Science of the Total Environment 409 (2010) 238–246

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Levels of brominated flame retardants and methoxylated polybrominated diphenyl ethers in eggs of white-tailed sea eagles breeding in different regions of Sweden

Ulrika Nordlöf [a,*], Björn Helander [b], Anders Bignert [b], Lillemor Asplund [b,c]

[a] Department of Applied Environmental Science (ITM), Stockholm University, SE-106 91, Stockholm Sweden
[b] Swedish Museum of Natural History, Department of Contaminant Research, SE-104 05 Stockholm, Sweden
[c] Environmental Chemistry Unit, Department of Materials and Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 13 July 2010
Received in revised form 24 September 2010
Accepted 27 September 2010
Available online 23 October 2010

Keywords:
White-tailed sea eagle
Predatory birds
Eggs
Polybrominated diphenyl ethers
Polybrominated biphenyl
Hexabromocyclododecane
Methoxylated polybrominated diphenyl ethers
Reproduction

ABSTRACT

Forty-four unhatched eggs from white-tailed sea eagle (*Haliaeetus albicilla*), collected in four regions in Sweden in 1992–2005, were analysed for contents of polybrominated diphenyl ethers (PBDEs), polybrominated biphenyl (PBB), hexabromocyclododecane (HBCD) and naturally occurring methoxylated polybrominated diphenyl ethers (MeO-PBDEs). Two freshwater areas—Lapland in the arctic zone (LAP) and inland lakes in central and southern Sweden (INL), and two brackish marine areas in the Baltic Sea—the south Bothnian Sea (SB) and the Baltic Proper (BP)—were chosen for comparison of the concentrations and congener distributions in white-tailed sea eagles with different diet and migratory patterns. The geometric mean (GM) concentrations (ng/g lipid weight (l.w.)) of $\sum_5$PBDE (BDE-47, -99, -100, -153, and -154) were 720 (LAP), 1500 (INL), 4 100 (SB) and 4 300 (BP), whereas BDE-209 was not detectable in any of the samples. The GM concentrations for HBCD content in LAP, INL, SB and BP were 60, 90, 150 and 140 ng/g l.w., respectively, whereas the corresponding values for BB-153 were 20, 30, 100 and 120 ng/g l.w. In general, the eggs from all four regions demonstrated similar patterns of PBDE congeners, with concentrations in descending order of BDE-47, -100, -99, -153 and -154. The $\sum_3$-MeO-BDEs (6-MeO-BDE47, 2′-MeO-BDE68, 5-Cl-6-MeO-BDE47) for these same regions (as above) were 80, 40, 340 and 240 ng/g l.w., respectively. $\sum_3$-MeO-BDEs for LAP and INL (freshwaters) were significantly different, whereas those for SB and BP were not. The presence of MeO-PBDEs in all of the inland samples indicates that there is an as-yet-unidentified source of these compounds in the freshwater ecosystem. Between the two more contaminated subpopulations from the Baltic Sea coast, SB showed significantly lower productivity than BP, but no correlation was found between productivity and PBDE, PBB and HBCD at the concentrations found in this study.

1. Introduction

White-tailed sea eagles (*Haliaeetus albicilla*) breeding in Sweden are exposed to several threats, including environmental pollutants. At the beginning of the 1970s the eagle populations inhabiting the Baltic Sea coast and surrounding regions were critically endangered, due to the effects of high levels of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-1,1-dichloroetane (DDT) and polychlorinated biphenyls (PCB) (Helander et al., 2002). After the use of these chemicals was banned in Sweden during the 1970s and a program designed to save the white-tailed sea eagles (WTSE) was initiated, their numbers have increased from 50 pairs nesting primarily on the southeast coast in 1970 to more than 500 pairs spread throughout the country today. Because of its top position in a primarily aquatic food web, the WTSE is still exposed to persistent chemicals in the environment and thus potentially threatened by other organohalogenated lipophilic substances, such as brominated flame retardants (BFRs).

The large and diverse groups of BFRs are used as their name implies, to reduce the risk of fire. One group of these chemicals, the polybrominated diphenyl ethers (PBDEs), is employed worldwide and consists of three major commercial technical products, the penta-, octa-, and decabromodiphenyl ethers, with different degrees of bromination. These compounds are added to a variety of materials, primarily polyurethane foam utilized in upholstered furniture, but also to small quantities in textiles and rubber (Ecb., 2000). Another additive BFR, hexabromocyclododecane (HBCD) consists of several stereoisomers (α, β, and γ) and is used primarily in expanded and extruded polystyrene foams employed for thermal insulation in buildings and other constructions (Ecb., 2008). A third class, introduced onto the market in the early 1970s consist of the polybrominated biphenyls (PBB), primarily the hexa-substituted isomer (Firemaster BP-6), which contains predominantly hexabrominated congener, BB-153 (Di Carlo et al., 1978; Hardy, 2002) and the use of which has now been banned in both North America and in Europe (de Wit, 2002). All three of these classes of BFRs are lipophilic,

* Corresponding author. Tel.: +46 8 674 7519; fax: +46 8 764 7638.
E-mail address: ulrika.nordlof@itm.su.se (U. Nordlöf).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.09.042

persistent compounds that can bioaccumulate (de Wit, 2002) and since they are not bound chemically to the material to which they are added, these compounds leak into the environment.

Among the biological effects reported for PBDE and HBCD is an irreversible neurobehavioral influence on neonatal mice, which can impair learning and memory (Eriksson et al., 2001, 2006). Moreover thyroid hormone homeostasis is altered by pentaBDEs in rats and mice (Darnerud, 2003; Legler, 2008). Studies on captive American kestrels (*Falco spaverius*) have also revealed thyroid effects, as well as immunotoxicity, reduced eggshell thickness and reproductive success, and altered behaviour (Fernie et al., 2005a,b, 2008). Furthermore, the body mass of European starlings (*Sturnus vulgaris*) exposed to PBDEs is decreased (Van den Steen et al., 2008). High levels of $\sum_9$ PBDE with a predominance of BDE -47, -99 and -100 have been detected in eggs from Norwegian White-tailed sea eagles (Herzke et al., 2005), while in Sweden high levels of PBDE in particular hexa- to decaPBDEs, as well as HBCD and BB-153, are present in eggs of Peregrine falcon (*Falco peregrinus*) (Lindberg et al., 2004; Johansson et al., 2009). Reproductive impairments have been reported at elevated concentrations in eggs of free-ranging ospreys (Henny et al., 2009) and peregrine falcons (Johansson et al., 2009).

To the best of our knowledge, methoxylated polybrominated diphenyl ethers (MeO-PBDEs) are not produced commercially. Nonetheless, Haglund et al. (1997) detected such compounds in the aquatic Baltic environment in 1997. Several studies from the southern hemisphere have revealed the presence of MeO-PBDEs in relatively high concentrations in marine sponges, indicating a natural origin (Kuniyoshi et al., 1985; Handayani et al., 1997). On the basis of their content of ^{14}C, investigation concluded that the 6-MeO-BDE47 and 2′-MeO-BDE68 detected in True's beaked whale (*Mesoploson mirus*) living in the North Atlantic Ocean are natural products (Teuten et al., 2005).

