	2.5
PCB 118	1.9	18.4	1.2	12.3
PCB 156	2.6	12.7	2.8	14.5
Chlorinated dibenzodioxins				
2,3,7,8 TCDD	3.5	3.5	5.4	5.6
1,2,3,7,8 PECDD	5.4	5.3	5.0	5.2
Chlorinated dibenzofurans				
2,3,7,8 TCDF	4.7	0.5	5.3	3.8
2,3,4,7,8 PECDF	3.2	1.5	2.8	1.5

Metals

Mercury and selenium were present in all 5 eggs collected from Hood Canal in 1992. Arsenic was not detected in 4 of the 5 eggs and was close to the limit of detection in the other egg. The geometric mean for the concentrations of mercury and selenium were 298 ng/g and 553 ng/g, respectively (Table 8).

Table 8: Concentrations (ng/g, fresh wet weight) of arsenic, mercury, and selenium in five bald eagle eggs collected from Hood Canal territories in 1992.

Territory	Arsenic	Mercury	Selenium
Brown Point	38	380	524
Squamish Harbor	ND[a]	297	551
Thorndyke	ND	456	682
Hazel Point	ND	322	704
Union	ND	142	372
Geometric Mean	**NC[b]**	**298**	**553**

[a] Not detected
[b] Not calculated

Contaminants in Blood

All of the eagle blood samples contained PCBs. The geometric means of the PCB concentrations in the nestling and adult blood samples were 50.4 ng/g and 209.2 ng/g, respectively. All of the adult blood samples and two of the five nestling blood samples contained detectable levels of p,p'-DDE (Table 9). The geometric mean of the concentrations of p,p'DDE in the adult blood samples was 44.9 ng/g. The geometric mean of the concentrations of p,p'DDE in the nestling blood samples was 10.2 ng/g. The concentration determined for the Toandos East nestling sample was likely low as shown by only 32 percent recovery of PCB congener 204.

Table 9: Concentrations of total PCBs and p,p'-DDE (ng/g) in adult and juvenile bald eagle blood samples from Hood Canal, 1995 - 1997.

Location	Year	Age	Status	Total PCBs	p,p'-DDE
Duckabush	1995	Nestling		56.7	<6.7
Dosewallips	1995	Nestling		47.6	<6.7
Waketickeh Cr.	1995	Nestling		47.3	<6.7
Union	1996	Nestling		NC[a]	74
Toandos East	1997	Nestling		NC	39
Red Bluff	1995	Adult	male, breeder	193.4	19.2
Pt. Whitney	1995	Adult	male, non-breeder	347.5	50.7
Indian Island	1995	Adult	female breeder	197.1	38.5
Broad Spit	1995	Adult	female non-breeder	144.7	32.7
Pt. Whitney	1996	Adult	male, non-breeder	NC	61.7
Bolton Peninsula	1996	Adult	male, breeder	NC	89.8

[a]NC - Scan of total PCBs not conducted

Contaminants in Eagle Carcasses

Concentrations of total PCBs and p,p'DDE detected in the three eagle carcasses are reported in Table10. The eagle collected near Quilcene had the highest PCB concentrations with 22,555.3 Fg/g in the muscle. The brain concentrations of DDE were low at 1.2 Fg/g.

Table 10: Concentrations of total PCBs and p,p'-DDE (ng/g, wet weight) measured in three bald eagles found dead in the Hood Canal area.

Location/ Tissue sampled	% Lipid	Total PCBs	p,p'-DDE
Indian Island (Immature)			
Muscle	1.5	6,949.5	2,439.8
Liver	2.8	6,905.0	2,144.6
Kidney	1.9	5,015.3	1,446.5
Brain	5.1	3,402.8	1,215.6
Quilcene Bay (Adult)			
Muscle	4.9	22,555.3	8,622.0
Liver	2.0	9,381.7	1,894.1
Kidney	0.9	6,487.7	1,327.6
Brain	5.3	6,722.7	1,180.4
Bremerton (Adult)			
Muscle	4.3	9,775.4	2,526.6
Liver	4.9	9,449.4	2,117.2
Kidney	2.3	5,579.8	936.1

Contaminants in Fish

PCBs and p,p'-DDE were present in all 6 composite fish samples. The geometric means of the total PCBs and p,p'-DDE were 109 ng/g and 1.6 ng/g, respectively (Table 11). The only dioxin and furan congeners detected in all six fish samples were 2,3,7,8-TetraCDD and Octachloro-dibenzofuran (OCDF), with geometric means of 0.6 pg/g and 3.2 pg/g, respectively (Table 12). Of all the dioxin-like congeners, Congeners 118 and 105 were present at the highest concentrations with geometric means of 1,840 pg/g and 525 pg/g, respectively. Congeners 167 and 156 were the only other mono-ortho-PCBs present in most of the fish samples (Table 12).

