Organochlorines and Mercury in Eggs of White-tailed Sea Eagles *Haliaeetus albicilla* in Norway 1974-1994

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ABSTRACT

Chemical pollution and breeding performance of the White-tailed Sea Eagle population in Norway were studied since 1974. The DDE and PCBs were the dominant pollutants. Only 3% of the eggs had DDE levels above a suggested critical level of 25 μ g/g for this species, while 4.5 % were above a suggested critical of 12 μ g/g level for the Bald Eagle. Maximum fresh weight levels of 51 μ g/g DDE and 248 μ g/g for sum-PCBs were detected. Both DDE and PCBs were significantly negatively correlated with shell thickness and thickness index, that were on the average 2 and 6% thinner than the pre-DDT norm from museum eggs respectively. The mean reproductive rate in the period seemed unaffected by pollutant levels. Inverse relations were found between latitude and DDE, PCBs and mercury. There were temporal decreases for DDE and PCB. Only 13% of the PCBs were mono-ortho coplanars, but they accounted for 87% of the dioxin (TCDD) equivalents. A surprisingly high maximum level of $4.2 \mu g/g$ of chlordanes was found, but moderate or low levels of mirex, HCHs, dieldrin and HCB were recorded. Mercury residues were well below proposed critical levels. It was concluded that pollution adversely affected the breeding performance of some pairs of the White-tailed Sea Eagle in Norway, but there was no effect at the population level.

INTRODUCTION

Studies of numerous raptor species have demonstrated that the detrimental effect of persistent organochlorines and heavy metals on their reproduction and survival is a problem of global significance (Newton 1979). The White-tailed Sea Eagle *Haliaeetus albicilla* suffered major declines

throughout most of its range in Europe during the 1950s and 60s. The productivity of the Swedish population declined through the 1960s and 70s (Helander 1975). In Finland, the population in the Gulf of Bothnia failed to reproduce in the early 1970s (Koivusaari et al. 1972a). At the same time, a dramatic drop in productivity was also noted in West Germany (Koeman et al. 1972), and there was concern in East Germany as to why so many eagles were found dead from poisoning and unknown reasons (Oehme 1966). In the 1970s, the productivity of the population in Lapland was still normal, while that of the Baltic population was dramatically reduced. Analyses of pollutants in unhatched eggs revealed moderate levels of organochlorines (OCs) in Lapland, and high levels in the Baltic population. In the Baltic, a significant negative correlation was found between reproductive success and residue levels in the eggs (Helander 1982). Since Ratcliffe (1970) reported the shell-thinning phenomenon, a massive amount of data from the field (Newton 1979), laboratory (Lundholm 1987) and semi-natural conditions (Lincer 1994) have pinpointed that p-p'DDE, a metabolite of DDT, is primarily responsible for the shell thinning effect.

The White-tailed Sea Eagle is classified as vulnerable on the Red Data List in Norway (Størkersen 1992), and throughout its range (Tucker & Heath 1994). Norway is a stronghold for the species, with a stable or increasing population of ca. 1500 pairs, which is approximately 40% of the European breeding population (Folkestad 1994). The contamination levels and reproductive performance of the population in Norway are therefore of great international concern. The White-tailed Sea Eagle is distributed along the Norwegian coast north of 59°N to the Russian border, 70°N, 31°E (Willgohs 1961). Since protection of the species was initiated in 1968, the population has increased and expanded its range on the south-western coast and into the fjords. A project was started in 1975 by World Wildlife Fund/Norway to monitor population status, reproductive success and pollutant levels through annual surveys. Since 1991 the project has been organised through the Norwegian Ornithological Society (NOF).

The White-tailed Sea Eagle is one of the bird species in Norway that has the highest organochlorine levels, and is well suited for monitoring of persistent pollutants (Nygård 1991). This study aims to clarify the extent of contamination by persistent organic compounds and mercury in the Norwegian White-tailed Sea Eagle population, as expressed by concentration in the eggs. The Norwegian coast is generally remote from major agricultural and industrial sources of pollutants. Does this result in pollutant levels that are lower here than in the rest of Europe? Furthermore, is there a decrease with increasing latitude? Is there a temporal trend and, if so, can it help us to explain the population expansion of the White-tailed Sea-Eagles in Norway?.

MATERIAL AND METHODS

Sixty-seven eggs from 59 clutches were collected for analysis between 1974 and 1994. The coast was divided into four sampling areas from south to north (Figure 1). The eggs were collected at the time of banding of the chicks, 6 to 9 weeks after hatching. The contents, removed through a hole drilled at the equator of the egg, were stored in glass jars cleaned with pure ethanol,

Figure 1. The distribution of the White-tailed Sea Eagle in Norway 1994, and its division into sampling regions. The four main areas where the samples were collected are circled.



with aluminium-lined lids. For comparison of shell thickness, pre-1947 eggs from the collections of the natural history museums of Oslo, Bergen, Stavanger, Trondheim and Tromsø, together with some eggs from private collections in Norway, were measured.