In the case of the Baltic Sea, MeO-PBDEs are present in cyanobacteria, red algae (*Ceramium tenuicore*) (Malmvaern et al., 2008) and sponges (*Ephydatia fluviatilis*) (Unger et al., 2009) and perhaps, other, as-yet-unidentified sources as well. These compounds are found in several species at different trophic levels, i.e., blue mussels (Asplund et al., 2007; Löfstrand et al., 2007), fish (Asplund et al., 1999; Sinkkonen et al., 2004), birds (Olsson et al., 2000; Sinkkonen et al., 2004) and seals (Haglund et al., 1997). The elevation in concentrations observed further up the food chain is indicative of biomagnification (Weijs et al., 2009).

The present study was designed to quantify the levels of both less and more highly brominated BDEs (BDE-47, -99, -100, -153, -154, and -209), HBCD, a hexaBB (BB-153) and three MeO-PBDEs (6-MeO-BDE47, 2′-MeO-BDE68, 5-Cl-6-MeO-BDE47) in the eggs of different WTSE subpopulations nesting in Sweden.

2. Materials and methods

2.1. Samples and sampling areas

The annual surveys of WTSE reproduction performed in Sweden since the mid-1960s (Helander et al., 2008) have facilitated access to unhatched (dead) eggs for research purposes. The eggs analyzed here originated from four different regions, illustrated in Fig. 1; (1) Lapland, 12 eggs collected 1994–2005 in freshwater habitats in the arctic zone of Sweden, (2) southern inland freshwaters, 12 eggs collected 1992–2005 in central and southern Sweden, (3) the southern coast of the Bothnian Sea, 12 eggs collected 1992–2004, and (4) the coast of the Baltic Proper, 8 eggs collected 1994–2001. The 44 eggs analysed came from 38 different eagle territories, with more than one egg from five of these territories. The lipids extracted from these eggs have been stored in *n*-hexane in glass ampoules in the darkness. Since only dead eggs were collected they may not represent random fresh eggs laid by the various populations. Thus, the sample is potentially biased, if PBDEs or other contaminants affected the hatching success of these eggs.

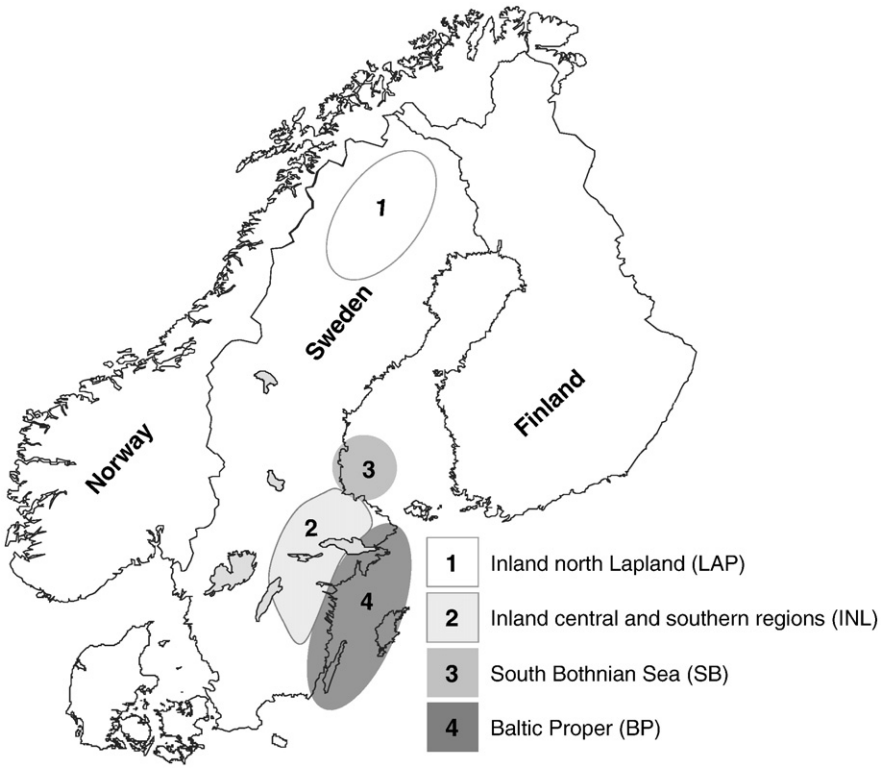

Fig. 1. The eggs analyzed originated from four subpopulations of White-tailed sea eagles, nesting in different parts of Sweden; 1) Freshwater lakes in the arctic zone of Lapland (LAP), 2) inland freshwater lakes in central and southern Sweden (INL), 3) the southern coast of the Bothnian Sea (SB) and 4) the coast of the Baltic Proper (BP).

Data on reproduction over a long range of years were available for all the eagle territories. Over the study period, the subpopulation in the south Bothnian Sea produced fewer young per pair and year than the subpopulation in the Baltic Proper. Productivity for the sampled females in this study was based on the number of young produced over a five-year period made up by the egg-sampling year +/− two years; if two five-year periods representing the same female were overlapping they were combined into one longer time period. Two females were represented by eggs from nine and ten years in between, respectively. In these cases, productivity for each five-year period around the egg-sampling year was used, to allow for responses to a change in concentrations over time.

2.2. Chemicals

All chemicals used were of analytical grade. Acetone of pesticide grade, *n*-hexane of HPLC grade, phosphoric acid of *pro-analysis* quality (Merck, Darmstadt, Germany), ethanol (99.5%) (Kemetyl AB, Haninge, Sweden), sulfuric acid and sodium chloride of *pro-analysis* quality, (VWR International, England) and diethyl ether of HPLC grade (Scharlau, Barcelona, Spain) were purchased from the sources indicated. PBDE standards used for identification were individual congeners 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5- and 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-99 and BDE-100), 2,2′,4,4′,5,5′- and 2,2′,4,4′,5,6-hexabromodiphenyl ether (BDE-153 and BDE-154) and decabromodiphenyl ether (BDE209) purchased from Accustandard (New Haven, USA). Firemaster BP-6 (recrystallized twice) 2,2′,4,4′,5,5′-hexabromobiphenyl (BB-153) (U.S. EPA) and hexabromocyclododecane (HBCD) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 6-methoxy-2,2′,4,4′-/2′-methoxy-2,3′,4,5′- and 5-Chloro-6-methoxy-2,2′,4,4-tetrabromo diphenyl ether (6-MeO-BDE47/2′-MeO-BDE68 and 5-Cl-6-MeO-BDE47) (Marsh et al., 2003) were kind gifts from Dr. Göran Marsh and Prof. Åke Bergman of the Environmental Chemistry Unit, Department of Material and Environmental Chemistry at Stockholm University. The internal standard, Dechlorane®603 was purchased from Hooker Chemicals Corp (Niagara Falls, NY, USA).

2.3. Extraction and cleanup

The liquid/liquid extraction with *n*-hexane/acetone and *n*-hexane/diethyl ether was performed (Jensen et al., 1983). An aliquot of the lipid extracts from each sample (corresponding to 15–50 mg lipid) dissolved in *n*-hexane, was carefully evaporated using nitrogen gas and the weight of the residue determined gravimetrically. The lipids were then re-dissolved in *n*-hexane and 252 ng of the internal standard dechlorane, added by weight. Following treatment with concentrated sulfuric acid, the remaining organic phase was reduced in volume to 100 µl under nitrogen gas.