Congeners 77 and 126 were the only non-ortho PCBs detected in all the fish samples (Table 12). Other dioxins, furans, mono-ortho-PCBs and non ortho-PCBs were below or near quantification limits.

The fish samples contained only small amounts of dioxin-like activity as measured by the H4IIE bioassay. The geometric mean of the TCDD-EQ for the 6 fish samples was 1.75 pg/g with a range of 0.1 to 7.1 pg/g (Table 11). The Thorndyke fish sample (from site number 1) was the only sample with a TCDD-EQ above 1.4 pg/g. A significant induction occurred in two of the three extracts tested from the Thorndyke sample; however, there was a large amount of variability associated with the overall sample mean which had a standard deviation of 8.0.

Table 11: Concentrations (ng/g, wet weight) of total PCBs and p,p'-DDE

Location	Site No.	Species	% Lipid	Total PCBs	p,p'-DDE	TCDD-EQ (pg/g)
Thorndyke[a]	1	Starry Flounder	2.0	157	3.5	7.1
Hazel Point	2	Starry Flounder	1.1	81	0.8	0.7
Tarboo	3	Starry Flounder	2.5	124	1.5	0.2
Tongue/Dabob	4	Starry Flounder	2.8	155	4.4	1.4
Union	5	Starry Flounder	2.3	74	0.9	0.8
Hazel Point	2	Greater Sculpin	1.7	92	1.0	0.3

[a]Average of replicates

Table 12: Concentrations (pg/g wet weight) of non-otho-PCBs, mono-ortho-PCBs, 2,3,7,8-TCDF, and OCDD.

Location	Fish[a]	77	126	118	105	167	156	2,3,7,8-TCDF	OCDD
Thorndyke[b]	F	16	10	2,190	570	200	190	1.1	3.5
Hazel Point	F	8	3	670	220	<110	<60	0.2	2.9
Tarboo	F	18	13	5,530	1,430	410	490	0.6	2.8
Tongue/Dabob	F	23	10	4,150	1,010	310	320	0.7	3.5
Union	F	9	3	580	180	<110	<60	0.3	2.9
Hazel Point	S	11	7	1,990	640	120	140	1.0	3.6
Geometric Mean		**13**	**7**	**1,840**	**525**	**145**	**125**	**0.6**	**3.2**

[a] Fish species were starry flounder (F) and great sculpin (S)
[b] Average of replicates

Contaminants in Sediments

Overall, concentrations of the organochlorine compounds were low in all the sediment samples. All of the sediment samples contained PCBs; however, concentrations of most of the PCB congeners were below the detection limit level of 0.3 ng/g, dry weight. The geometric mean of the concentrations of total PCBs (determined by adding the mono-ortho PCB and the congener specific PCBs) in the sediment samples was 16.9 ng/g (Table 12). Concentrations of the mono-ortho PCB congeners (123, 118, 114, 105, 167, 156, 157, and 189) were below detection limits in all the sediment samples except PCB congeners 118 and 167 (Table 12). All of the sediment samples, except one of the triplicate samples from Union, had DDE concentrations below the quantifiable levels of 1.4 to 1.7 ng/g. The geometric mean for the Union sample was 2.0 ng/g. Contaminant concentrations listed in the tables have not been normalized, however; percent total organic carbon for each sample is presented in Table 12.

Concentrations of dioxins and furans were below detection limits for most of the compounds in all the sediment samples. Concentrations of 2,3,7,8-TCDF was near the detection limit for all the samples except the Union sample which contained an average concentration of 2.5 pg/g. Similarly, concentrations of 1,2,3,4,6,7,8-HCDD were not detected or were slightly above detection limits at all the sites, except Union which contained an average concentration of 27.5 pg/g.

Table 13: Concentrations of PCBs (ng/g, dry weight), OCDD and OCDF (pg/g, dry weight), and percent total organic carbon in each sediment sample from Hood Canal, 1994.