Chemical analyses

The eggs were analysed at the Norwegian College of Veterinary Medicine/National Veterinary Institute in Oslo, Norway. Samples of egg were homogenised with an Ika Ultra Turrax 81. Approximately 1-2 g of each egg sample was weighed and extracted as described by Brevik (1978) with some modifications by Skaare et al. (1988). The procedures for packed column gas chromatography used before 1987 are described by Holt et al. (1979) and Barrett (1985). From 1988 on, capillary gas chromatography was used as described below. Some eggs were analysed using both chromatographic methods, and correction factors were thus obtained to make the methods comparable. Samples were automatically injected on a Carlo Erba, HRGC 5300 Mega Series gas chromatograph, equipped with a split/splitless injector, a 25 m/0.32 mm i.d. Ultra 1 column (Hewlett-Packard Comp.) and a Carlo Erba ⁶³Ni- electron capture detector. Hydrogen was used as carrier gas (2 ml/ min) and the split ratio was 1:30 of 2 μ l. The chromatographic data were calculated using the software Maxima 820 Chromatography Workstation (Millipore Waters) on an Olivetti PC M290. The blubber samples were analysed for 33 PCB-congeners (Σ PCB), IUPAC nos. (after Ballschmitter & Zell 1980): 28, 47, 52, 56, 66, 74, 87, 99, 101, 105, 110, 114, 118, 128, 132, 137, 138, 141, 149, 151, 153, 156, 157, 170, 180, 183, 187, 189, 194, 196, 199, 206 and 209 (Camebridge Isotope Laboratories, Woburn, Mass., USA); 5 DDT components and metabolites (*SDDT*): p,p'DDT, p,p'DDE, p,p'DDD, o,p'DDT and o,p'DDD (Supelco CPM); chlordanes (=·CHLs): heptachlor, heptachlor epoxide, cis-chlordane cis-nonachlor, oxychlordane and trans-nonachlor; hexachlorocyclohexane-isomers (= Σ HCH): α -HCH, β -HCH and γ -HCH and hexachlorobenzene, HCB. The PCB-128 and -187 were not separated on the GC-column used during the time of this study. Tetrachloronaphtalene (TCN) and PCB-112 were used as internal standards.

Analytical quality assurance

Percent recovery was calculated by adding a known amount of a PCB and/or pesticide standard to a sample of clean chicken egg. Recoveries ranged from 90 to 110 percent of added standard amount. Each sample preparation series of 24 samples includes four recoveries (two in the highest and two in the lowest standard concentration regions), two control samples and one blank. Each sample series on the GC includes a known standard followed by a pure cyclohexane for every tenth sample. Quantifications were carried out within the linear range of the detector. Linearity was determined routinely and varied between two and three times the concentrations of the highest standards (50-100 ng/ml). Detection limits were for PCB congeners 0.005 - 0.017 ng/g, for DDT components; 0.006 - 0.019 ng/g, for Σ CHLs; 0.005 - 0.007 ng/g, for HCHs; 0.003 - 0.015 μ g/g and for HCB; 0.002 ng/g. Non-detected components were assigned a value of zero. Control samples of seal blubber are included in each sample preparation series and the results from this sample are plotted on a control chart to test the reproducibility at the laboratory over time.

International reference materials (CRM 349 and 350, ICES cod liver oil and mackerel oil) are analysed regularly. The laboratory has participated in several intercalibration tests, organised by WHO/UNEP (The World Health Organisation/United Nations Environmental Programme, 1982 and 1992) and four steps of the ICES/IOC/OSPARCOM (International Council for Exploration of the Sea/ International Oceanographic Commission/ Oslo-Paris Commission) test on PCBs in marine material. Acceptable analytical quality was obtained in all tests.

The relationship between the first series (1974-1987) using GC with packed columns, vs. the later ones using capillary column technique, was tested on some selected eggs. Three White-tailed Sea Eagle eggs and nine of Merlin *Falco columbarius* were analysed using both methods. The results were in relatively good agreement. For DDE, Σ PCB and HCB respectively, the approximate capillary/packed ratios were found to be approximately 1.0, 0.9 and 1.4, and the results from packed columns were adjusted accordingly.

Measurements and corrections

All undamaged eggs were measured (length, breadth) with vernier calipers and weighed prior to emptying. The eggs were thoroughly washed with luke-warm water. The weight of the dried, empty eggshells was used after they reached a stable weight at room temperature. The thickness of whole eggs was measured through the blow-hole at the equator, taking a minimum of 4 measurements with a modified micrometer (Starrett Model 1010), and using the arithmetic mean. It was carefully checked to ensure that no remains of egg contents were present where the measurements were taken. When only fragments were available, ten pieces from each clutch were measured. Their average thickness was considered representative of the mean clutch value. A correction formula to compensate for lack of membranes was established, using data from shells with thickness measurements both with (T) and without membranes (t), expressed as a linear regression model.

$$T = 1.04 * t + 71 \mu m$$
, $(r = 0.92, p < 0.0001)$

Correction for the size of the blow-hole and eccentricity was done according to the formula;

SI =
$$(W_{/}(L*B))(1.25+0.27*D^2/(L*B) - 0.32*B/L),$$

where SI = shell index, Ws = mass of dried eggshell, L = length of shell B = breadth of shell, and D = hole diameter (Nygård, in manuscript). Correcting for loss of moisture was performed according to the method given by Stickel *et al.* (1973), originally developed for the Bald Eagle *Haliaeetus leucocephalus*. This involves the use of an empirical formula for the volume (V) of the egg; $V = 0.508*LB^2$

The net volume (Vn) is found by subtracting two times the shell thickness for the length and the breadth in the formula above. To compare data from eggs with different moisture content, a fresh weight correction factor, C, was calculated as follows, following Stickel *et al.* (1973):

$$C = W_p / W_f$$

where $W_p =$ weight of egg at time of preparation minus W_s , and fresh weight is obtained by the formula for net volume above; $W_f = Vn^* 1.0 \text{ g/cm}^3$ (assuming a factor of 1.0 for specific gravity). The corrected wet weight residue level for each egg was obtained by multiplying the uncorrected levels by the factor *C*. All the statistical tests on OCs are made on \log_{10} -transformed values of clutch averages, as residue levels from eggs usually need to be log-transformed to obtain a normal distribution (Blus *et al.* 1972).

