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Organohalogenated contaminants in nestlings of white-tailed eagles (*Haliaeetus albicilla)*: Plasma concentrations and possible influences on biomarkers of health

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Master thesis in Biology, BIO-3950, September 2014

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Abstract

Biomagnifying organohalogenated contaminants (OCHs) may have adverse effects on the health of birds, especially in marine avian top predators that accumulates high loads of such contaminants. Contaminants may impact the immune system by impairment of the humoral immunity and also influence the antioxidant enzyme activity. Moreover, developmental conditions may affect the shortening of telomeres, one of the main mechanism explaining cell senescence, and it is proven that birds in growth have their telomeres influenced by among other the amount of oxidative stress during their development.

To examine the potential effects of organohalogenated contaminants (OCHs) on physiological biomarkers of health, OCHs with different physiochemical properties were related to individual variations in humoral immunity (immunoglobulin Y levels), superoxide dismutase enzyme (SOD) activity, and telomere length in nestlings (n = 35) of white-tailed eagles (*Haliaeetus albicilla*) in northern Norway. Different organochlorines (OCs) and perfluoroalkylated substances (PFASs) were measured in blood plasma of 7-8 weeks old nestlings, demonstrating higher concentrations of the emerging contaminants (PFASs), notably PFOSli ($\bar{x}$ = 40.91 ng/g ww), compared to legacy contaminants (OCs).

There were no relationships between the contaminant loadings and plasma immunoglobulin Y (IgY) levels. Moreover, differences between years were found for the telomere length, which was not due to contaminant loadings, but more likely a result of the developmental conditions between the two years sampling took place. However, there were significant and negative relationships between the OC loadings and the super oxidative enzyme (SOD) activity (p<0.01). This suggest that legacy OCs are challenging the antioxidant capacity in nestlings of white-tailed eagles.

Abbreviations

AIC	Aikaike's information criterion
AMAP	Arctic Monitoring and Assessment Programme
ANOVA	analysis of variance
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immune absorbent assay
g	gram
GC-MS	gas chromatography mass spectrometry
IgY	immunoglobulin Y
LOD	limit of detection
ml	millilitre
ng	nanograms
OHCs	organohalogenated contaminants
OCs	organochlorines
PBDE	polybrominated diphenyl ethers
PCA	principal component analysis
PFASs	perfluoroalkylated substanses
RBC	red blood cells
ROS	reactive oxygen species
RSTD	recovery standard
SOD	superoxide dismutase
SRM	standard reference material
UHPLCMS/MS	ultrahigh pressure liquid chromatography triple-quadrupole mass spectrometry
µl	microlitre
WTE	white-tailed eagles
ww	wet weight

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Introduction

Organohalogenated contaminants (OHCs), such as organochlorines (OCs) and perfluoralkylated substances (PFASs), are chemicals emitted into the environment as pesticides, industrial products or by-products. They are of concern due to their potential for long-transport and resistance to biodegradation, and because of their negative effects on wildlife and humans (AMAP, 2004, AMAP, 1998). Species feeding at the top of the marine food chain accumulate particularly high levels of contaminants due to the biomagnification process (Borgå et al., 2004). While most OCs have shown decreasing trends over the last 25 years (Bignert et al., 1998), several PFASs are still in use and continue to accumulate in the environment (Smithwick et al., 2005b, Ahrens et al., 2011). Unlike the highly lipophilic organochlorines, which accumulate preferably in the adipose tissue, the PFASs mostly binds to the protein fraction of the blood and accumulate in the liver of exposed organisms (Giesy and Kannan, 2002, Haukås et al., 2007).

Determining how the organic pollutants affect free-ranging organisms is not strait forward, but such compounds have been linked to a wide range of biological effects including ecological impacts (Bustnes et al., 2008, Letcher et al., 2010). One approach to assess the impact of pollutants is to examine different biomarkers of health, a method commonly used to measure biological processes (Bourgeon et al., 2012, Handy et al., 2003). For example, studies of birds have shown that organic pollutants may adversely impact immune parameters (Bustnes et al., 2004, Grasman et al., 1996, Fairbrother et al., 2004). In a study of glaucous gulls (*Larus hyperboreus*), Bustnes et al. (2004) found negative relationships between OCs and antibody response in females, suggesting that OCs cause impairment to the humoral immunity. Moreover, organic pollutants may induce oxidative stress (Fernie et al., 2005), and American kestrels (*Falco sparverius*) exposed to a mix of polybrominated diphenyl ethers (PBDEs), showed higher hepatic oxidative stress than control birds (Fernie et al., 2005). Oxidative stress is defined as the imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the organism, which is potentially damaging to cellular proteins, lipids and DNA (Finkel and Holbrook, 2000, Sies, 1997). The damages induced by ROS can be reduced by endogenous (e.g. enzymes) and/or exogenous (e.g. vitamin E and C) antioxidant compounds. Antioxidant enzymes act as a first line of defence and include superoxide dismutase (SOD), a metalloenzyme that catalyse the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide (Finkel and Holbrook, 2000, Costantini, 2008). The antioxidant defence and damage repair systems are not fully developed in birds in early stages of development (Nussey et al., 2009), and exposure to stress during this time may have long-term effects (Gluckman and Hanson, 2006, Lindström, 1999). Telomeres, the non-coding nucleic acid sequences that cap the ends of the DNA chromosome, can be used as a way to study such effects (Hall et al., 2004). Telomeres shorten at

each cell division because of the problem of end-replication (Blackburn, 1991), and previous bird studies have shown that most telomere loss occurs early in life (Hall et al., 2004). While there are no studies reporting the effects of contaminants on telomere in birds, studies of growing immortal human skin keratinocytes (HaCaT) exposed to PCB153 showed significantly reduced telomerase activity and telomere length (Senthilkumar et al., 2012).

This study is part of a larger project which aims to assess the status of the white-tail eagle (*Haliaeetus albicilla*, hereafter referred to as WTE) population in northern Norway with regard to OHCs. The WTE is a top predator in the marine environment that feeds mainly on seabirds and marine fish. It is the largest raptor species in Northern Europe – with a female-biased sexual dimorphism, and is distributed from the east of Germany and Poland, across the entire Palaearctic, as well as Greenland (Wilgohs, 1961). In the 1960s, the WTE population nearly went extinct in the Swedish Baltics due to reproductive impairment reported to be linked to high contaminant concentrations (Helander, 1985, Helander et al., 1982).

The objective of the current study was to examine the relationships between individual loads in environmental contaminants and three biomarkers of health in WTE nestlings; i.e. the activity of SOD enzymes, telomere length; and the levels of plasma immunoglobulin Y (IgY). I examined the influence of legacy (OCs) and emerging (PFASs) OHCs in a single population located in Troms County, northern Norway. My study area, together with Lofoten and Vesterålen, is an important nesting area with approximately 1000 breeding pairs (Trond V. Johnsen, unpublished data). Concentrations of 15 OCs and 3 PFASs were quantified in the blood of WTE nestlings in two consecutive years. Humoral immunity was measured via plasmatic levels of IgY the major systemic antibody involved in the avian humoral immune response (Lundqvist et al., 2006), whereas the extent of oxidative stress was determined by the plasmatic activity of SOD and telomere length. On the basis of previous studies indicating negative correlations between different clinical-chemical parameters and OCs in raptor chicks from northern Norway (Sonne et al., 2010, Sonne et al., 2012), we predicted that individual WTEs with high loadings of OHCs should suffer reduced immunity with: (i) lower levels of IgY and higher oxidative stress via; (ii) decreased SOD activity; and (iii) shorter telomere length.

Materials and methods

Samples of 35 WTE nestlings were collected in Troms County (68-70°N, 15-22°E), northern Norway, between 19th of June and 9th of July in 2011 (*n* = 19) and 2012 (*n* = 16). A large number of nests were visited from late March to mid-May to record breeding activity and egg laying (*n* =83). Field observations of nests were performed from a distance with binoculars and telescope to avoid disturbance. The sampling took place at approximately eight weeks of age, i.e. a few days prior to fledging date. The nestlings were removed from the nests during sampling in order to collect data. Prior to blood sampling, morphological measurement were collected: body mass [g] with a spring balance; the length of the wing and skull [mm] with a sliding calliper. Blood samples (4.0-10.0 ml) were taken from the brachial vein using a heparin coated syringe. At the end of each sampling day the blood samples were centrifuged at 8000 rpm for 10 min, plasma and red blood cells (RBCs) were transferred to sterile Eppendorf tubes and stored at ÷ 20°C until analyses in the lab.