2.4. Instruments and instrumental conditions for analysis

The analytes were separated and detected employing gas chromatography/mass spectrometry (GC/MS) (the Hewlett Packard series 5890 II-Thermoquest SSQ 7000 system). Separation of HBCD, BDE-154 and BB-153 was achieved on a DB-5-MS fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness; J&W Scientific, Folsom, CA) with a GC temperature program of 80 °C for 2 min; increases of 25 °C/min to 200 °C; increases of 4 °C/min to 315 °C; and maintenance at 315 °C for 15 min. All of the other analytes were separated on the same type of column (15 m × 0.25 mm, 0.1 µm film thickness; J&W Scientific, Folsom, CA) at 80 °C for 2 min; increases of 20 °C/min to 200 °C; increases of 6 °C/min to 315 °C; and maintenance at 315 °C for 5 min. Injections were made in the splitless mode. The injector temperature maintained at 280 °C, the transfer line at 300 °C and the ion source 180 °C. Ammonia was the reagent gas and the MS was operated in the electron capture negative ionisation (ECNI), selected ion monitoring (SIM) mode. The ions, *m/z* 79 and 81 were monitored for all the target compounds, while the ions for the internal standard were *m/z* 237 and 239. Analytes were identified on the basis of their retention times relative to authentic standards.

2.5. Quality control

One procedural blank and one sample of internal reference material (a muscle homogenate from a free-living Swedish salmon included in the Swedish monitoring program) were analysed in parallel with each batch of samples to detect any possible contamination from solvent and glassware. The blanks, extracted along with the samples each year and subsequently stored, also allowed assessment of any background interference. The LRM values were within 2 standard deviation of the assigned mean value for the compound of interest. The variation of the method was calculated for the compounds from the LRM giving a relative standard deviation (RSD%) of 14–19% for the PBDEs and RSD of 22% for HBCD. Exposure of samples to UV-light was minimized by covering all lamps/windows in the laboratory with UV-protection film. In the case of GC/MS analysis, the samples and standards were analyzed in an order designed to eliminate memory effects and solvent were run following standards with high concentrations, as well as between certain of the actual samples. Solutions containing six to eight different concentrations of standard compounds were analysed twice along with the samples to obtain calibration curves. The limit of detection (LOD) was defined as a signal three times greater than the noise level and the limit of quantification (LOQ) as five times the noise level, calculated within the matrix. For BDE-47, -99, -100, -153, and -154, the MeO-BDEs and BB-153, LOD were estimated to 2 ng/g l.w. whereas for BDE-209 and HBCD the estimation were 11 and 13 ng/g l.w., respectively in the sample amount used. The actual instrument LOD for the BFRs is general in the 0.3 pg range, which correspond to LOD for the lowest standard used.

2.6. Statistical evaluation

Six of the 44 eggs sampled contained embryos that were at least half the length of hatchlings (>75 mm). To correct for an influence of lipid metabolism during embryo growth on the concentration values (which are expressed relative to lipid weight) those concentrations were adjusted by a factor equal to the *measured concentration value* × *75* × *embryo length in mm*$^{-1}$ (Helander et al., 2002). In a previous study, the variations in the residue concentrations of lipophilic organochlorines (DDE and PCB) for female sea eagles was considerably smaller within than the variations between individuals (Helander et al., 2002). Thus, when calculating geometric and arithmetic mean concentrations for the different regions, females represented by more than one egg (collected in different years) have been down weighted by using mean values for their eggs.

Since the concentrations of both PBDEs and MeO-PBDEs within each geographical region were skewed to the right, all values were transformed logarithmically to achieve normal distributions. Hotelling's T^2-test was employed for pair-wise comparisons between the regions. To achieve the desired p-value of 0.05 for repeated tests this value was set instead to 0.0083 (according to the Bonferroni-adjustment for 6 possible comparisons). To test for significant changes in concentrations over time and to test for effects of analysed contaminants on productivity, log-linear regression analysis was carried out. To identify non-linear trends (Fryer and Nicholson, 1993; Nicholson et al., 1998), a 7-point running mean smoother was applied and analysis of variance (ANOVA) was used to test if the smoother explains significantly more than (a) the overall mean concentration (a straight line) and (b) the log-linear regression line, considering the

Table 1
Number of eggs (*n*) extracted, with the lipid content (l.w.%). The geometric means (GM) and 95% confidence intervals, ranges (min–max values), arithmetic means (AM) and the numbers of samples with undetectable levels (n.d.), of six PBDE congeners, HBCD, BB-153 and three MeO-PBDEs, in eggs from white-tailed sea eagles breeding in four different regions of Sweden, Lapland (freshwaters), inland southern and central Sweden (freshwaters), the South Bothnian Sea (coastal) and the Baltic Proper (coastal). All values are presented as ng/g total lipid weight (l.w.).

Population (sampling period)	*n*	l.w. (%)	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	HBCD	BB-153	6-MeO-BDE-47	2′-MeO-BDE-68	5-Cl-6-MeO-BDE-47
Lapland (1994–2005)	*12*												
GM		4.7	280	120	130	90	50	*nd*	60	20	40	40	6.0
95% CI			180–430	80–180	90–200	60–130	30–70		40–90	10–30	20–70	20–80	5.0–7.0
Range (min–max)		2.9–6.6	80–1100	50–550	40–500	30–250	10–240		40–390	9.0–60	9.0–220	4.0–440	4.5–7.9
AM		4.8	360	160	170	110	60		80	30	80	80	6.2
													4[a]
Inland (1992–2005)	*12*												
GM		4.9	830	190	230	150	90	*nd*	90	30	20	12	6.7
95% CI			600–1100	140–260	170–310	120–200	70–120		80–110	20–50	10–30	9.0–20	5.0–8.0
Range (min–max)		3.2–6.0	370–2300	60–390	90–460	60–340	40–210		70–190	10–100	4.0–160	8.0–40	4.7–10
AM		5.0	950	220	260	170	100		100	40	40	20	7.0
													5[a]
South Bothnian Sea (1992–2004)	*12*												
GM		5.8	2500	500	400	340	230	*nd*	150	100	270	50	30
95% CI			1800–3500	320–770	320–540	260–450	180–290		110–210	80–130	170–420	40–80	20–40
Range (min–max)		3.2–18	1000–4800	200–1700	240–1100	180–1000	130–500		100–480	63–200	80–680	30–100	10–70
AM		6.5	2900	650	460	390	250		180	110	350	50	30
													11[a]
Baltic Proper (1994–2001)	*8*												
GM		5.1	2600	490	550	360	201	*nd*	140	120	180	40	10
95% CI			1700–4100	290–800	350–880	220–590	130–320		100–200	70–190	90–390	20–100	7.0–30
Range (min–max)		3.6–7.7	1400–8400	260–1600	280–2000	130–1000	90–580		80–310	50–430	70–1400	20–420	3.6–30
AM		5.2	3200	620	690	440	240		160	150	330	90	20
													5[a]

[a] Number of eggs in which 5-Cl-6-MeO-BDE-47 was detected.

loss of degrees of freedom related to the smoother (Hastie and Tibshirani, 1990).

Principal component analysis (PCA) was employed to examine the patterns of BFR and MeO-PBDE concentrations relative to the sum of all analytes, in order to achieve a better overview of both the differences in relative concentrations and the spread between the subpopulations. For this purpose the concentration values were transformed logarithmically to reduce the influence of extreme relative concentrations in individual specimens. In addition, the original PCA scores were converted to percentage values for each axis. The PCA figure is supplemented with a biplot showing loadings of the substances involved and the Hotelling's 95% confidence ellipses for the centre of gravity of the WTSE-populations investigated.