Location	Site No.	% TOC	PCB 118	PCB 167	Total PCBs[b]	OCDD	OCDF
Hazel Pt.	2	0.12	<0.35	<0.35	13.6	6.4	7.6
Tongue/Dabob	4	0.15	0.52	<0.35	17.0	6 NQ[c]	4 NQ
Tarboo	3	0.41	0.42	<0.35	17.2	15	6.5
Union[a]	5	0.89	0.69	0.66	25.7	194	16
Thorndyke	1	0.08	0.39	1.00	19.7	3 NQ	6 NQ
Dabob Bay	6	2.08	<0.35	<0.35	11.5	42	9 NQ

[a]Average of replicates

[b]Total PCBs are derived by adding the mono-ortho chlorinated PCBs and the congener specific PCBs.

[c]Not quantitated at specified average average concentration due to inaccurate ion ratio.

DISCUSSION

As was mentioned in the methods section, the methodology for the collection of bald eagle eggs changed during the study. In 1992 and 1993 when fresh eggs were collected, it was possible to target the bald eagle nests with the greatest failure rates. If environmental contaminants were the cause of the nest failures, we assumed that the nests with the greatest failure rates would also be the nests containing eggs with the highest contaminant concentrations. With the exception of the Union territory, eggs from the 1992 samples contained the highest concentrations of PCBs, had the highest TEQ values for both the avian- and mammalian-based TEQs and demonstrated the strongest dioxin-like activity (TCDD-EQs) as measured the H4IIE bioassay.

Total PCBs can be quantitated by comparing selected peak areas in a scan or by analyzing individual PCB congeners and summing their total concentrations. Traditionally, estimates of total PCBs were made using a scan. The concentrations estimated using the PCB scan are more comparable with past literature. Analyzing for the individual PCB congeners is a newer, more refined technique that removes confounding factors and interferences caused by co-eluding compounds. Total PCBs measured in this study exceeded values found in a nationwide study which correlated a level of #4.0 ppm total PCBs (fresh wet weight) with normal reproduction for bald eagles (Wiemeyer et al. 1993) and also exceeded the <6.0 ppm total PCB's (fresh wet weight) described for a healthy subpopulation of bald eagles in the Great Lakes region (Kubiak and Best, 1991). The PCB concentrations in eggs from the Hood Canal study are comparable to those found in bald eagle eggs from the Columbia River territories (Anthony *et al.* 1989, Buck *et al.* 1999) which also are experiencing depressed reproduction.

Bald eagle populations have generally experienced reproductive problems when eggshell thinning is 15-20 percent (Anderson and Hickey 1972). Mean eggshell thinning for the Hood Canal eagles was well below this level. Wiemeyer *et al.* (1993) reported that mean production increased when shell thinning was less than approximately 10 percent. Four out of the five eggs sampled outside the Hood canal area were thicker than the pre-DDT average.

Although bald eagles appear to be less sensitive to DDE-induced shell thinning than a number of other raptor species (Wiemeyer *et al*.1988), they are adversely affected by DDE. Wiemeyer *et al.* (1993) determined that bald eagle young production was normal when eggs contained <3.6 Fg/g DDE (wet weight), was nearly halved between 3.6 to 6.3 Fg/g, and halved again when concentrations exceeded 6.3 Fg/g. Because PCBs, p,p'DDE and dieldrin in addled eggs are inversely correlated to productivity, their individual effects are difficult to separate (Nisbet 1989, Bowerman *et al.* 1994). Only two eggs in this study exceeded the 3.6 Fg/g level. The egg collected from the Union nest in Hood Canal contained 4.3 Fg/g and one egg from the outer coast (Browns Point) contained 5.7 Fg/g. The rest of the eggs contained DDE values less than the 3.6 Fg/g threshold level. One young fledged from the Union nest the same year that the addled egg was collected. The mean concentration of p,p'-DDE measured in eggs collected from the lower Columbia River was higher with 5.63 Fg/g (fresh weight) compared to 1.92 Fg/g (fresh weight) in the Hood Canal eggs. Mean concentrations of DDE (presented as wet weight)

in eggs collected from six locations on the British Columbia coast (*Elliott et al.* 1996) were also higher than those measured in the Hood Canal study.

PCDDs and PCDFs are highly lipophilic, chemically stable environmental contaminants with low volatility, that bioaccumulate in fish and wildlife and biomagnify through food chains (Eisler 1986). Several PCDDs and PCDFs, as well as a few dioxin-like PCBs, have been shown to exert a number of common toxic responses similar to those observed for 2,3,7,8-TCDD, including reproductive deficits, dermal toxicity, immunotoxicity, teratogenicity, endocrine toxicity and carcnogenicity/tumor promotion (Ahlborg *et al.* 1993). The evidence of a common mechanism of action that causes similar effects in mammals and birds and for additivity of effects from exposure to PCDD, PCDF and PCB mixtures has led to the development of toxic equivalents (TEQs, Elliott *et al.* 1996b).