Mulhern and Reichel (1970) demonstrated that the rotting process will not cause significant breakdown of persistent pesticides like PCBs and DDT. Loss of lipid during incubation and putrefaction will, however, affect the organochlorine levels, as OCs are lipophilic (Connell & Miller 1984). Newton and Bogan (1978) showed that the OC residues approximately doubled in Sparrowhawk *Accipiter nisus* eggs between the first and the last fifth stage of development, the major increase taking place after the embryo was half-grown, due to loss of moisture and lipids. Peakall and Gilman (1979) showed that the lipid content in homogenised eggs containing embryo, yolk and albumen was approximately 60% of that of the fresh egg, the main reduction in lipid content taking place in the last half of the incubation period. Therefore, we report the values on a fresh weight basis. One extremely thick-shelled egg (more than 1 mm) from Vikna in 1990 was omitted from calculations involving shell thickness.

SPSS for Windows, v. 6.1.3 was used in the statistical tests (Norusis 1993).

RESULTS

Fat and dry matter content

The median fat content was 4.8 % (1.3-37%), and the median dry matter content was 19 % (11-76%). The wide range was due to some very dry eggs. By using the estimated net volume and the estimated weight of the dry matter, we were able to calculate the estimated original water content of the egg, giving a median value of 85%.

Residue levels

As the sophistication of the analytical techniques has improved during the course of the project, it has been possible to detect a wider range of pollutants, including more isomers and congeners of the main pollutants. However, to facilitate comparisons, some of the compounds detected in the samples collected later than 1987 were grouped to provide comparisons with older samples (the average contribution of the different compounds in recent material in parentheses): Σ HCHs (hexachlorocyclohexanes) is the sum of α - HCH (3.6%), β -HCH (96.4%) and γ -HCH, "lindane" (<0.01%); Σ CHLs is the sum of heptachlor epoxide (1.1%), oxychlordane (18.2%), cis-chlordane (16.6%), trans-nonachlor (37.7%), cis-nonachlor (24.3%) trans-chlordane (2.2%) and heptachlor (< 0.01%). DDE is p,p'-DDE alone, and it contributed the most of Σ DDT. Other members of the DDT group, like o,p'-DDD (0.6%), p,p'-DDD (1.5%), p,p'-DDT (0.4%) were detected at low levels, but were not included in the statistical treatments. Σ PCBs is the sum of all detected PCB congeners.

PCBs were the dominant organochlorine group, with a median level of 8.1 (range 1.6-248) μ g/g, fresh weight. In the present study, 33 of 209 possible PCB congeners were detected, but not all in each egg. Figure 4 shows the relative amounts of the detected congeners; among these were the mono-ortho-congeners with IUPAC-numbers 105, 114, 118, 156 and 157. These constituted on the average 13 % of the sum of PCBs in our sample. The median DDE level was 3.1 (range 0.56-69.0) μ g/g. The cyclodienes were represented by dieldrin (median 0.21, range 0.03-1.0 μ g/g) and chlordanes (median 0.79, range 0.03-3.9 μ g/g). Levels of HCB were low, with a median level of only 0.05 (range 0.01-1.28) μ g/g. Mirex was also detected (median 0.05, range 0.01 - 0.29 μ g/g). HCHs were also found in small amounts (median 0.02, range 0.01-0.22 μ g/g). The mercury levels were generally low; median 0.19 (range 0.04-0.60) μ g/g. The maximum pollutant loads recorded were from a clutch of two eggs with large embryos from Haram district, Møre og Romsdal county, collected in 1983. They contained 15 and 69 μ g/g DDE and 51 and 248 μ g/g Σ PCBs, respectively.

Inter- and intra-clutch variation

Eight clutches of two eggs were available, allowing for an analysis of variance of intra- vs. inter-clutch variation (ONEWAY, log values). The inter-clutch variation was significantly larger than intra-clutch for DDE, Σ PCBs, Σ HCHs, Σ CHLs and mercury levels (p < 0.05). For dieldrin and HCB the difference was not significant, but some of the results for these compounds were close to the detection limit, so analytical inaccuracy might have played a role here. We therefore regarded single eggs as representative of the whole clutch, i.e. the female organochlorine level in one breeding attempt.

Table 1. Geographical variation in organochlorine concentration in eggs of White-tailed Sea Eagle in Norway 1974-1994. The values are given as $\mu g/g$ on fresh weight basis based on clutch averages. A Spearman rank correlation-test between pollutant level and region is performed, and the r_s and p-values (two-tailed) are listed.