To test for difference in nestling brood size and productivity, contingency table analysis (G-test) was applied (Sokal and Rohlf, 1995).

3. Results

BDE-47, -99, -100, -153, and -154, HBCD, BB-153, 6-MeO-BDE47 and 2′-MeO-BDE68 were detected in all of the 44 eggs examined, whereas BDE-209 was not detected in any of these samples with the amount of lipid analyzed. 25 eggs contained 5-Cl-6-MeO-BDE47. The geometric mean concentrations (GM) with 95% confidence intervals, together with the arithmetic means (AM) and ranges for each subpopulation are presented in Table 1.

3.1. Brominated flame retardants

The concentrations of all compounds investigated (PBDE, HBCD and BB-153) were higher in eggs from the Swedish coast than in eggs from Lapland and eggs from southern inland freshwater lakes (Fig. 2). The levels in Lapland eggs differed significantly from those of all the other populations. The levels in southern inland freshwaters were significantly different from those in Lapland and the two Baltic Sea subpopulations, whereas the levels in the two coastal populations, Bothnian Sea and Baltic Proper, were not significantly different. The most abundant congener was BDE-47 followed by BDE-100, -99, -153 and -154, in all areas except the Bothnian Sea where the level of BDE-99 was higher than that of BDE-100, although not significantly so. The level of BDE-47 was several-fold higher along the Swedish coast than in the inland areas.

The geometric mean concentrations of the Σ_5 PBDE (BDE47, -99, -100, -153, and -154) on a lipid weight basis were as follows (with corresponding wet weight values given within brackets, based on an average lipid content of 5%): Lapland (arctic zone), 720 ng/g (36 ng/g); inland freshwater lakes (central and southern), 1500 ng/g (75 ng/g); south Bothnian Sea, 4100 ng/g (200 ng/g); Baltic Proper, 4300 ng/g (220 ng/g). The highest individual Σ_5PBDE, 13,600 ng/g (680 ng/g) was detected in an egg from the coast of the Baltic Proper. The geometric means for HBCD content in Lapland, inland freshwater lakes, the South Bothnian Sea and the Baltic Proper were 60, 90, 150 and 140 ng/g l.w. (3.0, 4.5, 7.5 and 7.0 ng/g w.w.), respectively; while the corresponding values for BB-153 were 20, 30, 100 and 120 ng/g l.w. (1.0, 1.5, 5.0 and 6.0 ng/g w.w.).

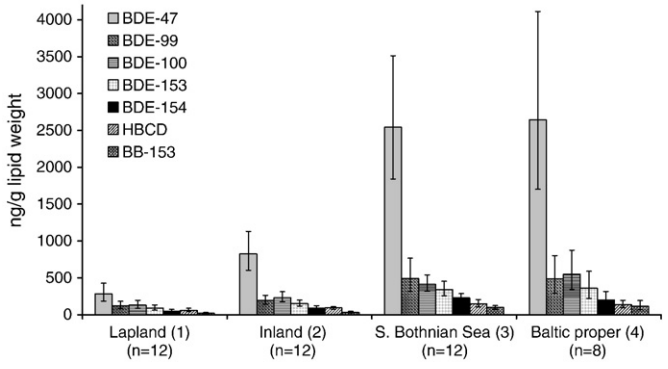

Fig. 2. The geometric mean concentrations (ng/g lipid weight) with 95% confidence intervals for the polybrominated diphenyl ethers BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154, hexabromocyclododecane (HBCD) and a hexa-polybrominatedbiphenyl (BB-153) in eggs from white-tailed sea eagles breeding in four different regions of Sweden, Lapland ((1), arctic zone, freshwater), inland southern and central Sweden ((2), freshwaters), the South Bothnian Sea ((3), coastal) and the Baltic Proper ((4), coastal).

3.2. Methoxylated polybrominated diphenyl ethers

The inland freshwater population exhibited significantly lower concentrations of MeO-BDEs than those found in Lapland and the two Baltic Sea regions, with no significant differences between these latter two regions. The samples from Lapland demonstrated significantly lower concentrations of these compounds than the coastal subpopulations. These comparisons were based on the levels of two of the three congeners, 6-MeO-BDE47 and 2′-MeO-BDE68, since these were detected in all of the eggs.

The geometric mean concentrations of the Σ_3-MeO-PBDE (6-MeO-BDE47, 2′-MeO-BDE68 and 5-Cl-6-MeO-BDE47) were as follows: Lapland, 90 ng/g l.w. (4.5 ng/g w.w.), inland freshwater lakes, 30 ng/g (1.5 ng/g), the south Bothnian Sea, 340 ng/g (17 ng/g) and the Baltic Proper, 240 ng/g (12 ng/g). The highest individual Σ_3-MeO-PBDE, 1800 ng/g (90 ng/g) was detected in an egg from the Baltic Proper that also had the highest levels of BFRs. With the exception of Lapland, the dominant congener was 6-MeO-BDE47 followed by 2′-MeO-BDE68, with 5-Cl-6-MeO-BDE47 being found in all regions but not in all individual eggs. In contrast to the lower content of BFRs in the eggs from Lapland compared to the inland freshwaters, the content of MeO-BDEs was significantly higher in the Lapland sample (Fig. 3). The sum of MeO-PBDE congener levels in the samples from the Swedish coast was several-fold higher than in those from the two inland subpopulations (Table 1).

3.3. Changes with time along the coast of the Baltic Sea

The 20 individual eggs from the coast of the Baltic Sea were examined for possible changes in the levels of PBDEs, BB-153 and HBCD during the period of 1992–2004 (Fig. 4). The smoother in the figure explained significantly more of the variation in concentration over time than the log-linear regression. All PBDEs showed the same temporal pattern, with a decrease in the first half of the time-series followed by an increase at the end. However, the increasing smoothers are based only on a few single eggs for the last three years. For HBCD and MeO-PBDEs, no trend was detected.

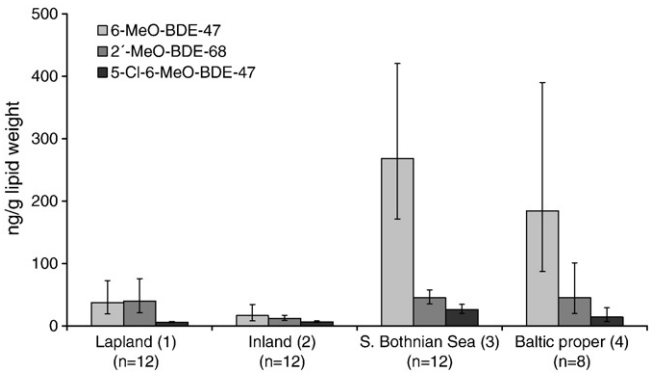

Fig. 3. The geometric mean concentrations (ng/g lipid weight) with 95% confidence intervals, for 6-MeO-BDE47, 2′-MeO-BDE68 and 5-Cl-6-MeO-BDE47 in eggs from white-tailed sea eagles breeding in four different regions of Sweden, Lapland ((1) arctic zone, freshwater), inland southern and central Sweden ((2) freshwaters), the South Bothnian Sea ((3) coastal) and the Baltic Proper ((4) coastal).