Since laboratory dose-response studies of bald eagle eggs have not been conducted, no-observable-adverse-effect-levels (NOAELs) for bald eagles have been based on numerous laboratory studies of other birds and field studies. Giesy *et al.* (1995) estimated a no- or lowest-observable-adverse-effect-level (NOEL/LOAEL) of 7 pg/g for TEQs in bald eagle eggs. Ellliott *et al.* (1996b) suggested using a no-observed-effect-level (NOEL) of 100 pg/g and a lowest-observable-effect-level (LOEL) of 210 pg/g $TEQs_{WHO}$ (includes planar PCBs) on a whole egg (wet weight) for bald eagle chicks.

The geometric mean of 2,3,7,8-TCDD for the Hood Canal bald eagles was 9.0 pg/g. This concentration is slightly above the NOEL/LOAEL of 7 pg/g estimated by Giesy *et al.* The geometric mean of 2,3,7,8-TCDD was higher in Lower Columbia River eagle eggs with 22 pg/g (Buck *et al.* 1999). Similarly, the geometric means of 2,3,7,8-TCDD were higher in eagle eggs from nests from four sites in British Columbia that receive industrial wastes (44 pg/g to 84 pg/g) and 15 pg/g from eggs collected at a reference location in British Columbia with little industrial activity other than lumber yarding (Elliott *et al.* 1996). The TEQ with planar PCBs calculated for the Hood Canal eagle showed that the TCDDs/TCDFs contributed less than 17 percent to the overall dioxin-like toxicity of the sample.

The geometric mean TEQ values (both avian- and mammalian-based including the planar PCBs) calculated for the Hood Canal eggs were higher than the LOEL suggested by Elliott *et al.* (1996b) with 258 pg/g and 315 pg/g, respectively. The geometric means for the TEQ values calculated for the eggs collected outside Hood Canal were close to the NOEL of 100 pg/g. The TEQs with planar PCBs calculated for the Lower Columbia River eggs and eggs from three of the British Columbia sites exceeded the threshold value of 210 pg/g.

Non-ortho and mono-ortho planar PCBs accounted for 81percent to 87 percent of the total dioxin-like toxicity found in the bald eagle eggs analyzed for this study. PCB 126 contributed the majority of the dioxin-like activity (45 percent to 49 percent). PCB 77 contributed the second greatest dioxin-like toxicity in the eggs for the A-TEQs . The greatest proportion of the TCDD-EQ in birds of the Great Lakes is contributed by the planar PCBs (Giesy et al. 1994).

PCB 126 contributed majority of the dioxin-like activity towards calculations of the TEQs in the Lower Columbia River (Buck *et al.* 1999). Two congeners, PCB 105 and PCB 126, accounted for more than 90 percent of the median estimated TCDD equivalents in Forster's tern eggs from Green Bay, Lake Michigan and Lake Poygan, Wisconsin (Kubiak et al. 1989). Elliott *et al.* (1996b) found concentrations of the toxic non-ortho PCBs 126>77>169>81>37 in bald eagle yolk sacs from British Columbia with the PCB congeners being the major contributors to TCDD-like toxicity. Results of their study compared to the pattern of contribution in common terns from the Netherlands where the PCB congeners 126 and 118 were the major contributors to TCDD-like toxicity (Bosveld *et. al 1994* in Elliott *et. al* 1996 b).

Hoffman *et al.* (1998) found that hatching success in American kestrels was affected at dose levels of PCB 126 that were 40- to 50-fold higher than in chickens. The approximate 50 percent lethal dose (LD50) for PCB 126 in chickens was 0.4 ng/g, 65 ng/g in kestrels, and 104 ng/g in terns. Hoffman *et al.* (1988) stated that high concentrations of PCB 126 found in bald eagle eggs are within the range of the LD50 (65 ng/g) in their study for American kestrels, but are nearly 20-fold higher than the lowest concentration tested in kestrels that caused significant edema and teratogenesis.