Plasma samples were subsequently used to assess organochlorine and perfluoralkylated concentrations, SOD activity and immunoglobulin levels. Red blood cells were used to assess telomere length and determine the sex. The nestlings were sexed following an adapted protocol by the IPHC-DEPE, France, described by Helander et al. (2007). Briefly, DNA was extracted from RBCs using a commercial kit (NucleoSpin® Blood QuickPure, Macherey Nagel, Germany) and the sections of the sex-linked chromo-helicase-DNA-binding gene (CHD-W and CHD-Z) were amplified by polymerase chain reaction (PCR) using primers 2550F/2718R [2550F (5'-GTT ACT GAT TCG TCT ACG AGA-3') / 2718 R (5'-ATT GAA ATG ATC CAG TGC TTG-3') (Fridolfsson and Ellegren, 1999)].

Chemical analyses

All chemical analysis of organochlorines and perfluoralkylated substances were carried out in the laboratories at The Norwegian Institute for Air research (NILU) in Tromsø, Norway.

OCs analysis

The method used has previously been described in detail by (Sandanger et al., 2004). All solvents used in the experiments were of Suprasolv® grade and were purchased from Merck-Schuchardt (Hohenbrunn, Germany).

In short, the plasma samples (0.50 - 1.05 g) were spiked with 100 µl internal standard solution (POP I 1:10 in iso-octane, 25 pg/µl) containing mass-labeled OHCs, followed by adding deionised

water saturated with ammonium sulphate and ethanol to denaturize proteins. The extraction was then conducted by phase separation using n-hexane two times as solvent, the supernatants transferred to a glass tube and by RapidVap (Rapid Vap; Labconco copr, Kansas city, MO, USA), evaporated to 0.2 ml. Prior to the gas chromatograph mass spectrometry (GC-MS) analysis, the extracts were run over a florisil column (0.150 - 0.250 mm; Merck, Darmstadt; Germany) to remove biological matrix and added a recovery standard (13C PCB 159). Finally the extracts were run on an Agilent Technology 7890 GC and Agilent Technology 5975C MSD equipped with a DB-5MS column (length 30 m, 0.25 µm film thickness, 0.25 mm inner diameter (i.d.); J&W Scientific, Folsom, USA). Helium (6.0 quality; Hydrogas, Porsgrunn, Norway) was used as a carrier gas at a flow rate of 1 ml/min. This instrumentation allows for the separation of target compounds based on differences in their volatility and interactions with the column stationary phase (Harris, 2010). The following temperature program was used: 70˚C for 2 minutes, then a steady increase of 15˚C/minute; until 180˚C, directly followed by an increase of 5˚C/minute up to 280˚C; where the temperature was held for 10 minutes. To quantify the substances, the internal standard method was used. Native ^{12}C and added ^{13}C-labeled equivalents were analyzed, representing all the groups of polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane **(**DDT), dichlorodiphenyldichloroethylene **(***p,p'*-DDE), hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), chlordane's and nonachlores. The labelled standards were used to produce a standard curve from which concentrations were calculated using following equations:

$C_{sample} = (C_{std} \times Area_{sample})/Area_{std,}$ where C_{sample} = represents concentration of the unknown sample, C_{std} = represent concentration of known standard, $Area_{sample}$ = represent known area from the GC-MS chromatogram of the sample, and $Area_{std}$ = is the known area on the GC/MS graph of the standard (wet weight).

Lipid content in the blood samples was analysed at UniLab AS, Tromsø, using enzymatic determination. The total lipid (TL) content was calculated from the amounts of free cholesterol (FC), total cholesterol (TC), triglycerides (TG) and phospholipids (PL), described by Akins et al. (1989):

$$TL = 1.677 \times (TC - FC) + FC + TG + PL \quad \text{(Equation 1)}$$

The amount of lipids was expressed in mg/dl and converted to lipid percentage of wet weight (ww) sample.

When analysing the relationships between on lipophilic OCs and biomarkers of health, we used wet weight (n/g) concentrations, since it is considered most relevant in studies (Henriksen et al., 1996). Hence, a basic assumption in toxicology is that: "The production of a response and the degree of response are related to the concentration of the agent of the agent at the reactive site" (Klassen and Eaton, 1991).

PFAS analysis

The method of PFAS analysis has previously been described by Powley et al. (2005). All solvents used in the experiments were of Lichrosolv® grade and were purchased from Merck-Schuchardt (Hohenbrunn, Germany).

0.20 ml of plasma were added 20 µl isotopically-labeled internal standards (0.1 ng/µl 13 C PFAS mix), which were used to calculate PFAS concentration. The internal standard were of >98% purity and were obtained from Wellington Laboratories Inc. The internal standard contained: MPFHxS ($PFHxS^{18}O_2$), MPFOS ($^{13}C_4PFOS$), MPFOA ($^{13}C_4PFOA$), MPFNA ($^{13}C_5PFNA$), MPFHxA ($^{13}C_2PFDA$), MPFUnDA ($^{13}C_2PFUnDA$) and MPFDoDA ($^{13}C_2PFDoDA$). 1 ml methanol was added followed by ultrasonic bath and centrifuged for sedimentation (2000 rpm). Next, the methanol supernatant mixed with 25 mg ENVI-Carb 120/400 (Supelco 57210-U) and 50 µl glacial acetic acid. The mix were then centrifuged (10 000 rpm) and the supernatant solution transferred to a vial were 20 µl recovery standard (RSTD 0.1 ng/µl RSTD in methanol (3.7-diMeo-PFOA) was added. The RSTD, which were used to measure percentage recovery, contained 3.7 - dimethyl- branched perfluorodecanoic acid (bPFDA). It was of 97% purity and was obtained from ABCR (Karlsruhe, Germany). Prior to the UHPLCMS/MS analysis an aliquot of 50 µl of the extract and 50 µl of 2 mM NH4OAc in water was transferred to an autosamplervial. PFAS were analyzed by ultrahigh pressure liquid chromatography triple-quadrupole mass spectrometry (UHPLCMS/MS) as previously described by (Hanssen et al., 2013a). The chromatograms were quantified for PFAS using the LCQuan software (version 2.6, Thermo Scientific). Quantification was done with the internal standard method with isotope labeled PFAS. An eight point calibration curve with a concentration range from 0.02 pg/µl to 10 pg/µl was used for quantification of PFAS.

Quality control

For every 10 sample one blank and a standard reference material were run to assure the quality and test the reproducibility and precision of the method. The blanks were treated identically to the other samples, except that no tissue was added. In the OC analyses some of the blanks were contaminated, witch lead to that limit of detection (LOD) was calculated from average LOD in the blank samples for these substances. Regarding the PFASs, none of the blanks were contaminated. Recovery of the mass labeled standard ranged between 51-105 % for the OCs and 60-90% for the PFASs.

For the OC and PFAS analysis a blood/serum standard was used (SRM 1958 fortified human serum and SRM 1957, non-fortified human serum respectively) from National Institute of Standards and Technology (NIST), Gaitherburg, MD, USA.

Biomarker analyses

SOD activity and plasma immunoglobulin Y were carried out in the laboratories at UniLab AS, in Tromsø. Analyses of telomere length were carried out at the IPHC-DEPE, France.

Superoxide dismutase (SOD) enzyme activity

SOD activity was assessed with a superoxide dismutase assay kit from Cayman Chemical, Ann Arbor, MI (Catalogue no.706002). The enzymatic activity of SOD was determined from plasma and the method utilizes tetrazolium salt to quantify superoxide radicals generated by xanthine oxidase and hypoxanthine. Briefly, plasma samples from the chicks were thawed on ice and diluted 1:5 with sample buffer (50 mM Tris-HCl, pH 8.0). After the addition of plasma sample and a chromogenic tetrazolium salt (free radical detector) onto 96-well plates, xanthine oxidase was added as an enzymatic source of superoxide anion ($O_2^{\cdot-}$). The samples were gently shaken to mix, thereafter incubated for 20 minutes at room temperature, and endpoint measurements were performed at 450 nm using a Victor3 multilabel plate reader (PerkinElmer, Turku, Finland). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutase of the superoxide radical. The standard curve was generated using quality controlled SOD standard (bovine erythrocyte). Each sample and standard was performed in duplicate and the results expressed in U/ml.