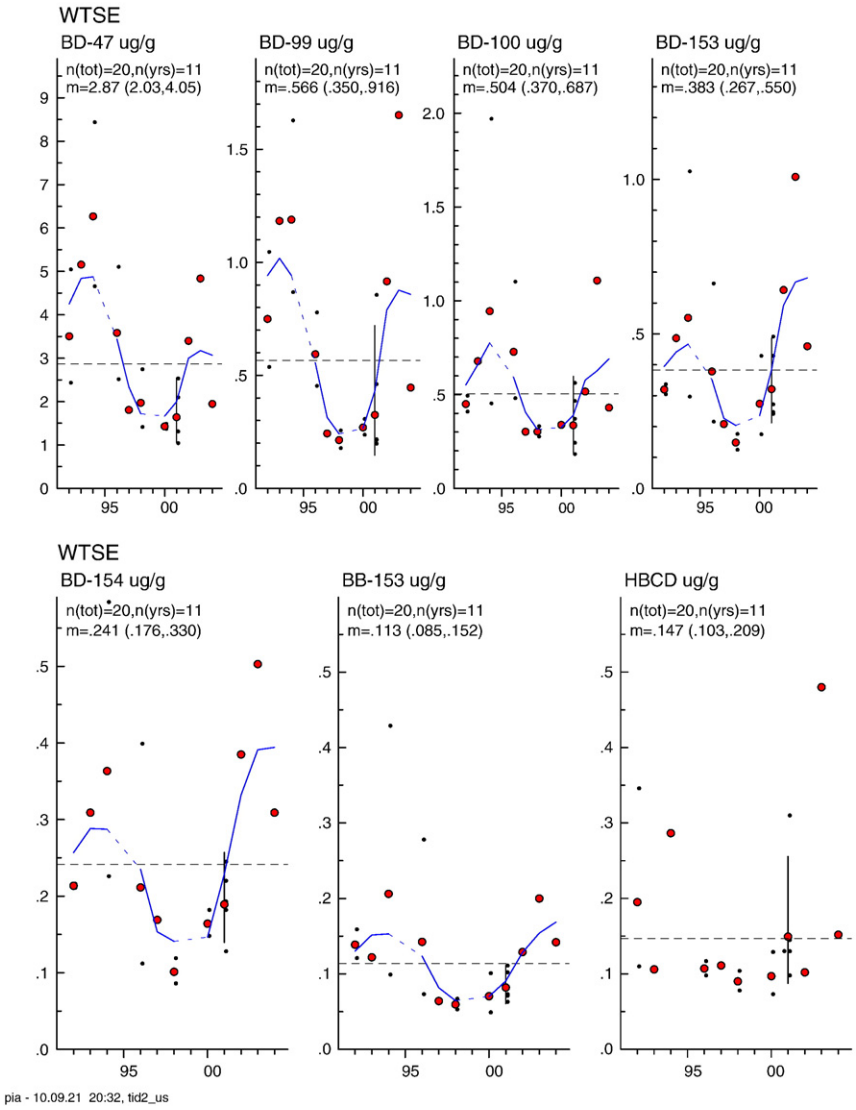

Fig. 4. Levels of the flame retardants BDE-47, -99, -100, -153, -154, BB-153 and HBCD in white-tailed sea eagle eggs from the coast of the Baltic Sea as function of time from 1992 to 2004 n(tot) = the total number of analyses performed; n(yrs) = the total number of years analyzed, m = the geometric mean for the entire period, with the 95% confidence in brackets. The geometric mean is also shown as a solid horizontal line. In 2001, 5 individual analyses were carried out and the 95% confidence interval for the geometric mean value for that year is depicted by a vertical bar. The solid blue lines indicate significant non-linear trends (i.e., this fitted line is more statistically significant than the mean or a log-linear regression).

3.4. Principal component analysis

In the PCA, the two first principal components, PC I and PC II, explained 46% and 20%, respectively, of the variation in the data. Fig. 5 shows the biplot of these first two components. The symbols represent the values for individual eggs from the different regions, while the lines represent the loading of each BFR or MeO-BDE. The centroids of each subpopulation are indicated by the larger symbols and the ellipses present the Hotelling's 95% confidence ellipses for each subpopulation.

The ellipses for the two coastal subpopulations overlap and no significance difference can be seen. The other subpopulations from Lapland and inland freshwaters contained a few values similar to those of the coastal populations, resulting in large confidence ellipses that overlap slightly. The Hotelling's T^2-test showed significant differences between the Lapland arctic freshwater, southern inland freshwater, and Baltic coastal populations ($p \leq 0.05$), but not between the two Baltic subpopulations.

3.5. Residue concentrations and reproduction

The mean productivity of the sampled females on the Baltic coast averaged 0.86 for the south Bothnian Sea and 1.27 for the Baltic Proper (based on 50 and 33 breeding years, respectively). This difference was statistically significant ($p \leq 0.015$, G-test). As shown in Fig. 2, the concentrations of PBDE congeners, HBCD and BB153 were almost identical in these subpopulations. Individual productivity for the females in the two Baltic subpopulations showed no significant relationship with Σ_5PBDEs range 1880–13 600 ng/g l.w. (94–680 ng/g w.w.), or with Σ_5 PBDEs together with HBCD and BB153 ($\sum$BFRs) over a range of concentrations from 2040 to 14400 ng/g, l.w. (100–720 ng/g, w.w.) ($r^2 = 0.04$).

4. Discussion

Mated sea eagles show strong site fidelity and typically use the same territory for breeding year after year, so a strong influence of

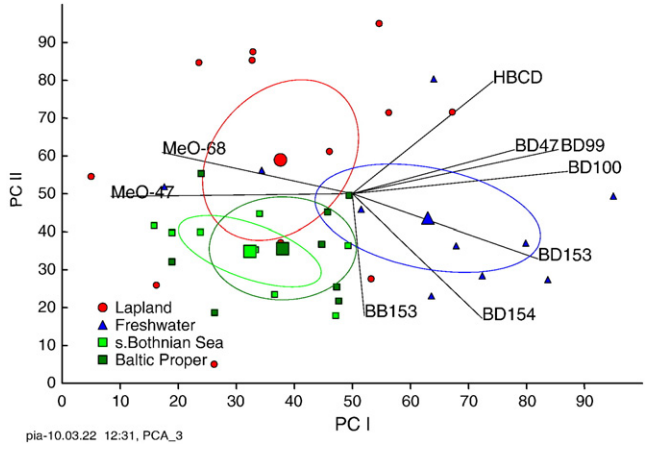

Fig. 5. Principal component analysis (PCA) of the levels BFRs and MeO-BDEs in the eggs of different subpopulations of white-tailed sea eagles. The symbols represent the components value for individual eggs from different regions and the lines represent the loading of each BFR or MeO-BDE. The centroids of each subpopulation are indicated by the larger symbols and the ellipses in this plot are the Hotelling's 95% confidence ellipses for each subpopulation. The first PC explains 46% and the second PC 20% of the total variation.