Region DDE	61°30'-63°N	63°-64°30'N	64°30'-66°N	66 ⁰ -69 ⁰ N	r _s	р
Mean	9.32	3.37	3.39	2.79		
Median	3.36	3.11	3.22	2.31	-0.25	0.055
S.D.	13.01	1.11	2.18	1.77		
N	9	6	23	21		
ΣPCBs						
Mean	39.75	8.56	8.31	6.75		
Median	15.03	8.84	6.61	5,33	-0.43	0.001
S.D.	43.78	1.55	6.05	3.95		
N	9	6	23	21		
НСВ						
Mean	0.186	0.094	0.056	0.051		
Median	0.126	0.083	0.041	0.034	-0.39	0.002
S.D.	0.211	0.062	0.046	0.042		
Ν	9	6	23	19		
ΣHCHs						
Mean	0.034	0.041	0.036	0.044		
Median	0.030	0.040	0.022	0.019	-0.37	0.026
S.D.	0.006	0.012	0.034	0.066		
Ν	3	4	18	11		
Dieldrin						
Mean	0.32	0.25	0.27	0.17		
Median	0.23	0.25	0.18	0.15	-0.40	0.077
S.D.	0.22		0.24	0.09		
Ν	3	1	10	6		
ΣCHLs						
Mean	1.17	1.41	0.60	0.94		
Median	0.93	1.14	0.62	0.63	-0.28	0.10
S.D.	0.71	0.71	0.52	0.91		
Ν	6	4	13	13		
Mirex						
Mean	0.147	-	0.037	0.037		
Median	0.128	-	0.030	0.034	-0.51	0.09
S.D.	0.101	-	0.024	0.016		
Ν	3	0	5	4		
Hg						
Mean	0.29	0.32	0.23	0.13		
Median	0.26	0.33	0.19	0.13	-0.58	< 0.001
S.D.	0.12	0.08	0.12	0.05		
Ν	9	6	24	21		

Table 2. Variation in organochlorine and mercury levels over time in eggs of White-tailed Sea Eagle in Norway 1974-1994. The values are median levels ($\mu g/g$) on a fresh weight basis, based on clutch averages. The predicted half-lives are based on curve-estimation by the SPSSPC ver. 6.1.3, using an exponential model $Y = b_0(e^{bt})$, where b_0 is a constant, b is a regression coefficient and t the year. (Spearman rank-correlation, of OC level *vs.* year, two-tailed significance).

										Predicted
	Period	1974-79	1980-84	1985-89	1990-94	r _s	р	b _o	b	half-life
DDE	Mean	4.6	6.1	4.0	2.6	-0.35	0.006	8.8E+35	0.0411	17 years
	Median	4.4	3.7	2.5	2.0					
	S.D.	1.1	9.0	5.7	1.4					
	Ν	6	15	18	20					
ΣPCBs	Mean	7.9	19.5	14.4	7.1	-0.29	0.024	8.5E+31	-0.036	19 years
	Median	7.4	10.2	5.3	6.6					
	S.D.	2.9	32.0	21.9	4.1					
	Ν	6	15	18	20					
HCB	Mean	0.063	0.110	0.078	0.061	-0.09	0.49			
	Median	0.069	0.049	0.046	0.051					
	S.D.	0.020	0.178	0.071	0.045					
	N	6	15	16	20					
ΣHCHs	Mean	0.016	0.033	0.050	0.043	0.18	0.28			
	Median	0.016	0.019	0.035	0.029					
	S.D.	0.001	0.042	0.039	0.048					
	N	4	7	5	20					
Dieldrin	Mean	0.194	0.293		0.180	0.22	0.33			
	Median	0.160	0.224		0.180					
	S.D.	0.090	0.238		0.003					
	N	6	12	0	2					
ΣCHLs	Mean		0.051	1.100	0.857	0.15	0.37			
	Median	•	0.051	0.700	0.731					
	S.D.	,	0.026	0.976	0.530					
	Ν	0	2	14	20					
Mirex	Mean				0.065	-0.48	0.11			
	Median		•		0.044					
	S.D.		•		0.068					
	N	0	0	0	12					
Hg	Mean	0.21	0.22	0.16	0.20	-0.12	0.34	3706150	-0.0085	62 years
	Median	0.23	0.22	0.15	0.18					
	S.D.	0.15	0.10	0.07	0.12					
	N	6	16	18	20					

Effects of developmental stage

Forty-four eggs were classified as infertile, 12 had less than half-grown embryos (defined as < 75 mm from head to tail) (Helander *et al.* 1982), and 9 had embryos that were half-grown or more. In five of the eggs, the stage was not noted. The effect of development on the dominating pollutants (DDE, Σ PCBs and Hg) was tested (Mann-Whitney). No significant effect was found on fresh weight basis (all p-values > 0.1), but the levels were higher in the > half grown group on a lipid weight basis (P < 0.03).

Variation in time and space

The eggs were divided into four groups based on geographical origin

Figure 2. Variation in shell thickness over time in eggs of White-tailed Sea Eagles in Norway 1850-1994. The thick line represents the median value, the box ends mark the 25th and the 75th percentile, and the whiskers indicate the largest and smallest values that are not outliers (i.e. values that are more than 1.5 box-lengths from 25th and 75th percentile).



along a gradient from south to north, encompassing the main sampling areas (Figure 1). All the compounds decreased with latitude, the most pronounced trends were found in mercury, $\Sigma PCBs$, and HCB (Table 3).

The temporal trends of organochlorine levels were illustrated by dividing the material into four approximate five-year periods from 1974 to 1994 (Table 2). A Spearman rank-correlation of pollutant level *vs.* year was performed, and there were significant trends for DDE ($r_s = -0.35$, n = 59, p = 0.006) and $\Sigma PCBs$ (rs = -0.29, n = 59, p = 0.024). The predicted half-lives, according to an exponential model, were 17 years for DDE, 19 years for $\Sigma PCBs$, and 62 years for mercury. The figures must be taken as crude approximations, due to the large variances.