Plasma immunoglobulin Y

Humoral immunity was assessed by using a sensitive ELISA (enzyme-linked immune absorbent assay) method. By the use of commercial antibodies reported in Martínez et al. (2003), the total amount of IgY was determined in the plasma of the WTE chicks. In order to calculate the linear range of the sigmoid curve for the chick plasma, ELISA plates were first coated with serial plasma dilutions (100µl) in carbonate-bicarbonate buffer (0.1 M, pH 9.6) and incubated overnight at 4°C. The dilution closest to the centre of its linear range was selected and used (Bourgeon et al., 2006). 96-well ELISA plates were filled with 100 µl of diluted serum samples (two samples per chick to 1/8000 in carbonate-bicarbonate buffer), incubated for 1 hour at 37°C and then overnight at 4°C. Next, the plates were washed; once with 200 µl solution of phosphate-buffered saline and Tween (PBS-Tween), before adding 100 µl of a blocking solution (5% powdered milk in PBS). After a second incubation at 37°C for 1 hour, the plates were washed with PBS-Tween buffer; and 100 µl of anti-chicken antibody, conjugated with peroxidase (Sigma A 9046, diluted 1: 250) were added. After incubation at 37°C for 2 hour, the plates were washed three times in PBS-Tween buffer, filled with 100 µl of a substrate solution: peroxide diluted in ABTS [2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] 1:1000 and

incubated for 1 hour at 37°C. The final absorbance was measured at 405 nm using a Victor3 multilabel plate reader (PerkinElmer, Turku, Finland) and subsequently used to assess total plasma IgY levels expressed in units of absorbance/arbitrary units.

Telomeres

Telomere measurements were conducted following the procedure described by Criscuolo et al. (2009). Briefly, DNA was extracted from 5 µl of red blood cells using a commercial kit "DNeasy® Blood and Tissue kit, Qiagen, Germany". Telomere and control gene PCR conditions were 10 minutes at 95°C followed by 30 cycles of 30 seconds at 56°C, 30 seconds at 72°C and 60 seconds at 95°C. qPCR amplicon sizes were determined after electrophoresis on a 1.5% agarose gel run in standard TBE (Tris/Borate/EDTA) buffer (90 V for 10 minutes and 130 V thereafter for 30 minutes) and using ethidium bromide staining. The method is based on the determination of a number of amplification cycles necessary to detect a lower threshold of fluorescent signal, the cycle number being proportional to the telomere length (T), or to the number of copies of a control gene (S). A ratio T:S of telomere repeated copy number (T) to single control gene copy number (S) was then calculated for each sample that will reflect relative inter and intra-individual differences in telomere length. Telomere and control gene amplifications were carried out in duplicate on each plate and twice on two different plates and the mean values of the four measurements of telomere and control gene were used to calculate the final T⁄S ratio for each sample. For each qPCR run, we confirmed that the amplification efficiency was between 95% and 105%, using the dilution curve calculation method (Larionov et al., 2005) and inter-plate standardization was achieved with a reference bird sample in each qPCR (Criscuolo et al., 2009).

Statistical analysis

Statistical analyses were carried out by using the statistical program R, version 3.0.3 (R Development Core Team 2014). Originally 27 organochlorine and 15 perfluoralkylated substances were measured, 11 of them were under detection limit and 13 were detected in less than 75% of the chicks and thus excluded (Table 1-3 provide descriptive statistics for biometric variables, contaminant concentrations and biomarkers, respectively). A principal component analysis (PCA) was used to investigate the relationship between the remaining 18 contaminants (15 OCs and 3 PFASs; see Appendix: Table A). The first two principal components (PCs) explained 71% and 11% of the variation, respectively. The analysis revealed a high degree of correlation between the majority of the OCs clustering along the PC1 axis, and a second cluster of two of the PFASs along the PC2 axis of the PCA plot (Appendix: Figure A). Due to a high degree of correlation between the compounds and to reduce the number of contaminants in further effect analyses, we selected the following compounds

based on their commonness and physiochemical properties (Bustnes et al., 2013, Eulaers et al., 2013): HCB, oxy-chlordane, *p,p'*-DDE, PCB153, perfluorooctane sulfonate (PFOSli) and perfluoroundecanoate (PFUNA). To control for potentially systematic differences in contaminant loadings between the chicks, we included sex and year as factors in an analysis of variance (ANOVA). Moreover, inter-annual differences, such as food/prey availability and weather conditions, may also impact the health status of the chicks emphasising the importance of controlling for year in the effect analyses.

We also tested the potential influence of sex and year on biomarker responses, and the different body measurements (body mass, wing length and skull). Since female and males differed significantly in body mass (Table 4; $R^2 = 0.48$), sex was excluded from further analysis to avoid problems with collinearity (Zuur et al., 2009). Furthermore, due to missing body mass measurements for 6 individuals and as wing length significantly explained the variance in body mass [Pearson's product-moment correlation (r) = 0.38, df = 27, $p < 0.05$[1]], wing length was used as a proxy for body size in the effect analyses.

The chicks were sampled from 27 nests, 8 of them with 2 nestlings, the remaining with 1 nestling. Nests may represent interesting biological processes including parental investments and diet variance due to habitat differences. However, due to the low number of nests with 2 chicks (70% of the nests contained only 1 nestling), I did not assess between-nest variability in my analyses, which could have been performed using linear mixed effect models (Zuur et al., 2009).

The impact of contaminants on the biomarkers of health was analysed using linear models with the biomarkers as response variables and the selected contaminants HCB, Oxy-chlordane, *p,p'*-DDE, PCB153, PFOSli and PFUNA (Bustnes et al., 2013, Eulaers et al., 2013), wing length, (a proxy for the chicks developmental stage) and year as predictor variables. The contaminants were tested individually to reveal any differences in response to the different chemical properties. From the pool of models, one model was selected for each biomarker and per compound based on the second-order Akaike's Information Criterion (AICc) values and I selected the simplest model with a $\Delta AIC < 1.5$ (Matthiopoulos, 2011; see Appendix: Table C). Assumptions of constant variance and approximate normal distribution of residuals were determined through plots of residuals against fitted values and normal quantile-quantile plots (Zuur et al., 2009).

[1] A simple linear regression model, with body mass as the response, produced the following results: Intercept = 3019.82 (SE = 730.20, p < 0.01); $\beta_{wing\ length}$ = 3.83 (SE = 1.78, p = 0.04); and $F_{(1,27)}$ = 4.641 (p = 0.04).

Results

Biological characteristics

A summary of the biological characteristics can be found in Table 1. There were no significant differences in body mass, wing and skull lengths between 2011 and 2012 (Table 4). Between the sexes, there were significant differences in both body mass and skull length with females being on average 18 % heavier than males (females body mass: $\bar{x}$ = 5057.50 g ± 101.45 SE, *n* = 10; males body mass: $\bar{x}$ = 4289.47 g ± 130.62 SE, *n* = 19), and females having skulls 6 % longer than males (females: $\bar{x}$ = 134.75 mm ± 1.87 SE, *n* = 13; males: $\bar{x}$ = 127.28 mm ± 1.16 SE, *n* = 22; Table 4).

OHC concentrations

The chemical analysis of plasma assessed the presence of several major classes of contaminants. The compounds a-HCH, o,p'-DDT, *p,p'*-DDT, o,p'-DDD, o,p'-DDE, PFBS, PFBa, PFPA, PFHxA, PFHpA and PFTEA were below the detection limit and therefore not reported. b-HCH, g-HCH, t-chlordane, *p,p*'-DDD, PCB 52, PCB 101, PCB 194, PFHxS, PFOSbr, PFOA, PFDcA, PFdoA and PFTRA were detected in less than 75% of the chicks and were excluded from the statistical analyses (Appendix: Table A). OCs made up the majority of compounds, whereas the PFASs were found in the highest concentrations; $\bar{x}$ = 40.91 ± 5.75, ng/g ww for the PFOSli (Table 2). Of the measured OCs, PCB153 had the highest concentrations ($\bar{x}$ = 11.98 ± 1.77 ng/g ww). The mean concentration of ∑OC was 41.34 ± 5.66 (ng/g ww) where ∑PCB represented 85% of that sum ($\bar{x}$ = 35.06 ± 4.96 (ng/g ww; Table 2; Figure1). No significant differences in OHC levels were found between the years except for PFUNA that differed significantly between the years with plasma levels being 47% higher in 2011 than in 2012 (2011: $\bar{x}$ = 6.58 ng/g ww ± 0.75, *n* = 19; 2012: $\bar{x}$ = 4.46 ng/g ww ± 0.63, *n* = 16; Table 4). See Figure 1 for a visualization of the percentage composition for each compound.