Bald eagles and other bird species can bioaccumulate mercury to elevated concentrations that cause toxic effects (Wiemeyer *et al.* 1984, Wood *et al.* 1996, Wolfe *et al.* 1998). Wiemeyer *et al.* (1993) reported that adverse effects of mercury on bald eagle reproduction might be expected when eggs contain more that 0.5 Fg/g (wet weight) mercury. Thompson (1996) reported that values of 0.5 to 2.0 Fg/g (wet weight) mercury were sufficient to cause adverse effects on birds. Concentrations of mercury in all the eggs from Hood Canal were well below these levels. Concentrations of selenium in the Hood Canal eggs were also well below levels known to cause biological impacts to birds (Skorupa and Ohlendorf 1991).

Detectable levels of PCBs and DDE in blood samples from nestling eagles indicated that they were being exposed through their prey. When comparing differences in residue concentrations between plasma and whole blood, plasma should contain about twice the concentration in whole blood (Wiemeyer *et al.* 1989). Bowerman *et al.* 1994 analyzed blood plasma samples while whole blood samples were analyzed by Anthony *et al.* (1993) and in our study. The mean concentration of PCBs (50 ng/g) measured in blood from the Hood Canal nestlings was higher than the mean concentration of 40 ng/g in nestling blood samples from the Lower Columbia River (Anthony et al. 1993) and 24 ng/g from the interior areas of Michigan, Minnesota, Ohio, Ontario and Wisconsin (Bowerman *et al.* 1994). However, it was lower than the mean PCB concentration of 183 ng/g for nestlings from the Great Lakes shoreline nests. The mean concentration of DDE residues in nestling blood from this study was comparable to the levels measured by Bowerman *et al.*(1994) from the interior nests, and lower than the Great Lake nestlings (61 ng/g) and nestlings from the Lower Columbia River (50 ng/g).

It is unknown whether or not the eagles carcasses collected in the Hood Canal area were from resident or transient birds. For this reason, correlations cannot be made between contaminant

burdens in the carcasses and potential sources of contamination in Hood Canal. Frenzel and Anthony (1989) collected 10 eagle carcasses in the vicinity of the Klamath Basin wintering area, Oregon and California. They found the concentrations of organochlorines in brains of necropsied eagles were far below those associated with death. The highest concentration of DDE and PCBs in the brains were 24.0 and 35.0 ppm (wet weight), respectively. Mean concentration of DDE in the whole carcass tissues was 2.61 ppm (wet weight) and mean concentration of PCBs was 2.23 ppm (wet weight). Concentrations of DDE and PCBs in the one brain sample for this study were lower. Concentrations of DDE in two the birds from this study were at comparable levels to the Klamath Basin eagles, but the PCB levels in all three of the eagles were higher.

Bald eagles are migratory birds and forage in different areas which complicates identifying areas of contaminant exposures. Watson and Pierce (1998a) monitored the movements of 6 adult bald eagles captured in the Hood Canal area via satellite telemetry. Between May and August, eagles migrated northward along coastal and interior British Columbia, as far as southeast Alaska. Evidence suggested that the migration was in response to summer and fall spawning runs of coastal salmon. The two breeding adults eagles monitored returned to breeding territories following a migration northward and other than 5 short (<75 km) and brief (#4 days) excursions from breeding territories just before and after migration, they remained on territories the rest of the year. The use of coastal British Columbia and southeast Alaska by migrant eagles in the fall appears to be the norm for other breeding populations from western Washington (Watson and Pierce 1998b in Watson and Pierce 1998a). Therefore any contaminant exposure in the British Columbia and Alaska should also be occurring to local populations in Washington and would not be totally unique to Hood Canal eagles (Watson and Pierce 1998a).

Hood Canal bald eagles are exposed to environmental contaminants in their prey. In 1993, prior to our sampling fish for this study, Watson *et al.* (1996) conducted a bald eagle foraging study in the Hood Canal area. They identified 308 prey items to class with fish accounting for 85 percent and birds for15 percent of the prey items. Only 16 fish were identified to species, with 11 of those being starry flounder. Fish comprised 91 percent of prey captured by eagles in a separate study on Hood Canal (Watson and Pierce 1998a) and in the Columbia River Estuary, fish accounted for 90 percent of prey captured by eagles and 71 percent of the prey remains found in nests (Watson *et al.* 1990). In comparison, Knight *et al.* (1990) collected prey remains from nests in Washington and found that birds comprised 78 percent of all prey remains at the Olympic Peninsula nests (which were located along the northern and western coasts of the Peninsula and did not include the Hood Canal nests on the west edge). Although fish contained low concentrations of organochlorine and PCB residues, fish-eating birds appeared to be the major source of elevated contaminant levels in bald eagles nesting near Lake Superior (Kozie and Anderson 1991).