local and regional contamination should be to expect. Differences in the food composition and patterns of migration of the WTSE will also be reflected in the levels of contaminants in their eggs. During the summer, WTSEs nesting along the coasts of the Bothnian Sea and the Baltic Proper prey mainly on fish and seabirds (Helander, 1983), and the same goes for WTSEs nesting in the southern inland region. During the winter season most of their food is seabirds and carrion. In Lapland, before the ice breaks up in early spring, WTSEs feed largely on carrion from herbivores (reindeer *Rangifer tarandus* and moose *Alces alces*), whereas the summer diet consists of fish, birds and mammal carrion. Territorial adult eagles in the coastal areas examined here (3 and 4 in Fig. 1) and around southern and central inland freshwater lakes (study area 2) are essentially stationary all year round and thus produce their eggs from locally acquired lipid and protein. In contrast, as food becomes largely unavailable to WTSE in the arctic when waters freeze during winter, the territorial adults breeding in Lapland (study area 1) move south in late autumn. Resightings of ringed WTSEs indicate that adults migrating from Lapland often overwinter in the same places from year to year (Helander unpubl.). Many of these ringed Lapland birds spend time from November into March in central and southern Sweden, inland as well as on the Baltic Sea coast, and some have been found also on the Norwegian coast. Most of these birds leave the wintering areas no later than during February and return to their breeding sites in Lapland during March. Eggs are laid from late March to mid-April in Lapland (Helander, 1985). These habits would imply that the WTSE breeding in Swedish Lapland could be referred to as an "income" breeder, since it spends 3–4 weeks on the breeding area before egg laying (Elliott et al., 2007), rather than a "capital" breeder, that uses energy stores accumulated at an earlier time, in the wintering area (Yates et al., 2010). It is not clear, though, to what extent the WTSEs use also energy stores from the wintering grounds for egg production. These eagles are known (from resightings of ringed birds) to stop and feed on migration, and the distance from Lapland to wintering grounds in central and southern Sweden is just 1000 to 1500 km, a relatively short distance. It seems reasonable to assume that more than a little of the energy stores gained in the wintering areas would still be retained upon arrival on the breeding grounds. Whether the lipid and protein used for egg production stems mainly from energy acquired on the breeding grounds prior to laying, or integrates recent intake with stored energy reserves, is not known in this species. The fact that residue concentrations in eggs from the same female in different years tend to be similar (Helander et al., 2002) seems to imply a conservative influence of stored reserves. If so, one would expect that residues of persistent contaminants picked up over a longer time period would be reflected in the eggs.

4.1. Brominated flame retardants

In general, all regions except the South Bothnian Sea show similar patterns of BFR congeners, with levels from high to low in the order BDE-47, -100, -99, -153, and -154. In the case of the Bothnian Sea, the level of BDE-99 tended to be higher than that of BDE-100, although not significantly so. The concentrations in eggs from Lapland and the southern inland freshwater lakes were lower than along the Baltic coast. A probable explanation for the higher concentrations in the coastal samples would be a stronger contamination by these substances in the generally more polluted Baltic Sea than in the freshwater lakes of Sweden, but at present no supporting data relevant to this proposal are available.

Similar levels and congener patterns of PBDEs, with the decreasing order of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154 as shown here have been reported in WTSE eggs from Norway (Herzke et al., 2005). Eggs of guillemot (*Uria aalge*) breeding on Stora Karlsö in the Baltic Proper exhibit a congener pattern similar to that of the WTSE in this study, with highest levels of BDE-47 followed by BDE-99 and BDE-100 (Sellstrom et al., 2003). Guillemots in the Baltic Sea are mostly stationary, feeding exclusively on fish, mainly on herring.

In contrast, the congener pattern in eggs of free-ranging peregrine falcons in Sweden has a different pattern, containing relatively more of the more highly brominated congeners. In this case the major congener was BDE-153, followed by BDE-99, BDE-100 and HBCD, with lower concentrations of BDE-47 and BDE-154 (Lindberg et al., 2004). These peregrine falcon eggs also contained BDE-183 and BDE-209, which have also been detected in peregrine falcon nesting in Greenland (Vorkamp et al., 2005), Norway (Herzke et al., 2005) and Sweden (Johansson et al., 2009).

It has been shown previously that in WTSE, as well as in other predatory birds such as the peregrine falcon, only the α-isomer of HBCD is present, indicating stereo-specific accumulation and/or metabolism of this compound (Janak et al., 2008). The geometric mean (GM) concentrations of HBCD in the eggs from Lapland and from the freshwater populations were similar (60 versus 90 ng/g l.w., respectively); whereas the corresponding values for the coastal populations were approximately two-fold higher (150 ng/g l.w. in the South Bothnian sea and 140 ng/g l.w in the Baltic Proper), which is half the level detected in peregrine falcon (Johansson et al., 2009). In the case of BB-153 the GM concentration in Lapland eggs was less than 25% of that in the falcon eggs, whereas the coastal populations (100–120 ng/g l.w.) demonstrated concentrations similar to those of the falcon (Johansson et al., 2009).

The differences in BFR patterns may reflect consumption of different prey, since falcons feed primarily on both aquatic and terrestrial birds. Another factor that may be of significance in this connection is that peregrine falcons migrate to and spend the winter in western and southern Europe, where high levels of BFRs have been found in the sediments of estuaries (Law et al., 2006). More highly brominated PBDEs are less soluble in water and bind with high affinity to particles that can be filtrated by bottom-living animals and thereby biomagnified further up the falcon food chain. However, since pattern of PBDE congeners in different species is fundamentally different, it seems unlikely that habitat and diet can completely explain the differences in the BFR pattern. Terrestrial organisms may be exposed to PBDEs from different sources and/or may metabolise these compounds in other ways than aquatic species, so that birds feeding in terrestrial environments and on other birds may be more highly exposed to the higher brominated congeners than marine species (Law et al., 2003).

4.2. Methoxylated polybrominated diphenyl ethers

MeO-PBDEs, a natural product in the marine environment, may also be formed by metabolic hydroxylation and subsequent methylation of PBDEs. MeO-PBDEs have been found in different primary producers in the Baltic Sea, including cyanobacteria, red algae (Malmvaern et al., 2008; Malmvarn et al., 2005) and sponges (Unger et al., 2009). One possible route of metabolic formation might involve *O*-biomethylation of the corresponding halogenated phenols, in a manner analogous to the formation of simple anisoles from phenols (Allard et al., 1987).

Pike living in Lake Bolmen in the south of Sweden have been shown to contain levels of the most abundant (6-MeO-BDE47) and the second most abundant (2′-MeO-BDE68) MeO-BDEs that are equal to or greater than the levels of individual PBDEs (BDE47, -99, -100, and -153) and the most frequent congener was 6-MeO-BDE47 (Kierkegaard et al., 2004). In this same study, herring in the Bothnian Sea exhibited higher levels than herring living in other parts of the Baltic Sea. Moreover, in an earlier investigation of blood samples from WTSE nestlings, 6-MeO-BDE47 was the congener detected in highest levels (Olsson et al., 2000).

Here, the highest levels were found in WTSE eggs from the coastal areas indicating a specific source in the Baltic Sea. The levels in eggs collected in Lapland may reflect the migration of these birds in the wintertime to the south and/or coastal areas of Sweden or to the Atlantic coast of Norway. Samples of Arctic char (*Salvelinus alpinus*) caught in 2005 in Lake Abiskojaure in Lapland analysed at ITM exhibited a pattern opposite to that of pike from the south of Sweden, i.e., with higher levels of 2′-MeO-BDE68 (15 ng/g l.w.) than of 6-MeO-BDE47 (4 ng/g l.w.) (Unpublished data). In pike from Lake Storvindeln in Lapland, 2′-MeO-BDE68 was also the predominant congener (Kierkegaard et al., 2004). The presence of MeO-PBDEs in all of the inland samples indicates that there is an as-yet-unidentified source of these compounds in the freshwater ecosystem as well.