Biomarkers of health

A summary of the biological characteristics can be found in table 3. While there was no differences between the sexes in any of the biomarkers of health, telomeres differed significant

between the years with initial telomere length being 30 % shorter in 2012 than in 2011 (2011: $\bar{x}$ = 1.37 ± 0.09, *n* = 19; 2012: $\bar{x}$ = 0.96 ± 0.07, *n* = 16; Table 5).

Superoxide dismutase (SOD)

The best model predicting SOD activity was the simplest model that included only the effect of the contaminants (Appendix: Table B). All the OCs (HCB, Oxy-chlordane, *p,p'*-DDE and PCB153) were statistical significantly and negatively related to the SOD activity (Table 6; Figure 2). On the contrary, PFASs did not show any significant relationship to with the enzyme activity; suggesting that the two groups of OHCs had different influence on the physiology of birds.

Plasma immunoglobulin Y (IgY)

There were no statistically significant relationship between the contaminants and plasma IgY levels (humoral immunity; Table 6; Appendix: Table B).

Telomeres

The best model predicting telomere length included year in addition to the different contaminants, a result due to a significant difference in telomere length between the years (Table 5). However, the results showed no statistically significant relationship between telomere length and the different contaminants (Table 6; Appendix: Table B).

Table 1. Descriptive statistics [mean ± standard error, median and range (min-max)] for biometric variables and plasma lipid content (%) in white-tailed eagle (*Haliaeetus albicilla)* chicks from Troms, Norway. The sample size was 29 for body mass and 35 for wing length, skull length and lipid %.

	Mean ± SE	Median	Min – Max
Body mass (g)	4554.31 ± 104.13	4600.00	2875.00 – 5550.00
Wing length (mm)	405.20 ± 9.94	399.00	256.00 – 525.00
Skull length (mm)	130.05 ± 1.17	129.90	115.10 – 150.70
Lipids %	0.89 ± 0.04	0.83	0.56 – 1.48

Table 2. Descriptive statistics [mean ± standard error, median and range (min-max)] for contaminant concentrations (ng/g ww) analysed blood plasma from white-tailed eagle (*Haliaeetus albicilla)* chicks in Troms, Norway. The sample size was 35.

	Mean ± SE	Median	Min – Max
OCs			
HCB	2.018 ± 0.166	1.830	0.615 – 4.390
c-chlordane	0.126 ± 0.012	0.117	0.046 – 0.403
oxy-chlordane	1.483 ± 0.197	1.060	0.337 – 5.900
t-Nonachlor	1.021 ± 0.103	0.798	0.317 – 2.620
c-Nonachlor	0.462 ± 0.052	0.333	0.153 – 1.300
p,p'-DDE	1.167 ± 0.175	0.748	0.151 – 4.240
PCB28	0.117 ± 0.009	0.103	0.050 – 0.268
PCB99	1.548 ± 0.207	1.060	0.226 – 5.200
PCB105	0.944 ± 0.120	0.736	0.140 – 3.160
PCB118	3.470 ± 0.466	2.620	0.488 – 11.800
PCB138	9.191 ± 1.233	6.740	1.500 – 30.100
PCB153	11.978 ± 1.765	8.060	1.560 - 44.500
PCB180	4.803 ± 0.748	2.770	0.678 - 18.000
PCB183	0.888 ± 0.133	0.606	0.086 – 3.340
PCB187	2.123 ± 0.278	1.610	0.401 – 7.140
∑ PCB	35.061 ± 4.959	24.305	
∑ OC	41.337 ± 5.664	29.191	
PFASs			
PFOSli	40.914 ± 5.746	34.302	0.262 -187.653
PFNA	3.108 ± 0.488	2.281	0.131 – 13.499
PFUNA	5.609 ± 0.525	5.761	1.059 – 14.015
∑ PFAS	49.631 ± 6.759	42.343	

Table 3. Descriptive statistics [mean ± standard error, median and range (min-max)] for biomarker responses from white-tailed eagle (*Haliaeetus albicilla)* chicks in Troms, Norway. Superoxide dismutase (SOD) activity was measured in U/ml, immunoglobulin Y (IgY) were measured in absorbance unit and telomere length was assessed using T/S ratio. The sample size was 35 for SOD and telomeres and 25 for IgY.

	Mean ± SE	Median	Min – Max
SOD	0.797 ± 0.034	0.823	0.316 – 1.125
IgY	0.510 ± 0.026	0.459	0.313 – 0.856
Telomeres	1.179 ± 0.068	1.093	0.534 – 2.308

Table 4. Parameter estimates for biological profiles and plasma organochlorines (OCs) and perfluoralkylated compounds (PFASs) of white-tailed eagle (*Haliaeetus albicilla*) chicks in Troms, Norway. The values were calculated using analysis of variance (ANOVA). The sample size was 29 for body mass and 35 for wing length, skull length, HCB, Oxy-chlordane, *p,p'*-DDE, PCB153, PFOSli and PFUNA.

Parameter	Estimate	SE	*t*-value	*p*-value	Parameter	Estimate	SE	*t*-value	*p*-value
Body mass (g) ($F_{(1,27)}$ = 2.49, R^2 = 0.08, p = 0.13)					**Body mass (g)** ($F_{(1,27)}$ = 24.54, R^2 =0.48, p = <0.001)				
Intercept	4760.70	163.80	29.07	<0.001	Intercept	4241.70	109.30	38.82	<0.001
Year (2012)	-359.00	227.70	-1.58	0.13	Sex (♀)	878.80	177.40	4.95	<0.001
Wing length (mm) ($F_{(1,33)}$ = 3.48, R^2 = 0.10, p = 0.07)					**Wing length (mm)** ($F_{(1,33)}$ = 0.01, R^2 = <0.001, p = 0.92)				
Intercept	421.63	13.03	32.37	<0.001	Intercept	404.46	12.73	31.78	<0.001
Year (2012)	-35.94	19.27	-1.87	0.07	Sex (♀)	2.01	20.88	0.10	0.92
Skull length (mm) ($F_{(1,33)}$ = 0.26, R^2 = 0.01, p = 0.62)					**Skull length (mm)** ($F_{(1,33)}$ = 12.79, R^2 = 0.28, p = <0.01)				
Intercept	130.61	1.61	81.31	<0.001	Intercept	127.28	1.27	100.05	<0.001
Year (2012)	-1.21	2.38	-0.51	0.62	Sex (♀)	7.46	2.09	3.58	<0.01
HCB (ng/g ww) ($F_{(1,33)}$ = 1.1, R^2 = 0.03, p = 0.30)					**HCB (ng/g ww)** ($F_{(1,33)}$ = 0.24, R^2 = 0.01, p = 0.63)				
Intercept	2.18	0.23	9.67	<0.001	Intercept	1.95	0.21	9.23	<0.001
Year (2012)	-0.35	0.33	-1.05	0.30	Sex (♀)	0.17	0.35	0.49	0.63
Oxy-chl (ng/g ww) ($F_{(1,33)}$ = 3.28, R^2 = 0.09, p = 0.08)					**Oxy-chl (ng/g ww)** ($F_{(1,33)}$ = 0.01, R^2 = <0.001, p = 0.93)				
Intercept	1.80	0.26	6.96	<0.001	Intercept	1.47	0.25	5.83	<0.001
Year (2012)	-0.69	0.38	-1.81	0.08	Sex (♀)	0.04	0.41	0.09	0.93
***p,p'*-DDE (ng/g ww)** ($F_{(1,33)}$ = 1.85, R^2 = 0.05, p = 0.18)					***p,p'*-DDE (ng/g ww)** ($F_{(1,33)}$ = 0.03, R^2 = <0.001, p = 0.87)				
Intercept	1.38	0.23	5.90	<0.001	Intercept	1.19	0.22	5.32	<0.001
Year (2012)	-0.47	0.35	-1.36	0.18	Sex (♀)	-0.06	0.37	-0.16	0.87
PCB153 (ng/g ww) ($F_{(1,33)}$ = 2.52, R^2 = 0.07, p = 0.12)					**PCB153 (ng/g ww)** ($F_{(1,33)}$ = 0.06, R^2 = <0.01, p = 0.81)				
Intercept	14.49	2.34	6.19	<0.001	Intercept	12.32	2.26	5.46	<0.001
Year (2012)	-5.51	3.47	-1.59	0.12	Sex (♀)	-0.92	3.70	-0.25	0.81
PFOSli (ng/g ww) ($F_{(1,33)}$ = 0.06, R^2 = <0.01, p = 0.81)					**PFOSli (ng/g ww)** ($F_{(1,33)}$ = 0.82, R^2 = 0.02, p = 0.37)				
Intercept	39.60	7.91	5.01	<0.001	Intercept	44.92	7.27	6.18	<0.001
Year (2012)	2.88	11.70	0.25	0.81	Sex (♀)	-10.78	11.92	-0.90	0.37
PFUNA (ng/g ww) ($F_{(1,33)}$ = 4.42, R^2 = 0.12, p = 0.04)					**PFUNA (ng/g ww)** ($F_{(1,33)}$ = 0.51, R^2 = 0.02, p = 0.48)				
Intercept	6.58	0.68	9.68	<0.001	Intercept	5.32	0.67	7.98	<0.001
Year (2012)	-2.11	1.01	-2.10	0.04	Sex (♀)	0.78	1.09	0.71	0.48

Table 5. Parameter estimates for plasma biomarkers of white-tailed eagle (*Haliaeetus albicilla)* chicks in Troms, Norway. The values calculated using analysis of variance (ANOVA). The sample size was 35 for SOD and telomeres and 25 for IgY.