It was evident that both time- and regional effects were acting simultaneously. Accordingly, an analysis of variance was performed (on the pollutants with adequate samples), to elucidate their partial contributions to the pollutant levels (Table 3). The analysis strongly indicated that regional effects were dominant over time effects. There were strong significant effects of region for $\Sigma PCBs$, HCB and mercury, near significant for DDE (p = 0.052). Corrected for the effect of region, there were no significant changes over time for any of the pollutants, although decreasing trends for DDE (p = 0.08)

Figure 3. Shell thickness, T, (mm) in relation to the levels of DDE and \cdot PCBs (mg/g) in Sea-Eagle eggs in Norway 1974-94. The regression equations were T = 628.7 - 78.4 logDDE (Rsq = 0.100, p = 0.06); T = 661.4 - 69.8 logPCB (Rsq = 0.139, p = 0.002).



Table 3. The simultaneous effects of period and region on pollutant levels in White-tailed Sea Eagle eggs in Norway 1974-94. (2-way ANOVA, based on log values, two-tailed significance levels).

			DDE					ΣPCBs		
Source of	Sum of	DF	Mean	F	р	Sum of	DF	Mean	F	р
variation	squares		square			squares		square		
Main effects	1.24	6	0.21	2.25	0.05	3.27	6	0.55	6.11	< 0.001
Period	0.65	3	0.22	2.34	0.08	0.60	3	0.20	2.25	0.09
Region	0.70	3	0.23	2.25	0.07	2.64	3	0.88	9.85	< 0.001
Explained	1.24	6	0.21	2.25	0.05	3.26	6	0.54	6.11	<0.001
Residual	4.78	52	0.09			4.64	52	0.09		
			HCB					Hg		
Source of	Sum of	DF	Mean	F	р	Sum of	DF	Mean	F	р
variation	squares		square			squares		square		
Main effects	2.10	6	0.35	2.77	0.02	1.53	6	0.26	5.47	< 0.001
Period	0.22	3	0.07	0.57	0.64	0.10	3	0.03	0.71	0.55
Region	2.06	3	0.69	5.44	0.003	1.36	3	0.45	9.73	<0.001
Explained	2.10	6	0.35	2.77	0.02	1.53	6	0.26	5.47	<0.001
Residual	6.32	50	0.13			2.47	53	0.05		

and $\Sigma PCBs$ (p = 0.09) were found. For the other pollutants the material was too unevenly represented in the different categories to permit proper analysis.**Correlations between pollutants and shell thickness**

Shell thickness and shell thickness index were negatively correlated with the major pollutants, but correlations were significant only for DDE, Σ PCBs, and HCB (Table 4). The relationships between DDE and Σ PCB levels and eggshell thickness are shown in Figure 3. Σ PCB levels were slightly

Table 4. Correlation coefficients (Pearsons) between the different pollutants and shell thickness variables in eggs of White-tailed Sea Eagle in Norway 1974-94. (Log values of OCs and Hg, two-tailed significance levels).

	DDE	ΣPCBs	HCB	ΣHCHs	Dieldrin	ΣCHLs	Mirex	Mercury	Shell index
Shell index	-0.38	-0.38	-0.43	-0.24	-0.24	-0.26	0.15	-0.16	
Ν	(52)	(52)	(50)	(29)	(23)	(28)	(8)	(53)	
	P=0.006	P=0.01	P=0.002	P=0.208	P=0.29	P=0.18	P=0.71	P=0.24	
Shell thicknes	s -0.32	-0.37	-0.41	-0.13	-0.21	-0.14	-0.16	-0.23	0.90
Ν	(66)	(66)	(63)	(38)	(24)	(39)	(12)	(67)	(110)
	P=0.010	P=0.002	2P=0.001	P=0.44	P=0.32	P=0.41	P=0.62	P=0.06	P<0.001

Table 5. Shell thickness and thickness index in eggs of White-tailed Sea Eagle in Norwa	y
1947-94. The thinning is based on median values. The groups are tested by Mann-Whitney	Ū
test, one-tailed significance levels.	

	1850-1946	1947-73	1974-79	1980-84	1985-89	1990-94	1947-94
Whole eggs							
Shell thickness (μ m)							
Mean	619	582	591	594	628	590	600
Median	606	571	602	585	611	579	593
S.D.	60	46	40	76	77	79	71
	N=29	N=6	N=10	N=16	N=18	N=19	N=69
Relative to pre-1947 (%)		-5.8	-0.7	-3.5	0.8	-4.5	-2.2
р		0.08	0.09	0.04	0.38	0.03	0.045
Fragments							
Shell thickness (μ m)							
Mean		546	579	585	557	586	576
Median		546	585	579	567	592	576
S.D.			35	92	36	46	59
	N=0	N=1	N=21	N=24	N=22	N=20	N=88
Relative to pre-1947(%)		-9.9	-3.5	-4.5	-6.4	-2.3	-5.0
Shell index							
Mean	3.17	3.03	2.97	3.04	3.26	2.99	3.06
Median	3.16	2.95	2.92	2.96	3.15	2.94	2.98
S.D.	0.30	0.23	0.20	0.38	0.35	0.31	0.31
	N=28	N=6	N=9	N=14	N=12	N=16	N=57
Relative to pre-1947(%)		-6.6	-7.6	-6.3	-0.3	-7.0	-5.6
р		0.13	0.04	0.046	0.27	0.015	0.024

Figure 4. The average relative amounts in % of the detected PCB congeners in eggs of White-tailed Sea Eagles in Norway 1987-94. Black columns represent the mono-ortho congeners known to have dioxin-like toxicity.



better correlated than DDE with shell thickness.