4.3. Changes with time along the coast of the Baltic Sea

The changes over time of the sea eagle eggs collected at the coast of the Baltic Sea do not appear to follow the overall pattern as seen for two other species in the Baltic Sea: guillemot and herring. In guillemot eggs collected at Stora Karlsö in the Baltic Sea, between 1969–1997 concentrations of BDE-47, BDE-99 and BDE-100 were analysed. The concentrations increased with a peak in the mid1980s, whereupon a declining trend is seen (Sellstrom et al., 2003). The concentrations have continued to decrease in guillemot eggs during recent years, and in herring muscle tissue, BDE-47 shows the same pattern (Bignert et al., 2010). For HBCD a significant increase in the guillemot can be seen whereas for herring the trend is decreasing (Bignert et al., 2010). In the sea eagle eggs in this study, the temporal pattern with a decrease followed by an increase in PBDE concentrations at the end of the period is different from the other two species. However, the sample size is too small to draw any conclusions from these observations.

4.4. Principal component analysis

The PCA illustrated in Fig. 5 indicates the implication of migration and food patterns. The coastal populations (areas 3 and 4 in Fig. 1) form a group, which is also in the case for the inland freshwater population, except for three points that are scattered. These scattered values originated from birds nesting in the inland regions closest to the coastline, and may reflect the influence of their occasional consumption of coastal prey. In the case of Lapland, the pattern is even more widely spread, which seems to agree with the fact that these birds spend the winter in different places far away from Lapland, and implies that they include energy reserves acquired in the wintering grounds for egg production. Concentrations of PBDEs and HBCD in reindeer and moose—the principal food of WTSEs in Lapland prior to egg-laying—are very low (below or near the detection limits of 0.01–0.1 ng/g, w.w.; Danielsson et al., 2008). As also illustrated in this plot, the levels of MeO-BDEs in the Baltic are higher than in the inland lakes. The levels of the two MeO-BDEs exert a strong influence on the PCA of the coastal groups.

4.5. PBDEs and reproduction

No indication of an effect on productivity of WTSEs was found at the concentrations of BFRs presented in this study. In a study of 120 North American osprey nests (Henny et al., 2009) observed normal reproduction at Σ_{14}PBDE concentrations <1000 ng/g on a wet weight basis in eggs from 114 nests, but a significant reduction in productivity by almost 50% in six nests with eggs containing >1000 ng/g. The congener pattern in the osprey eggs resembles the pattern in WTSE eggs in this study, with a strong dominance of BDE47. The same five BDEs as in this study made up about 95–99% of the Σ_{14}PBDEs in the osprey eggs, and BDE-209 was not found. The Σ_5PBDEs in the WTSE eggs from the Baltic coast averaged [GM] 4100 and 4300 ng/g l.w., equal to about 200 and 220 ng/g on a wet weight basis. This is five times below the threshold indicated for osprey. The two most highly contaminated eggs in the WTSE sample contained 9100 and 13,700 ng/g l.w. (450 and 680 ng/g w.w.). Captive American kestrels (*Falco sparverius*) fed a commercial PBDE product attained levels averaging [AM] 467 ng/g Σ_{12}PBDEs (w.w.) which exerted a negative influence on reproduction (Fernie et al., 2009). Among free-ranging Swedish peregrine falcons, the geometric means of Σ_{14}PBDEs in two subpopulations were 2500 and 3100 ng/g (l.w.) (Johansson et al., 2009). A significant negative correlation between the $\sum$PBDE in eggs and productivity of 15 individual peregrine females was found, but an influence of other contaminants in the eggs could not be excluded. As mentioned above, the reported congener pattern in the Swedish falcon eggs is different from that reported from WTSE (Herzke et al., 2005, this study), and from osprey (Henny et al., 2009), with a predominance in falcon eggs of BDE153, 99 and 100 and the presence of BDE-209. The proportion of BDE47 in Swedish WTSE eggs is 10-fold higher than in the eggs from Swedish peregrines. Such differences in congener patterns, as well as possible differences between species in their response to specific congeners, are important to take into account in population studies when comparing and interpreting effects and estimate threshold levels.

Acknowledgments

Ulla Eriksson at ITM is gratefully acknowledged for her valuable technical support. Joseph de Pierre at the Institution of Biophysics and Biochemistry at Stockholm University is acknowledged for his help to improve the language. We thank Åke Englund for his assistance with collecting dead sea eagle eggs from the south Bothnian Sea coast. FORMAS provided financial support for this project as a part of an attempt to preserve the White-tailed sea eagle in Sweden.

References

Allard A, Remberger M, Neilson A. Bacterial O-methylation of halogen-substituted phenols. Appl Environ Microbiol 1987;53:839–45.

Asplund L, Athanasiadou M, Sjodin A, Bergman A, Börjeson H. Organohalogen substances in muscle, egg and blood from healthy Baltic salmon (*Salmon salar*) and Baltic salmon that produced offspring with the M74 syndrome. Ambio 1999;28:67–76.

Asplund L, Nylund K, Eriksson U, Bignert A. Monitoring of PBDE, Methoxylated PBDE and PCB in blue mussels from the Swedish coast line. Organohalogen Compd 2007;69:1713–6.

Bignert A, Danielsson S, Nyberg E, Asplund L, Eriksson U, Berger U, et al. Comments concerning the National Swedish Contaminant Monitoring Programme in marine biota. Report to the Swedish Environmental Protection Agency, 2010-04-01; 2010. 156 pp.