Parameter	Estimate	SE	*t*-value	*p*-value	Parameter	Estimate	SE	*t*-value	*p*-value
SOD (U/ml) ($F_{(1,33)}$ = 0.47, R^2 = 0.01, p = 0.50)					**SOD (U/ml)** ($F_{(1,33)}$ = <0.001, R^2 = <0.001, p = 0.99)				
Intercept	0.78	0.05	16.70	<0.001	Intercept	0.80	0.043	18.34	<0.001
Year (2012)	0.05	0.07	0.69	0.50	Sex (♀)	<-0.001	0.07	-0.01	0.99
IgY(absorbance unit) ($F_{(1,23)}$ = 0.01, R^2 = <0.01, p = 0.91)					**IgY (absorbance unit)** ($F_{(1,23)}$ = 0.07, R^2 = <0.01, p = 0.80)				
Intercept	0.51	0.04	12.27	<0.001	Intercept	0.50	0.04	12.47	<0.001
Year (2012)	-0.01	0.06	-0.12	0.91	Sex (♀)	0.02	0.06	0.26	0.80
Telomeres (T/S ratio) ($F_{(1,33)}$ = 11.87, R^2 = 0.26, p = <0.01)					**Telomeres (T/S ratio)** ($F_{(1,33)}$ = 0.08, R^2 = <0.1, p = 0.78)				
Intercept	1.37	0.08	16.95	<0.001	Intercept	1.16	0.09	13.34	<0.001
Year (2012)	-0.41	0.12	-3.45	<0.01	Sex (♀)	0.04	0.14	0.28	0.78

Table 6. Factors affecting biomarkers of health in white-tailed eagle (*Haliaeetus albicilla*) chicks in Troms, Norway calculated by linear regression models. The predictor variables in the statistical models were contaminants (ng/g wet weight), wing length and year, were selected based on the lowest second-order Akaikes Information Criterion (AICc; Appendix: Table B). The sample size was 35 for SOD and telomeres and 25 for IgY.

Parameter	**Estimate**	**SE**	***t*-value**	***p*-value**
SOD ($F_{(1,33)}$ = 9.50, R^2 = 0.22, p = 0.004)				
Intercept	0.99	0.07	14.13	<0.001
HCB	-0.10	0.03	-3.08	**0.004**
SOD ($F_{(1,33)}$ = 11.44, R^2 = 0.26, p = 0.002)				
Intercept	0.93	0.05	19.10	<0.001
Oxy-chl	-0.09	0.03	-3.38	**0.002**
SOD ($F_{(1,33)}$ = 16.57, R^2 = 0.33, p = <0.001)				
Intercept	0.93	0.04	21.70	<0.001
p,p'-DDE	0.11	0.03	-4.07	**< 0.001**
SOD ($F_{(1,33)}$ = 9.33, R^2 = 0.22, p = 0.004)				
Intercept	0.91	0.05	19.38	<0.001
PCB153	-0.01	<-0.01	-3.05	**0.004**
SOD ($F_{(1,33)}$ = 1.10, R^2 = 0.03, p = 0.30)				
Intercept	0.75	0.05	14.08	<0.001
PFOSli	<0.01	<0.01	1.05	0.30
SOD ($F_{(1,33)}$ = 0.29, R^2 = 0.01, p = 0.59)				
Intercept	0.83	0.07	11.60	<0.001
PFUNA	-0.01	0.01	-0.54	0.59
IgY ($F_{(1,23)}$ = 1.23, R^2 = 0.05, p = 0.28)				
Intercept	0.44	0.07	6.02	<0.001
HCB	0.03	0.03	1.11	0.28
IgY ($F_{(1,23)}$ = 0.30, R^2 = 0.01, p = 0.59)				
Intercept	0.49	0.05	9.42	<0.001
Oxy-chl	0.01	0.03	0.55	0.59
IgY ($F_{(1,23)}$ = 0.43, R^2 = 0.02, p = 0.52)				
Intercept	0.49	0.05	9.99	<0.001
p,p'-DDE	0.02	0.03	0.65	0.52
IgY ($F_{(1,23)}$ = 0.64, R^2 = 0.03, p = 0.43)				
Intercept	0.48	0.05	9.84	<0.001
PCB153	<0.01	<0.01	0.80	0.43
IgY ($F_{(1,23)}$ = 0.04, R^2 = <0.01, p = 0.84)				
Intercept	0.52	0.05	10.68	<0.001
PFOSli	<-0.01	<0.01	-0.21	0.84
IgY ($F_{(1,23)}$ = 0.12, R^2 = 0.01, p = 0.73)				
Intercept	0.49	0.07	7.12	<0.001
PFUNA	<0.01	0.01	0.35	0.73
Telomeres ($F_{(2,32)}$ = 6.68, R^2 = 0.29, p = 0.004)				
Intercept	1.53	0.16	9.71	<0.001
HCB	-0.07	0.06	-1.17	0.25
Year	-0.44	0.12	-3.62	**0.001**
Telomeres ($F_{(2,32)}$ = 7.31, R^2 = 0.31, p = 0.002)				
Intercept	1.51	0.12	12.17	<0.001
Oxy-chl	-0.08	0.05	-1.51	0.14
Year	-0.47	0.12	-3.81	**<0.001**
Telomeres ($F_{(2,32)}$ = 6.32, R^2 = 0.28, p = 0.005)				
Intercept	1.44	0.12	12.45	<0.001
p,p'-DDE	-0.06	0.06	- 0.91	0.37
Year	-0.44	0.12	- 3.55	**0.001**
Telomeres ($F_{(2,32)}$ = 5.94, R^2 = 0.27, p = 0.006)				
Intercept	1.41	0.12	11.80	<0.001
PCB153	<-0.01	0.01	-0.53	0.60
Year	-0.43	0.13	-3.43	**0.002**
Telomeres ($F_{(2,32)}$ = 6.45, R^2 = 0.29, p = 0.004)				
Intercept	1.30	0.11	12.12	<0.001
PFOSli	<0.01	<0.01	1.01	0.32
Year	-0.42	0.12	-3.49	**0.001**
Telomeres ($F_{(2,32)}$ = 5.77, R^2 = 0.26, p = 0.007)				
Intercept	1.35	0.16	8.41	<0.001
PFUNA	<0.01	0.02	0.13	0.90
Year	-0.41	0.13	-3.14	**0.004**