Shells of intact eggs were on the average 2.2% thinner, and shell index 5.6% lower, in the period after 1947 (the time when DDT was introduced on a large scale in the western world) up to the present, compared to older material (Table 5). The difference is significant (for shell thickness, p = 0.045, for shell index, p = 0.038). However, when dividing the material into approx. five-year periods after 1974, some variability appears. Eggs were on average 5-7% thinner between 194784. Between 1985-89 thickness levels were near normal, but after 1990 they were 5-7% thinner than pre-DDT levels. The shell thickness and index varied in parallel, with the exception of 1974-79, when the thickness was considerably less reduced than the index (Table 5). Fragments of eggshell collected in the nest after 1974 were on the average 3.6 % thinner than whole eggs (p = 0.03, df = 157, Mann-Whitney U). However, the significance was weakened when the shell thickness was corrected for region (ANOVA, DF = 1,3,153, F = 2.7, p = 0.10). The shell thickness varied significantly with region (controlled for the effect of period, ANOVA, DF = 1,3,154, F = 2.99, p = 0.033.), the more thick-shelled eggs being laid in the north.

Pollutant levels and reproduction

The reproductive rate of the Norwegian breeding population has been

Figure 5. The relationship between DDE levels in eggs (fresh weight) and breeding parameters in White-tailed Sea Eagles in Norway 1974-93. Reproduction data from Folkestad (in manuscript). The Spearman rank correlations (two-tailed significance) between reproductive rate (RR, the number of large young per occupied territory), breeding success (BS, the ratio of successful nests to occupied territories) in relation to mean logDDE levels (fresh weight, mg/g) and year, were; BS vs logDDE; r = 0.029, p = 0.91, BS vs year; r = -0.14, p = 0.58, RR vs logDDE; r = 0.005, p = 0.98, RR vs year; r = -0.36, p = 0.12, logDDE vs year; r = -0.28, p = 0.23.



quite stable during 1974-93, varying between 0.5 and 0.9 large chicks per occupied territory (mean 0.65) and the nest success between 0.3 and 0.7 (mean 0.46) (Folkestad in manus.). No significant correlations were found between shell thickness or pollutant levels in the eggs, and reproductive parameters (Spearman rank test, p > 0.05). The general temporal decrease in pollutant levels had no discernible effects on nest success and reproductive output (Figure 5).

DISCUSSION

Fat and dry matter

Our results on fat and dry matter content were quite similar to those reported by Helander *et al.* (1982) from Sweden. Their sample of 60 eggs had a mean value of 4.6% for undeveloped eggs, compared to our median 4.8%. In fertile eggs with embryos 10-75 mm the Swedish eggs' fat content averaged 4.0%, while ours were 4.8%. The estimated original water content of the Swedish sample was 85.4%, while in our sample the median value was

85%. As expected, eggs from Sweden and Norway seem to agree very well with regard to fat and water content.

Effects of laying sequence and embryo development

Some studies on other species have shown a slight increase in OCs in eggs during the laying sequence (e.g. Mineau (1982) in Herring Gulls *Larus argentatus*), while others have found no systematic trend (e.g. Newton & Bogan 1978). However, we had no way to determine the laying sequence of the eggs, except when there were two, and they both had developing embryos. The relatively small difference in pollutant levels between the two eggs in a clutch in our sample, as supported by other authors, makes us believe in the use of eggs as an appropriate monitoring tool for environmental pollutants. They also have a relatively fixed chemical composition, and may be argued to represent the pollution status of the female at the egg-laying stage.

Previous studies of the effect of development stage on pollutant levels have shown an increase of levels after the embryos have been half-developed (Newton & Bogan 1978). This increase has been shown to be in parallel with the decrease of lipids in the egg (Peakall & Gilman 1979). Helander *et al.* (1982) found that there was a linear relationship between DDE and PCB residues and embryo length in yolk and muscle from embryos in White-tailed Sea Eagle eggs in Sweden. Our study confirmed this.

Time trends

Little recent information on pollutant levels in eggs of White-tailed Sea Eagle of neighbouring populations from later years were available. However, we were able to look at time trends in its food items in the same time period, and at species occupying a similar trophic level in the same habitats. The decrease over time for OCs in our sample of eggs of White-tailed Sea Eagle was paralleled by a general decrease in levels of $\Sigma PCBs$, DDE, HCB, $\Sigma HCHs$ and oxychlordane between 1983 and 1993 in six seabird species from northern Norway and the Barents Sea region (Barrett et al. 1996). In our sample there was a tendency for many pollutants, especially $\Sigma PCBs$, to reach the highest levels in 1980-84, with an overall decreasing trend from 1974-94. A similar trend was found in Herring Gull eggs from the Lofoten islands, where levels of $\Sigma PCBs$ in 1983 were two times those of 1973, and three times those of 1993 (Barrett et al. 1996). Interestingly, Johansen et al. (1994) found an increase of Σ PCBs in human milk in Norwegian mothers from 1970 to 1979, followed by a decrease from 1982 to 1991. There may a general decreasing tendency of $\Sigma PCBs$ in the North Atlantic water masses, which eventually leads to lower levels in the biota. PCBs declined in Fulmars Fulmarus glacialis, Kittiwakes Rissa tridactyla and Brünnich's Guillemots Uria lomvia breeding in the eastern Canadian Arctic 1967-87 (Elliot et al. 1992). Helander (1994a)

reported that the levels of DDE in all eggs from the 1980s and 90s were below 600 μ g/g (lipid weight, i.e. ca. 28 μ g/g f.w.), while in the 1960s and 70s more than two-thirds of the eggs contained > 600 μ g/g.

