Polybrominated Flame retardants in eggs from Swedish White-Tailed Sea Eagles *(Haliaeetus albicilla)*

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Abstract
Brominated flame retardants (BFRs) used to suppress or inhibit the risk of fire consist of several classes such as polybrominated biphenyl ethers (PBDE), hexabromocyclododecane (HBCD) and polybrominated biphenyls (PBB). These substances are found in the environment to different extents. The common feature for these organohalogenated chemicals are that they are lipophilic, persistent and bioaccumulate to higher levels in the food chain. The present study shows the congener pattern and the contamination levels of PBDE, HBCD and BB-153 in white-tailed sea eagle (WTSE) hatching in four different areas of Sweden; inland Lapland, inland freshwater lakes, along the coast of south Bothnian Sea and the coast of the Baltic Proper. The BFRs analyzed for were detected in all 44 selected eggs, except BDE-209, which was non-detectable. The highest levels were detected for BDE-47 and the levels decreased for higher brominated PBDEs with BDE-154 showing the lowest value. Similar levels as BDE-154 were also detected for HBCD and BB-153.

Introduction
The white-tailed sea eagle *(Haliaeetus albicilla)* in Sweden has suffered from several threats including environmental pollutants and the species was almost extinct in the beginning of the 1970s due to high levels of DDT and polychlorinated biphenyls (PCB). The use of these chemicals was banned in Sweden in the 1970s and a program to save the white-tailed sea eagles was started. This program has led to a recovery of the WTSE in Sweden. Due to its top position in the food chain, the WTSE is a vulnerable species and may be potentially threatened from other organohalogenated lipophilic substances such as BFRs. The BFRs are a large diverse group of chemicals used to suppress or inhibit the risk of fire. PBDEs are one class of BFR used, consisting of three major commercial products, penta- (PentaBDE), octa- (OctaBDE), and decabromodiphenyl ethers (DecaBDE) based on the degree of bromination. These are used in different types of materials such as polyurethane for use in upholstered furniture but also in textiles, plastics and electrical components [1]. HBCD consists of several stereoisomers and is another BFR mainly used for expanded and extruded polystyrene for thermal insulation foams in buildings and constructions [2]. A third class of BFR, not as commonly used as the others is polybrominated biphenyls (PBB). The primary product (BP-6) in use in the 1970s was HexaBB, which contains BB-153 as the dominant component [3]. All three BFR groups are lipophilic, persistent compounds that can bioaccumulate [4]. One biological effect among others that has been observed for both lower brominated PBDE and HBCD is that they show irreversible neurobehavioral effects in neonatal mice, which can affect the learning and memory functions [5, 6]. A consequence of the broad use of substances not chemically bound to the material is that they might be released into the environment to such levels that they can be seen as environmental contaminants. The present study was aimed to quantify brominated flame-retardants in WTSE eggs from Sweden and to study differences in congener patterns among eggs from different parts of the country.

Materials and Methods
Samples and sampling areas
Since the mid 1960s, annual surveys during spring and summer to study the reproductive success of WTSE has facilitated the collection of dead eggs for investigation. The eggs in this study represent females from four different subpopulations. A total number of 44 eggs collected between 1991-2004 were selected. The investigated populations are; Lapland (inland in the North of Sweden**,** 12 eggs**)**, inland freshwater lakes (middle and south of Sweden, 12 eggs), the southern coast of the Bothnian Sea (12 eggs), and the coast of the Baltic Proper (8 eggs).

Extraction, clean up and analysis
All the samples had previously been extracted due to earlier investigations of PCB and DDT levels in the eggs. The extraction method used was a wet-wet extraction with n-hexane: acetone developed by Jensen et al [7]. The rest of the lipids were stored in glass ampoules dissolved in n-hexane. In this study, 15-50 mg of this material was used and further cleaned with concentrated sulphuric acid. Analysis of the substances was performed using GC/MS (Hewlett Packard series II 5890/Finnigan SSQ 7000 system) equipped with a quadropole, operating in the electron capture negative-ionisation (ECNI) mode using selected ion monitoring (SIM), scanning for the negative bromide ions, *m/z* 79, 81 and with ammonia as the reagent gas. Separation of BDE-47, -99, -100, -153, -209, was done on a DB-5-MS fused silica capillary column (15 m, 0.25 mm i.d, 0.1 μm film thickness) whereas to separate HBCD and BDE-154 from BB-153, a longer DB-5-MS fused silica capillary column (30m, 0.25 mm i.d., 0.25 μm film thickness) was used. The injector temperature was held at 280°C. Two different GC temperature programs were used depending on the column length. The transfer line was held at 300°C and the temperature in the ion source was 180°C.