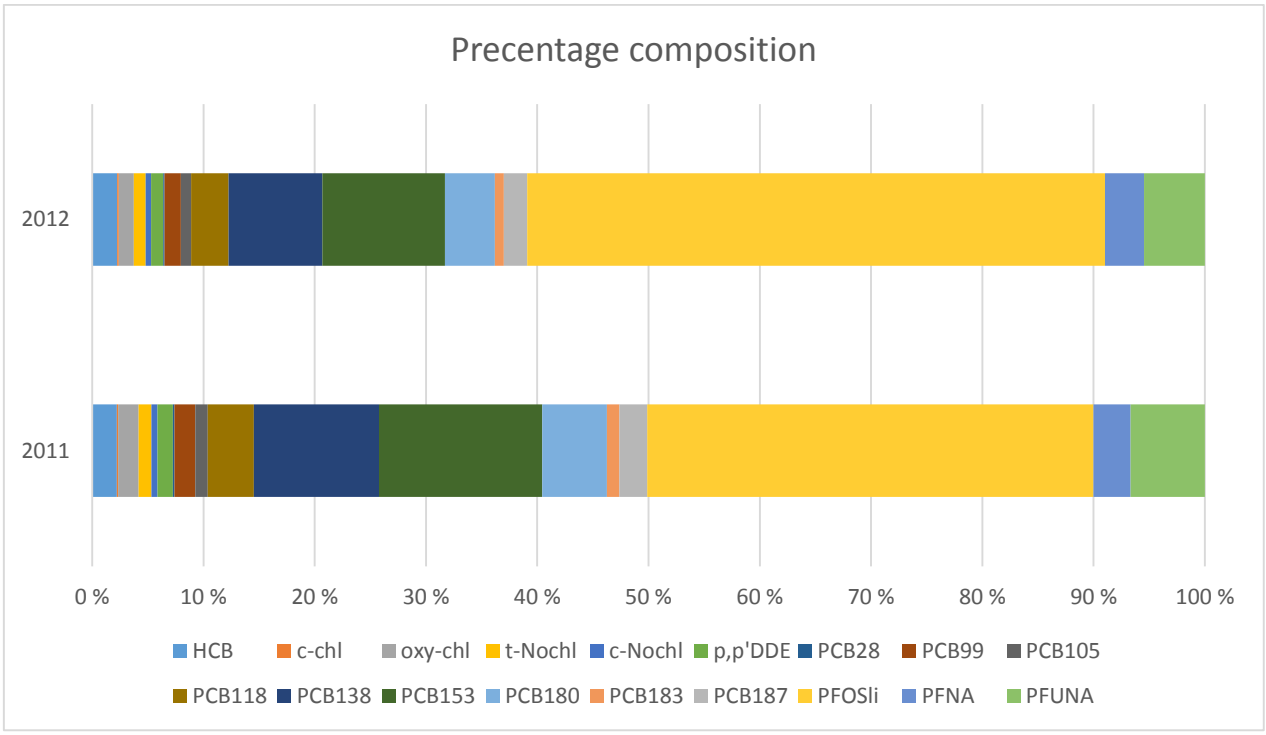

Figure 1. Percentage composition of total OC and PFAS burden in white-tailed eagle (*Haliaeetus albicilla)* chicks in 2011 and 2012 located in Troms, Norway. The sample size was 35.

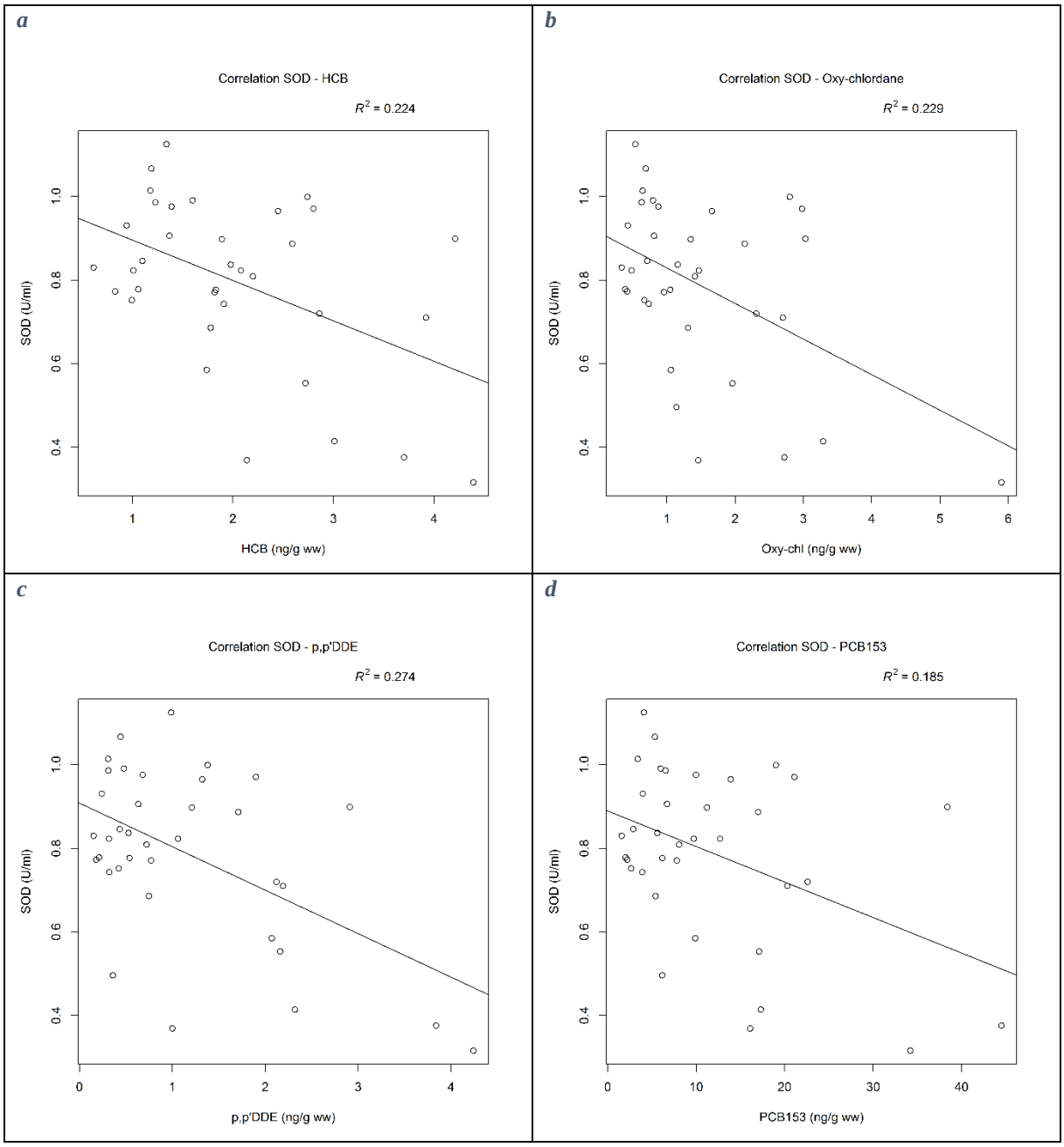

Figure 2. Relationships between superoxide dismutase enzyme (SOD) activity and plasma concentrations of the following organochlorine compounds: a) HCB, b) oxy-chlordane, c) *p,p'*-DDE, and d) PCB153 in white-tailed eagle (*Haliaeetus albicilla*) chicks in Troms, Norway (see Table 6 for details).

Discussion

The present study investigated the relationships between organohalogenated contaminants (OHCs) and physiological parameters in chicks of the WTE in northern Norway, a marine top predator species that has previous been endangered by environmental contaminants in some areas (Helander et al., 1982, Helander, 1985). Over two years, both short- (oxidative stress) and long-term (humoral immunity and telomere length) markers of exposure were examined.

As predicted, we found negative correlations between plasma concentration of an antioxidant enzyme, superoxide dismutase (SOD), and organochlorines. The most heavily polluted chicks showed lower SOD activity, further indicating a challenged antioxidant capacity. Contrary to our predictions, however, we did not find any relationship between OHC concentrations and humoral immunity (i.e., plasma immunoglobulin Y levels) or telomere length. Telomere lengths, however, differed between the years, regardless of OHC concentrations.

Sexual and annual variation in body size

As anticipated, female biased sexual dimorphism with female exhibiting significantly higher body mass and longer skull length than males. However, we did not find any significant differences between the years for any of the biometric variables; body mass, wing length and skull length.

Variations in concentrations and patterns of OHC levels

Data on contaminants in raptors from northern Norway is scare, but comparison to other marine birds in Troms, such as the great black-backed gulls (*Larus marinus*) and the lesser black-backed gulls (*Larus fuscus*; Bustnes et al., 2006), show that WTE nestlings in this study have lower concentrations of OCs. Nevertheless, if we compare the loadings with a terrestrial raptor from the same area, the northern goshawk (*Accipiter gentilis*), both OCs and PFOS loadings were 2-3 times higher in the WTE nestlings. However, in comparison to Bald eagle (*Haliaeetus leucocephalus*) breeding near the Mississippi river in Upper Midwestern, United States (Route et al., 2014), WTE nestlings in this study had 20 times lower PFOS burdens.