Danielsson S, Odsjö T, Bignert A, Remberger M. Organic contaminant in moose (*Alces alces*) and reindeer (*Rangifer tarandus*) in Sweden from the last twenty years. Swedish Museum of Natural HistoryReport nr 7; 2008.
Darnerud PO. Toxic effects of brominated flame retardants in man and in wildlife. Environ Int 2003;29:841–53.
de Wit CA. An overview of brominated flame retardants in the environment. Chemosphere 2002;46:583–624.
Di Carlo F, Seifter J, DeCarlo V. Assessment of the hazards of polybrominated biphenyls. Environ Health Perspect 1978;23:351–65.
Ecb. Penta bdpereport 015, Risk-Assessment Report. Vol. 05, CAS# 32534-81-9. 2000. http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/penta_bdpereport015.pdf.
Ecb. Hexabromocyclododecane, Risk-Assessment Report. CAS#25637-99-4. 2008. http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/hbcddreport044.pdf.
Elliott JE, Morrissey CA, Henny CJ, Inzunza ER, Shaw P. Satellite telemetry and prey sampling reveal contaminant sources to Pacific Northwest ospreys. Ecol Appl 2007;17:1223–33.
Eriksson P, Jakobsson E, Fredriksson A. Brominated flame retardants. A novel class of developmental neurotoxicants in our environment? Environ Health Perspect 2001;109:903–8.
Eriksson P, Fischer C, Wallin M, Jakobsson E, Fredriksson A. Impaired behavior, learning and memory in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 2006;21:317–22.
Fernie KJ, Mayne G, Shutt JL, Pekarik C, Grasman KA, Letcher RJ, et al. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. Environ Pollut 2005a;138:485–93.
Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, et al. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). Toxicol Sci 2005b;88:375–83.
Fernie KJ, Shutt JL, Letcher RJ, Ritchie JI, Sullivan K, Bird DM. Changes in reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to environmentally relevant levels of the polybrominated diphenyl ether mixture, DE-71. Toxicol Sci 2008;102:171–8.
Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. Environ Sci Technol 2009;43:2124–30.
Fryer R, Nicholson MD. The power of a contaminant monitoring programme to detect linear trends and incidents. J Mar Sci 1993;50:161–8.
Haglund P, Zook DR, Buser HR, Hu J. Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. Environ Sci Technol 1997;31:3281–7.
Handayani D, Edrada RA, Proksch P, Wray V, Witte L, Van Soest RW, et al. Four new bioactive polybrominated diphenyl ethers of the sponge *Dysidea herbacea* from West Sumatra, Indonesia. J Nat Prod 1997;60:1313–6.
Hardy ML. The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. Chemosphere 2002;46:757–77.
Hastie TJ, Tibshirani RJ. Generalized additive models. London: Chapman and Hall; 1990.
Helander B. Food ecology of the White-tailed Sea Eagle *Haliaeetus albicilla* in Sweden. "Reproduction of the White-tailed Sea Eagle *Haliaeetus albicilla* (L.) in Sweden, in relation to food and residue levels of organochlorine and mercury compounds in the eggs". Thesis, Stockholm University, Stockholm, 1983.
Helander B. Reproduction of the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. Holarctic Ecol 1985;8:211–27.
Helander B, Olsson A, Bignert A, Asplund L, Litzen K. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. Ambio 2002;31:386–403.
Helander B, Bignert A, Asplund L. Using raptors as environmental sentinels: monitoring the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. Ambio 2008;37:425–31.
Henny CJ, Kaiser JL, Grove RA, Johnson JL, Letcher RJ. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. Ecotoxicology 2009;18:802–13.
Herzke D, Berger U, Kallenborn R, Nygard T, Vetter W. Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. Chemosphere 2005;61:441–9.
Janak K, Sellstroem U, Johansson AK, Becher G, de Wit CA, Lindberg P, et al. Enantiomer-specific accumulation of hexabromocyclododecanes in eggs of predatory birds. Chemosphere 2008;73:S193-20.
Jensen S, Reutergårdh L, Jansson B. Analytical methods for measuring organochlorines and methyl mercury by gas chromatography. FAO Fish Tech Pap 1983;212:21–33.
Johansson AK, Sellstroem U, Lindberg P, Bignert A, de Wit CA. Polybrominated diphenyl ether congener patterns, hexabromocyclododecane, and brominated biphenyl 153 in eggs of peregrine falcons (*Falco peregrinus*) breeding in Sweden. Environ Toxicol Chem 2009;28:9-17.
Kierkegaard A, Bignert A, Sellstrom U, Olsson M, Asplund L, Jansson B, et al. Polybrominated diphenyl ethers (PBDEs) and their methoxylated derivatives in pike from Swedish waters with emphasis on temporal trends, 1967–2000. Environ Pollut 2004;130:187–98.
Kuniyoshi M, Yamada K, Higa T. A biologically active diphenyl ether from the green alga *Cladophora fascicularis*. Experientia 1985;41:523–4.
Law RJ, Alaee M, Allchin CR, Boon JP, Lebeuf M, Lepom P, et al. Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. Environ Int 2003;29:757–70.
Law RJ, Allchin CR, De Boer J, Covaci A, Herzke D, Lepom P, et al. Levels and trends of brominated flame retardants in the European environment. Chemosphere 2006;64:187–208.
Legler Juliette. New insights into the endocrine disrupting effects of brominated flame retardants. Chemosphere 2008;73:216–22.
Lindberg P, Sellstrom U, Haggberg L, de Wit CA. Higher brominated diphenyl ethers and hexabromocyclododecane found in eggs of peregrine falcons (*Falco peregrinus*) breeding in Sweden. Environ Sci Technol 2004;38:93–6.
Löfstrand K, Malmvärn A, Haglund P, Bignert A, Bergman Å, Asplund L. PBDD, MeO-PBDE, OH-PBDE and brominated phenols in blue mussels from the Swedish coast line. Organohalogen Compd 2007;69:287–90.
Malmvaern A, Zebuehr Y, Kautsky L, Bergman A, Asplund L. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic Sea. Chemosphere 2008;72:910–6.
Malmvarn A, Marsh G, Kautsky L, Athanasiadou M, Bergman A, Asplund L. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. Environ Sci Technol 2005;39:2990–7.
Marsh G, Stenutz R, Bergman A. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers—natural products and potential polybrominated diphenyl ether metabolites. Eur J Org Chem 2003:2566–76.
Nicholson MD, Fryer R, Larsen JR. Temporal trend monitoring: robust method for analysing contaminant trend monitoring data. ICES Tech Mar Environ Sci 1998;20:1-22.
Olsson A, Ceder K, Bergman A, Helander B. Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds—an evaluation. Environ Sci Technol 2000;34:2733–40.
Sellstrom U, Bignert A, Kierkegaard A, Haggberg L, de Wit CA, Olsson M, et al. Temporal trend studies on tetra- and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from the Baltic Sea. Environ Sci Technol 2003;37:5496–501.
Sinkkonen S, Rantalainen AL, Paasivirta J, Lahtipera M. Polybrominated methoxy diphenyl ethers (MeO-PBDEs) in fish and guillemot of Baltic, Atlantic and Arctic environments. Chemosphere 2004;56:767–75.
Sokal RR, Rohlf FJ. Biometry. The principles and practice of statistics in biological research3nd Ed. . New York: Freeman & Co.; 1995.
Teuten EL, Xu L, Reddy CM. Two abundant bioaccumulated halogenated compounds are natural products. Science 2005;307:917–20.
Unger M, Asplund L, Haglund P, Malmvarn A, Arnoldsson K, Gustafsson O. Polybrominated and mixed brominated/chlorinated dibenzo-p-dioxins in sponge (*Ephydatia fluviatilis*) from the Baltic Sea. Environ Sci Technol 2009;43:8245–50.
Van den Steen E, Eens M, Covaci A, Dirtu AC, Jaspers VLB, Neels H, et al. An exposure study with polybrominated diphenyl ethers (PBDEs) in female European starlings (*Sturnus vulgaris*): toxicokinetics and reproductive effects. Environ Pollut 2008;157:430–6.
Vorkamp K, Thomsen M, Falk K, Leslie H, Moller S, Sorensen PB. Temporal development of brominated flame retardants in peregrine falcon (*Falco peregrinus*) eggs from South Greenland (1986–2003). Environ Sci Technol 2005;39:8199–206.
Weijs L, Losada S, Das K, Roosens L, Reijnders PJH, Santos JF, et al. Biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in harbour seals and harbour porpoises from the Southern North Sea. Environ Int 2009;35:893–9.
Yates MA, Fuller MR, Henny CJ, Seegar WS, Garcia J. Wintering area DDE source to migratory white-faced ibis revealed by satellite telemetry and prey sampling. Ecotoxicology 2010;19:153–62.