Statistic evaluation
The 44 analysed eggs, were laid by 36 different females. An analysis of the components of variance showed significantly smaller variance within females than among females ($p < 0.001$). Therefore the individual results from eggs laid by the same female were replaced by their mean concentrations. The concentrations within each geographical region were skewed to the right and to achieve normal distribution, the 36 concentrations were ln-transformed. MANOVA was used to test for differences among geographical regions considering all BDE-congeners (except BDE-209), BB-153 and HBCD. Hotelling´s T^2-test was used to carry out pair-wise comparisons between regions. For these comparisons the significance level was adjusted to 0.0083, accounting for repeated tests (Bonferroni-adjustment).

Results and Discussion
All the substances, BDE-47, -99, -100, -153, -154, HBCD and BB-153 analysed for were detected in all 44 eggs except for BDE-209 which was non-detectable in any of the eggs with the lipid amount used. The mean concentration for each respective population can be seen in Table 1. The arithmetic mean concentrations of the Σ_5 PBDE (BDE-47, -99, -100, -153, -154) were: Lapland, 900 ng/g lipid weight (lw), inland freshwater lakes, 1800 ng/g lw, south Bothnian Sea, 4900 ng/g lw and the Baltic Proper, 5200 ng/g lw. The highest individual Σ_5PBDE was 13700 ng/g lw in an eagle from the coast of the Baltic Proper.

Table 1. Arithmetic mean value (AM), standard deviation (SD), Range (min –max values) and geometric mean (GM) for 6 PBDE congeners, PBB-153 and HBCD in eggs from white-tailed sea eagles hatching in four different areas in Sweden; Lapland, inland fresh water lakes, along the coast of south Bothnian Sea and the Baltic Proper. All values are presented as ng/g lipid weight.

Population	*n*		*Lipid weight(%)*	*BDE47*	*BDE99*	*BDE100*	*BDE153*	*BDE154*	*BDE 209*	*BB 153*	*HBCD*
Lapland	12	**Mean (AM)**	**4.8**	**370**	**163**	**172**	**114**	**66**	**nd**	**28**	**83**
		SD	1.1	278	136	129	68	60		19	97
		Range	**2.9-6.6**	**79-1077**	**53-553**	**44-504**	**27-252**	**13-236**		**9-61**	**35-386**
		Mean (GM)	4.7	294	130	136	95	50		22	64
Inland Fresh Water lakes	12	**Mean (AM)**	**5.0**	**1002**	**233**	**275**	**178**	**108**	**nd**	**40**	**103**
		SD	0.9	587	118	129	84	50		23	39
		Range	**3.2-6.0**	**366-2330**	**55-439**	**95-456**	**55-339**	**36-207**		**10-96**	**72-189**
		Mean (GM)	4.9	865	202	244	160	96		35	97
Bothnian Sea	12	**Mean (AM)**	**6.5**	**3047**	**667**	**485**	**407**	**261**	**nd**	**113**	**185**
		SD	4.0	1510	467	240	234	110		46	122
		Range	**3.2-17.7**	**1041-4835**	**198-1651**	**243-1108**	**208-1008**	**128-503**		**63-200**	**98-480**
		Mean (GM)	5.8	2689	521	438	362	243		105	159
Baltic Proper	8	**Mean (AM)**	**5.2**	**3221**	**615**	**691**	**444**	**244**	**nd**	**152**	**157**
		SD	1.1	2425	474	578	296	170		132	90
		Range	**3.6-7.7**	**1372-8440**	**256-1628**	**276-1970**	**125-1026**	**86-584**		**53-429**	**78-310**
		Mean (GM)	5.1	2645	487	551	361	201		117	139

BDE-47 was several times higher along the Swedish coast than in the inland areas, which can be explained by the fact that the spread of these substances has not reached the lakes and the north of Sweden to the same extent but it might also depend on the food composition from different areas and the migration patterns of WTSE. The concentrations for all substances were higher in eggs from the Swedish coast than in eggs from Lapland and the freshwater lakes. Lapland eggs differed significantly from all the other populations at a significance level of $p<0.05$. The freshwater lakes population was significantly separated from the Lapland and Bothnian Sea populations ($p<0.05$) whereas the two coastal populations (Bothnian Sea and Baltic Proper) were not significantly separated.