The majority of compounds, i.e. 15 of 42 tested for, found in the chicks were OCs, whereas PFASs, notably PFOSli, were found in the highest concentrations. Higher concentration of PFOSli than legacy OCs have previously been documented in livers of songbirds (Hoff et al., 2005b) and freshwater fish from Belgium (Hoff et al., 2005a), and Canadian polar bears (Ursus maritimus; Smithwick et al., 2005a). Many PFAS compounds are not banned and still in high demands due to

their widespread use in industrial and consumer products applications, suggesting that increasing environmental concentrations may be expected (Ahrens et al., 2011, Buck et al., 2011). Despite the observed sexual dimorphism in size, we found no significant differences in contaminants concentrations between the males and the females. Likewise, we found no significant differences in OHC levels between the years except for PFUNA, whose concentrations were significantly higher in 2011 compared to 2012. This inter-year difference may be due to these contaminants being released at different rates between the years. It may also result from inter-annual differences in food availability and/or distance of nest to source areas.

Plasmatic biomarkers of health

The health status of birds may be influenced by both natural and anthropogenic stress factors, which may affect different physiological biomarkers. In the current study, none of the biomarkers assessed was significantly affected by sex, and IgY and SOD did not vary significantly between years. Only initial length of telomeres was significantly shorter in 2012 than in 2011, which could be interpreted as chicks hatched in 2012 being of poorer quality than chicks hatched in 2011. Another finding that might indicate annual variation in the state of the chicks was the fact that wing length was shorter in 2012 than in 2011 (even though this difference was just nearly statistical significant).

Indeed, telomeres that shorten at each cell division are thought to play an important role in cellular senescence and aging. Conditions during the embryonic and initial post-hatching periods are known to affect the length of the telomeres (Hall et al., 2004). For example, lesser black-backed gulls (*Larus fuscus*) chicks that were large at hatching tended to have shorter telomere length, suggesting that embryonic growth could have affected telomere attrition (Foote et al., 2011). Additionally a study done on European shag (*Phalacrocorax aristotelis*) and the wandering albatross (*Diomedea exulans*) showed that individuals hatched late in the season had greater telomere loss than early hatchlings (Hall et al., 2004). The latter results could be linked to nutritional stress as a consequence of lower parental quality and/or seasonal changes in food supply.

Relationships between contaminants and biomarker of health

We observed significant relationship between oxidative stress parameters (SOD) and the OCs (HCB, Oxy-chlordane, *p,p'*-DDE, PCB153). More precisely, we reported a decrease in SOD activity in the individuals with the highest loadings of OCs. This result may express a negative influence of contaminants on the WTE chicks through the reduction of their antioxidant capacity.

Accordingly, Costantini (2008) proposed that; oxidative stress may rise as a result of lowered antioxidant levels. Nevertheless, the physiological and biochemical machinery underlying oxidative

stress is very complex, and an assessment of markers of oxidative damage, in addition to multiple markers of antioxidant defence, is needed to obtain meaningful insight. For example, the reactive oxygen species (ROS) production burden is largely counteracted by an intricate antioxidant defence system including enzymatic scavengers such as SOD, catalase and glutathione peroxidase. In addition may the system be affected by the metabolism and physical state of the individuals; e.g.; production of ROS may increase with the metabolic rate of an organism (Finkel and Holbrook, 2000). This may be especially important to chicks at early developmental stages, when growth is rapid and nutrient demand is high (Hanssen et al., 2013b, Janeway et al., 1999).

Previous studies have shown decreased SOD enzyme activity in relation to OCs, such as in juveniles of East Pacific green turtles (*Chelonia mydas*), where enzymatic antioxidant activity showed a negative relationship with pesticide concentrations (Labrada-Martagón et al., 2011). In addition, the latter study also reported that the activities of several enzymes (CAT, t-SOD and Mn-SOD) correlated positively or negatively with the concentrations of trace elements. Both increases and decreases in the activity of antioxidant enzymes were also documented in some fish species exposed to biodegradable compounds like aromatic hydrocarbons (reviwed by van der Oost et al., 2003). In waterbirds living in the Büyük Menderes River (Turkey), antioxidant enzyme activity was positively correlated to liver concentrations of OC pesticides (Kocagöz et al., 2014). The initiating events leading to activation of pathways in response to oxidants are incompletely understood (Finkel and Holbrook, 2000). Moreover, increasing enzyme levels may have an energetic cost being unaffordable to all organisms, thus forcing individuals in rapid growth and/or in a bad condition to trade investment in self maintenance over countering oxidative stress (Stier et al., 2014).

Contrary to OCs, PFASs showed no significantly relationship to the SOD activity. This might be due to the different chemical properties of the two contaminant groups. PFASs accumulate mainly in protein-rich tissues, and may therefore induce different effects than the lipophilic OCs (Giesy and Kannan, 2002). How the mechanisms through which PFASs affect the birds are still poorly understood, but it has been suggested that some of the dominating compounds interfere with mitochondrial bioenergetics and fatty-acid binding proteins (reviewed by Lau et al., 2004), and that they cause developmental effects in young birds due to disruption of thyroid hormone homeostasis (Nost et al., 2012).

The other biomarkers tested; plasma IgY and telomere length, were not affected by any of the contaminants. Several hypotheses may explain such lack of significant relationships in my study. Firstly, the combination of relatively low loads of contaminants in chicks compared to adults, and the moderate sample size in this study may hinder the detection of any negative effects (i.e. I lack statistical power to document such weak effects). For example, breeding adult glaucous gulls (*Larus hyperboreus*) from Bjørnøya, in which organochlorine pollution was suggested to influence immunocompetence (Bustnes et al., 2004), had loadings ten to hundred times higher than my study species. Secondly, the small sample size of our study reduces the inter-individual variances in OHC

and biomarker levels further preventing potential significant statistical relationships. Finally, birds at early development stages may have underdeveloped immune systems, which may have prevented them from responding similar to adult birds (Janeway et al., 1999).

This study documented that legacy contaminants (OCs) in WTE nestlings may negatively affect their antioxidative enzyme activity. Although PFOSli (an emerging compound) was found at higher concentrations than OCs, it did not show any significant relationships with any of the biomarkers suggesting that PFASs are less prone to influence stress markers in wild birds than OCs, at least when concentrations are comparable.

It is challenging to determine how reduced enzyme activity influences the nestlings. Nevertheless, this finding is of concern as it affects, already at low OHC concentrations, the antioxidant system in young individuals. However, more studies are needed to document the long-term effects of OHCs on biomarkers of health as well as the ecological effects of OHCs on breeding and survival probabilities of WTE chicks.

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Appendix

Table A. List of organochlorine (OCs) and perfluoralkylated compounds (PFASs) with levels of detection.

	Detection				Detection		
OCs	> 75%	< 75%	< LOD	**PFASs**	> 75%	< 75%	< LOD
α-hexachlorocyclohexane (a-HCH)			X	Perfluorobutane sulfonate (PFBS)			X
β-hexachlorocyclohexane (b-HCH)		X		Perfluorohexane sulphonate (PFHxS)		X	
γ-hexachlorocyclohexan (g-HCH)		X		**Perfluorooctane sulphonate (PFOSli)**	**X**		
Hexachlorobenzene (HCB)	**X**			Perfluorooctane sulphonate (PFOSbr)		X	
trans-chlordane (t-chlordane)		X		Perfluorobutanoate (PFBa)			X
cis-chlordane (c-chlordane)	X			Perfluoropentanoate (PFPA)			X
oxy-chlordane	**X**			Perfluorohexanoate (PFHxA)			X
trans-Nonachlor (t-Nonachlor)	X			Perfluoroheptanoate (PFHpA)			X
cis-Nonachlor (c-Nonachlor)	X			Perfluorooctanoate (PFOA)		X	
o,p'-dichloro-α,α-diphenyl-β,β,β-trichloroethane (*o,p'*-DDT)			X	Perfluorononanoate (PFNA)	X		
p,p'-dichloro-α,α-diphenyl-β,β,β-trichloroethane (*p,p'*-DDT)			X	Perfluorodecanoate (PFDcA)		X	
o,p'-dichlorodiphenyldichloroethane (*o,p'*-DDD)			X	**Perfluoroundecanoate (PFUNA)**	**X**		
p,p'-dichlorodiphenyldichloroethane (*p,p'*-DDD)		X		Perfluorododecanoate (PFDoA)		X	
o,p'-dichlorodiphenyldichloroethylene (*o,p*'-DDE)			X	Perfluorotridecanoate (PFTRA)		X	
***p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE)**	**X**			Perfluorotetradecanoate (PFTeA)			X
Polychlorinated biphenyls							
PCB 28	X						
PCB 52		X					
PCB 99	X						
PCB 101		X					
PCB 105	X						
PCB 118	X						
PCB 138	X						
PCB 153	**X**						
PCB 180	X						
PCB 183	X						
PCB 187	X						
PCB 194		X					