Geographical differences

Our data indicate a strong inverse relation between latitude and the major OC pollutants and with mercury. The latitudinal relation for OCs was shown for Harbour Porpoise *Phocoena phocoena* in the North Atlantic (Kleivane *et al.* 1995), where DDT, HCHs and PCB levels were higher in Danish waters and decreasing on the Norwegian coast. However, chlordane metabolites were highest in the group collected in Finnmark, the northernmost county in Norway. Bernhoft and Skaare (1994) report that the blubber levels of PCBs in Harbour Seals *Phoca vitulina* from the southern coast were about five times that of animals from the north-eastern coast. Barrett *et al.* (1996) found a decreasing gradient from north Nordland to East Finnmark of PCBs, HCB, DDE and Hg. This supports our findings; a south to north decrease, although their gradient starts where ours ends. An alternative explanation for the gradient could be different diets between populations, but no data are available to test this possibility. The increase in shell thickness from south to north suggests a natural clinal variation.

Implications of DDE and PCBs for survival and reproduction

Few recent data are published on pollutants in European White-tailed Sea Eagle populations. More data, however, are published on its close American relative, the Bald Eagle, although it is inadvisable to assume equal sensitivities. The sensitivity to DDE is suspected to differ between species in this genus (Helander 1994a). As pollutant levels vary over time, one cannot compare populations from different countries from different time periods. Other factors, such as age and nutritional status of the birds, may influence these levels. Another confounding factor is that most of the eggs represent different birds from different localities, with differing diets. Furthermore, there may be differences in analytical methods and their quality between laboratories, for instance Σ PCBs may differ between investigations. Most laboratories have gone through substantial changes and improvements in their methods during this twenty-year period.

No more than 3% of all our eggs had concentrations of DDE > 25 μ g/g fresh weight, a suggested lower end of the critical level for successful reproduction (Helander 1994a). However, > 48% had DDE levels > the suggested critical level of 3.8 μ g/g DDE (wet weight) for the Bald Eagle in North America 1969-79 (Wiemeyer *et al.* 1984). Nisbet & Risebrough (1994) analysed a data-set of Bald Eagles from 1984 to 1991, and came up with a proposed critical level of 12 μ g/g. Only 4.5% of the eggs from Norway

exceeded this value (we have adjusted the wet weight values to fresh weight (f.w. = w.w. * 0.8) for comparisons). It is intriguing that the two data-sets for the Bald Eagle gave such different results. We find it highly improbable that the sensitivity of the species should have changed so rapidly. The apparent difference in sensitivity implied by the data between the congeneric White-tailed Sea Eagle and the Bald Eagle is surprising. It is more likely that more than one factor is involved, but that the primary effect is from DDE. The other factors may have changed over time. In this context it is interesting to note that our data show that shell thinning correlated better with ΣPCB than DDE. Since DDE and other OCs are usually highly correlated, a PCB effect, or a synergistic PCB-DDE effect, is not easily detectable (Nisbet & Risebrough 1994). A careful examination of the contribution of other OCs on productivity is needed, but so far adequate data-sets are lacking.

The reproductive success in an uncontaminated population of Sea Eagles in Sweden before the DDT period was 1.32 young per territorial pair (Helander 1994b), but this was in a thinned-out (persecuted) population with no density-dependent effects. The average productivity in Norway from 1974-93 was 0.65 young per territorial pair (A.O. Folkestad, in manuscript). This is higher than in the neighbouring population in the Swedish Baltic during 1965-78 (0.32), but roughly equivalent to what was found in Swedish Lapland in the same period (0.55, Helander 1983). The DDE and PCB levels there (5.7 and 10 μ g/g f.w., calculated from lipid weight values in Helander *et al.* 1982) were similar to the Norwegian eggs in the same period. Swedish Lapland has very little local contamination, while the Baltic, due to its low water exchange, has had serious pollution problems, and contaminants were the main factor responsible for lowered productivity (Helander 1985). However, the situation has greatly improved during later years, following the rather rapid drop-off in DDE levels in the Sea Eagle eggs from Baltic Sweden (Helander 1994a). The improvement seems to be more rapid than in the Norwegian population.

Surprisingly low levels were found in two White-tailed Sea Eagle eggs from Denmark in 1979-80 (1-2 μ g/g of DDE and PCBs w.w, Dyjk *et al.* 1988). However, serious problems in the Baltic population were reported in the former East Germany, where DDE levels were 40-68 μ g/g w.w. in 1966-69.(Oehme 1971), and in Schleswig-Holstein, where 8 eggs had a median value of 12 μ g/g DDE in 1969-71(Koeman *et al.* 1972). The global nature of the problem, including the Arctic, is underlined by the levels in five eggs from Greenland, that had median levels of 4 μ g/g DDE, and 5 μ g/g PCBs (calculated from original data on lipid basis) (Wille 1976).