The contaminant concentrations in the fish composites collected for this study were similar to background levels measured by WDFW for the Puget Sound Ambient Monitoring Program (PSAMP). The PSAMP was designed to monitor the average or “ambient” conditions in Puget

Sound. English sole and rockfish sampled in the non-urban bays (which included Hood Canal) accumulated lower concentrations of PCBS than fish from the and near-urban or urban bays (PSWQAT 1998). Exposure to PCB-contaminated sediment was believed to be the main factor associated with PCB accumulation in English Sole (PSWQAT 2000). The average concentration of the sum of Arochlors 1248, 1254 and 1260 in English sole muscle tissue from Hood Canal between 1991 and 1996 was 23 ng/g (PSWQAT 1998).

Chemicals that are relatively insoluble in water, including PCBs, PCDDs and PCDFs, accumulate through the food chain beginning with the uptake from sediments. Based on the low concentrations of PCBs and compounds with dioxin-like activity in the six sediment samples collected for this study, an obvious pathway of contaminants from the sediments to fish was not identified. The Effects Range Low (ER-L) value of 22.7 ng/g is intended to represent concentrations of total PCBs toward the low end of the effects range, below which adverse biological effects are rarely observed (Long *et al*. 1995). Total PCB concentrations measured in samples from all the sites, except Union with an average of 25.7 ng/g, were below the ER-L value. The total PCB values reported in Table 12 were not corrected for background concentrations as measured by procedural blanks and the blanks had an average concentration of 13 ng/g for total PCBs. This value was almost half the value reported for the Union sample so it is possible that the Union sample is reported at a concentration slightly higher than its true value. Concentrations of PCBs were not detected in sediment samples collected in Hood Canal as part of the Puget Sound Ambient Monitoring Program (PSWQAT 1988, Llanso *et al.* 1998?). A study of sediment conditions in Lynch Cove Estuary at the tip of Hood Canal in the south found that all levels of contaminants analyzed for (including PCBs and a number of metals) were below state standards, with many being undetectable (Clifford 1996).

RESEARCH IMPLICATIONS

Although this study was conducted over 6 years, it provides a one-time evaluation of contaminants affecting the Hood Canal bald eagles. Concentrations of PCBs and compounds with dioxin-like activity were elevated at levels of concern in the eggs from the Hood Canal nests. Overall, the PCB concentrations in the eggs collected early in the study were at higher levels than those collected later. Although two nests were sampled during two different years, it is not possible to compare the eggs collected during the two sampling years to evaluate if conditions are improving or decreasing because of the short time period between when they were sampled and small sample size of two. Also, both of the nests had a year of inactivity between the time when they were first sampled and the second time. New nests continue to be established in the Hood Canal area. As older birds with substantial contaminant burdens are replaced by younger birds, we would expect to see a decrease in the persistent environmental contaminants in eggs if a constant or new source of the environmental contaminants available to the eagles is not present.

Monitoring productivity of the Hood Canal nests needs to be continued to evaluate if the Hood Canal area is meeting recovery productivity goals. If the number of young produced per occupied territory per year or the nest success rate continues to dramatically fluctuate or if they remain consistently lower than the statewide values, we recommend re-initiating a contaminants study of the Hood Canal bald eagles. Contaminant concentrations could be compared to those measured for this study to evaluate if contaminants are potentially causing the decreased productivity and if the levels of the contaminants measured in this study are changing. Evaluations of other environmental contaminants that were not analyzed for in this study, but which could be potentially adversely affecting the Hood Canal bald eagles, should also be considered if a contaminants study is reinitiated. Evaluations of other factors, such as disturbance and habitat alterations affecting either nesting territories or prey, possibly should also be included if productivity is impacted.

ACKNOWLEDGMENTS

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APPENDICES

Appendix A
Congener-Specific analysis of PCBs

The congeners which were not identified separately and include a single concentration value are listed together. The PCB congener identification numbers follow that of the International Union of Pure Applied Chemistry.

004, 010	044	087	135,144,124	178
005,008	045	091	136	179
006	046	092	137	180
007,009	047	095	138	182,187
016, 032	048	097	141	183
017, 015	049	099	146	185
018	051	101	147	189
019	052	105	151	191
020,033	053	107	153	193
022	056,060	110	156	194
024,027	063	114	157, 201	196,203
025	064	118	158	197
026	066	119	167	198
028	067	123,149	170,190	199
029	070,076	128	171,202	200
031	074	129	172	205
040	081	130	173	206
041	082	131,122	174	207
042	083	132	176	208,195
043	084	134	177	209