The WTSE is a top predator in the aquatic food chain and the food composition varies depending on the area and season. During summer, the eagles along the coasts of the Bothnian Sea, the Baltic Proper and inland freshwater lakes prey mainly on fish and seabirds, whereas in the winter season seabirds and carrion makes up most of the food. In early spring in Lapland, before the ice breaks up, WTSE rely largely on carrion from reindeer, whereas in summer the diet there is mainly fish and seabirds. Territorial adult eagles along the coast and at inland freshwater lakes are mostly stationary birds, whereas the birds from Lapland move in September-November to central and southern Sweden and return to Lapland in March-April. Some of the Lapland birds may stay over winter along the Norwegian coast[8].

The dominant PBDE congener in all areas was BDE-47 followed by, -99, -100, -153, -154 and HBCD were somewhat higher than BB-153 in most areas, Fig 1. This follows a similar pattern presented in a recent study of WTSE in Norway **[9]**.

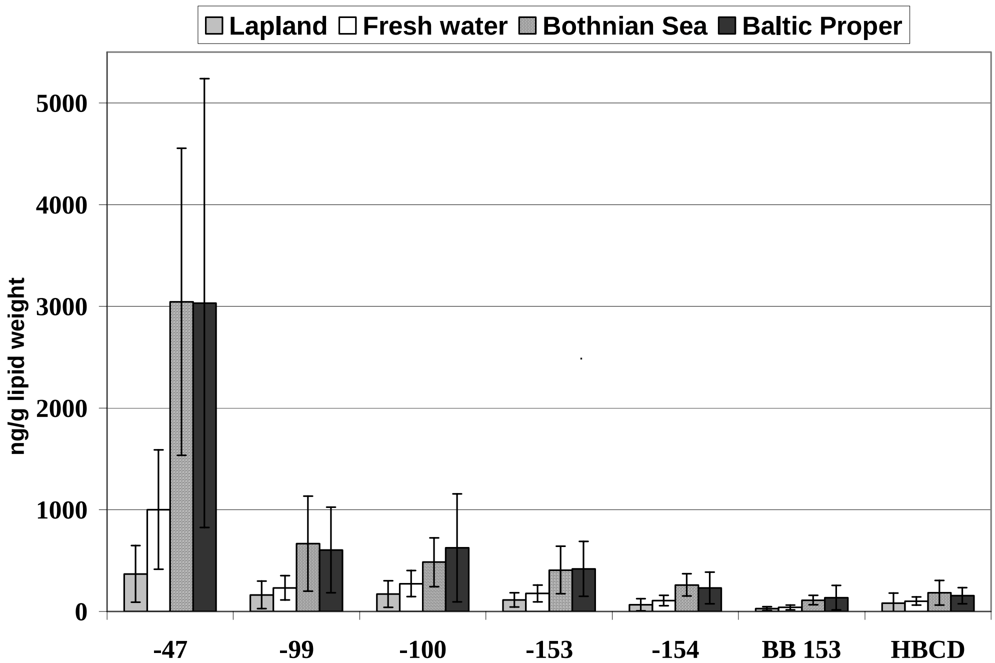

Figure 1. Arithmetic mean concentrations presented as ng/g lipid weight with standard deviations bars for polybrominated diphenyl ethers – 47, -99, -100, -153, -154, polybrominated biphenyl (BB-153) and hexabromocyclododecane (HBCD) in eggs from white-tailed sea eagles hatching in four different areas in Sweden; Lapland, inland freshwater lakes, along the coast of south Bothnian Sea and the Baltic Proper.

The congener pattern in the eggs of the wild peregrine falcon populations in Sweden showed a different pattern, with BDE-153 as the dominant congener followed by BDE-99, BDE-100 and HBCD, whereas lower concentrations of BDE-47, -154 were detected [10, 11]. These eggs also contained BDE-183 and BDE-209. The difference in BFR pattern may be explained due to different prey, as falcons feed on other birds both from the aquatic and terrestrial food web but may also be related to peregrine falcons winter migration to central/south of Europe where there might be a higher degree of contamination from these substances. Differences in metabolism may also play a role in explaining the differences between the two species.

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