Shell thinning

Most Sea Eagle populations in the west have gone through a period of considerable shell thinning. Compared to museum samples from the time before DDT was put into use (1947), eggs from Sweden in the period 1951-1976 averaged 17 % thinner than eggs from 1861-1920 (Odsjö & Helander 1977). In former East Germany, average shell thinning in 1954 -1978 was 21 %, compared to pre-DDT levels (Oehme 1980). In Finland, a sample of eggshell fragments from the Quarken area in 1967-71 were 14.5% thinner than reference material from 1884-1935 (Koivusaari et al. 1972b). The average shell thinning in the Norwegian population (2-6%) is well below the proposed critical population level of 15 % (Newton 1979). Merlin eggs in Norway showed an average decrease of shell thickness of 12% and a decrease of shell index of 10% during 1947-95, 1960-80 being two decades of severe shell thinning, with on the average 15-17% lowered shell index (Nygård et al. 1994; Nygård, in manuscript). Compared to the present study, the levels of DDE in the Merlin were 2-3 times higher and the PCB levels were 6-10 times lower. In contrast to the White-tailed Sea Eagle, the Merlin is a terrestrial predator and a migratory species; therefore it may be exposed to a completely different mix of environmental pollutants. In the 1980s and 90s many raptor species in Norway, including the Peregrine Falcon Falco peregrinus and the Goshawk Accipiter gentilis were showing improvement in shell thickness (Nygård 1991). There is now a general trend of lowered DDE stress and subsequent shell thickness recovery.

The PCBs

The significance of the PCB levels, which are about twice those of the DDE levels, is unclear. The different PCB congeners vary greatly in their toxicity. Their individual toxicities have been estimated by Safe (1990), in units of toxic equivalency factors (TEFs) of 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD). The non- and mono-ortho coplanar PCBs are approximate stereoisomers of TCDD, and have similar abilities to induce hepatic aryl hydrocarbon hydroxylase (Safe 1987). In our sample of eggs, a moderate proportion (13% on the average) were mono-ortho coplanars but, using TEFs, they were estimated to account for 87% of the total PCB toxicity. IUPAC no 118 and 105 alone accounted for *ca*. 55 and 12.7%, very similar to the 48 and 13.4% found in samples of breast-muscle in White-tailed Sea Eagles found dead in Poland (Falandysz *et al.* 1994).

The almost identical pattern of PCB homologues and congeners of Otter tissue (Christensen & Heggberget 1995) and White-tailed Sea Eagle eggs in Norway is striking. This may imply that the relative amount of the different PCB congeners of the coastal food-web of Norway is relatively uniform, and will be accumulated to relatively similar levels in a coastal predator. An alternative explanation is that the Sea Eagle and the Otter eat similar food. There are many observations of the Sea Eagle as a kleptoparasite of the Otter (Willgohs 1961; Love 1983). Staven (1994) found that the food composition of the Sea Eagles in a study area on the central coast of Norway was almost exclusively fish, deep-water species believed taken from Otters or as offal from commercial fisheries. The proportion of fish in the diet in earlier studies (Willgohs 1961, 1984) was apparently underestimated; new video-studies have revealed no trace of small fish in the nest after they were digested by the young (Staven 1994).

It is interesting that Lincer (1994) reported possible synergism between PCB and DDE in an experiment involving feeding of the American Kestrel *Falco sparverius* with different mixtures of PCBs and DDE. In Sweden, Helander (1994a) reported no effects of PCB and Hg levels in eggs on reproductive success of White-tailed Sea Eagles. Similar to our findings, Nisbet (1989) found a stronger correlation between PCB and shell thickness than for DDE, using a non-parametric regression procedure, but DDE was the parameter most closely associated with productivity, followed by oxychlordane (a cyclodiene). This is interesting in the light of the relatively high levels of chlordane found in our study.

Other organochlorines and mercury

One ought to look more closely upon the chlordane group, where a maximum of 4.2 μ g/g on a fresh weight basis was recorded. We know of no other bird data that show such high levels of this particular OC compound, which has no known use in Scandinavia (Wachtmeister & Sundström 1986). The environmental significance of HCB is probably low. Dieldrin is very toxic, and has probably played a role in the population declines of raptor species including Golden Eagles Aquila chrysaetos in Scotland (Lockie & Ratcliffe 1964; Newton & Galbraith 1991). Newton (1988) found that populations of Sparrowhawk Accipiter nisus and Common Kestrel Falco *tinnunculus* declined when liver levels of the birds contained > 1 μ g/g dieldrin, and when Peregrine Falcon eggs exceeded 0.7 μ g/g wet weight (approx. 0.55 $\mu g/g$ f.w.). In the present study, three eggs (5%) had levels above 0.5 $\mu g/g$ f.w. Thus there is no reason to believe that dieldrin has affected population numbers in Norway. The levels of mirex and Σ HCHs were very low, and probably of negligible biological significance. The occurrence of mirex is in itself interesting, as it is principally known as a compound used for ant control and as a fire retardant in North America (Eisler 1985). Thus the global nature of the organochlorine pollution is illustrated.

The mercury levels are below what is believed to be critical levels, as they are no higher than in Sea Eagles in Sweden with normal productivity

CONCLUSIONS

The results imply that the pesticide burden in the eggs of White-tailed Sea Eagle in Norway is generally below accepted critical levels. It seems that the Norwegian breeding population is less affected by long-range pollutants than its neighbouring conspecifics in the Baltic, although some pairs have reduced breeding success, potentially due to pesticides. The dominating gradient is a decreasing trend of the major pollutants from south to north. There is a decreasing trend over time for DDE and $\Sigma PCBs$. The White-tailed Sea Eagle in Norway was only moderately affected by OCs. The shell thinning, believed to be caused by DDE in the females, has probably had a negligible effect on the productivity of the population. It is believed that most of the pollutant load originates from sources outside Norway, as we have found compounds like mirex in the eggs, with no known use in this part of the world. Finally, we regard the White-tailed Sea Eagle as a good monitor of environmental pollution on the Norwegian coast.

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