Table B. Model selection in the effects of OCs and PFASs on biomarkers of health, a) Superoxide dismutase (SOD) activity, b) immunoglobulin Y (IgY) and c) telomeres. The predicting variables presented OHCs (ng/g wet weight), wing length and year.

a) SOD

HCB (R^2=0.22)	OHC	Wing	Year	OHC × Wing	OHC × Year	K	AICc	ΔAICc
Mod1	X	X	X	X	X	7	-12.62	3.47
Mod2	X	X	X	X		6	-13.08	3.01
Mod3	X	X	X			5	-11.19	4.90
Mod4	X	X	X		X	6	-15.21	0.88
Mod5	X	X				4	-13.76	2.33
Mod6	X		X			4	-13.59	2.51
Mod7	X					**3**	**-16.09**	**0.00**
Oxy-chl (R^2=0.26)								
Mod1	X	X	X	X	X	7	-9.19	8.47
Mod2	X	X	X	X		6	-11.37	6.29
Mod3	X	X	X			5	-13.01	4.65
Mod4	X	X	X		X	6	-12.33	5.32
Mod5	X	X				4	-15.74	1.91
Mod6	X		X			4	-15.16	2.50
Mod7	X					**3**	**-17.66**	**0.00**
***p,p*'DDE (R^2=0.33)**								
Mod1	X	X	X	X	X	7	-16.19	5.30
Mod2	X	X	X	X		6	-17.88	3.60
Mod3	X	X	X			5	-16.44	5.04
Mod4	X	X	X		X	6	-18.98	2.50
Mod5	X	X				4	-19.17	2.31
Mod6	X		X			4	-18.94	2.55
Mod7	X					**3**	**-21.48**	**0.00**
PCB153 (R^2=0.22)								
Mod1	X	X	X	X	X	7	-12.03	3.92
Mod2	X	X	X	X		6	-13.57	2.38
Mod3	X	X	X			5	-10.91	5.04
Mod4	X	X	X		X	6	-15.15	0.80
Mod5	X	X				4	-13.63	2.32
Mod6	X		X			4	-13.39	2.56
Mod7	X					**3**	**-15.95**	**0.00**
PFOSli (R^2=0.03)								
Mod1	X	X	X	X	X	7	1.99	10.38
Mod2	X	X	X	X		6	-1.05	7.34
Mod3	X	X	X			5	-3.87	4.52
Mod4	X	X	X		X	6	-1.11	7.28
Mod5	X	X				4	-5.92	2.47
Mod6	X		X			4	-6.28	2.11
Mod7	X					**3**	**-8.39**	**0.00**
PFUNA (R^2=0.01)								
Mod1	X	X	X	X	X	7	0.74	8.29
Mod2	X	X	X	X		6	-1.07	6.48
Mod3	X	X	X			5	-3.70	3.85
Mod4	X	X	X		X	6	-1.28	6.27
Mod5	X	X				4	-5.84	1.70
Mod6	X		X			4	-5.28	2.26
Mod7	X					**3**	**-7.55**	**0.00**

b) IgY

HCB (R^2=0.05)	OHC	Wing	Year	OHC × Wing	OHC × Year	K	AICc	ΔAICc
Mod1	X	X	X	X	X	7	-8.93	9.03
Mod2	X	X	X	X		6	-8.85	9.11
Mod3	X	X	X			5	-12.04	5.92
Mod4	X	X	X		X	6	-12.05	5.91
Mod5	X	X				4	-15.20	2.76
Mod6	X		X			4	-15.13	2.83
Mod7	X					**3**	**-17.96**	**0.00**
Oxy-chl (R^2=0.01)								
Mod1	X	X	X	X	X	7	-9.08	7.90
Mod2	X	X	X	X		6	-8.59	8.40
Mod3	X	X	X			5	-11.18	5.81
Mod4	X	X	X		X	6	-12.01	4.97
Mod5	X	X				4	-14.33	2.65
Mod6	X		X			4	-14.13	2.85
Mod7	X					**3**	**-16.98**	**0.00**
***p,p*'DDE (R^2=0.02)**								
Mod1	X	X	X	X	X	7	-7.68	9.44
Mod2	X	X	X	X		6	-8.18	8.94
Mod3	X	X	X			5	-11.25	5.87
Mod4	X	X	X		X	6	-10.50	6.62
Mod5	X	X				4	-14.41	2.71
Mod6	X		X			4	-14.27	2.85
Mod7	X					**3**	**-17.12**	**0.00**
PCB153 (R^2=0.03)								
Mod1	X	X	X	X	X	7	-9.32	8.03
Mod2	X	X	X	X		6	-8.34	9.00
Mod3	X	X	X			5	-11.45	5.90
Mod4	X	X	X		X	6	-10.65	6.69
Mod5	X	X				4	-14.60	2.74
Mod6	X		X			4	-14.50	2.85
Mod7	X					**3**	**-17.34**	**0.00**
PFOSli (R^2=0.002)								
Mod1	X	X	X	X	X	7	-4.42	12.29
Mod2	X	X	X	X		6	-8.31	8.40
Mod3	X	X	X			5	-10.92	5.78
Mod4	X	X	X		X	6	-7.64	9.06
Mod5	X	X				4	-14.01	2.70
Mod6	X		X			4	-13.86	2.85
Mod7	X					**3**	**-16.71**	**0.00**
PFUNA (R^2=0.005)								
Mod1	X	X	X	X	X	7	-4.91	11.88
Mod2	X	X	X	X		6	-8.82	7.97
Mod3	X	X	X			5	-11.16	5.63
Mod4	X	X	X		X	6	-7.80	8.99
Mod5	X	X				4	-14.28	2.51
Mod6	X		X			4	-13.93	2.86
Mod7	X					**3**	**-16.79**	**0.00**

c) Telomeres

HCB (R^2=0.29)	OHC	Wing	Year	OHC × Wing	OHC × Year	K	AICc	ΔAICc
Mod1	X	X	X	X	X	7	40.57	8.63
Mod2	X	X	X	X		6	37.43	5.49
Mod3	X	X	X			5	34.60	2.66
Mod4	X	X	X		X	6	37.51	5.57
Mod5	X	X				4	43.24	11.30
Mod6	X		X			**4**	**31.94**	**0.00**
Mod7	X					3	41.38	9.44
Oxy-chl (R^2=0.31)								
Mod1	X	X	X	X	X	7	39.17	8.19
Mod2	X	X	X	X		6	36.26	5.28
Mod3	X	X	X			5	33.66	2.68
Mod4	X	X	X		X	6	36.58	5.60
Mod5	X	X				4	43.27	12.29
Mod6	X		X			**4**	**30.98**	**0.00**
Mod7	X					3	41.48	10.50
***p,p*'DDE (R^2=0.28)**								
Mod1	X	X	X	X	X	7	40.42	7.91
Mod2	X	X	X	X		6	37.70	5.20
Mod3	X	X	X			5	35.18	2.68
Mod4	X	X	X		X	6	38.11	5.61
Mod5	X	X				4	43.40	10.89
Mod6	X		X			**4**	**32.51**	**0.00**
Mod7	X					3	41.59	9.08
PCB153 (R^2=0.27)								
Mod1	X	X	X	X	X	7	41.50	8.40
Mod2	X	X	X	X		6	38.40	5.30
Mod3	X	X	X			5	35.80	2.70
Mod4	X	X	X		X	6	38.36	5.26
Mod5	X	X				4	43.21	10.11
Mod6	X		X			**4**	**33.10**	**0.00**
Mod7	X					3	41.47	8.37
PFOSli (R^2=0.29)								
Mod1	X	X	X	X	X	7	38.58	6.28
Mod2	X	X	X	X		6	36.82	4.52
Mod3	X	X	X			5	34.74	2.44
Mod4	X	X	X		X	6	35.77	3.47
Mod5	X	X				4	43.15	10.85
Mod6	X		X			**4**	**32.30**	**0.00**
Mod7	X					3	41.01	8.71
PFUNA (R^2=0.26)								
Mod1	X	X	X	X	X	7	42.11	8.73
Mod2	X	X	X	X		6	38.98	5.60
Mod3	X	X	X			5	36.10	2.72
Mod4	X	X	X		X	6	38.98	5.59
Mod5	X	X				4	42.56	9.17
Mod6	X		X			**4**	**33.38**	**0.00**
Mod7	X					3	40.24	6.86

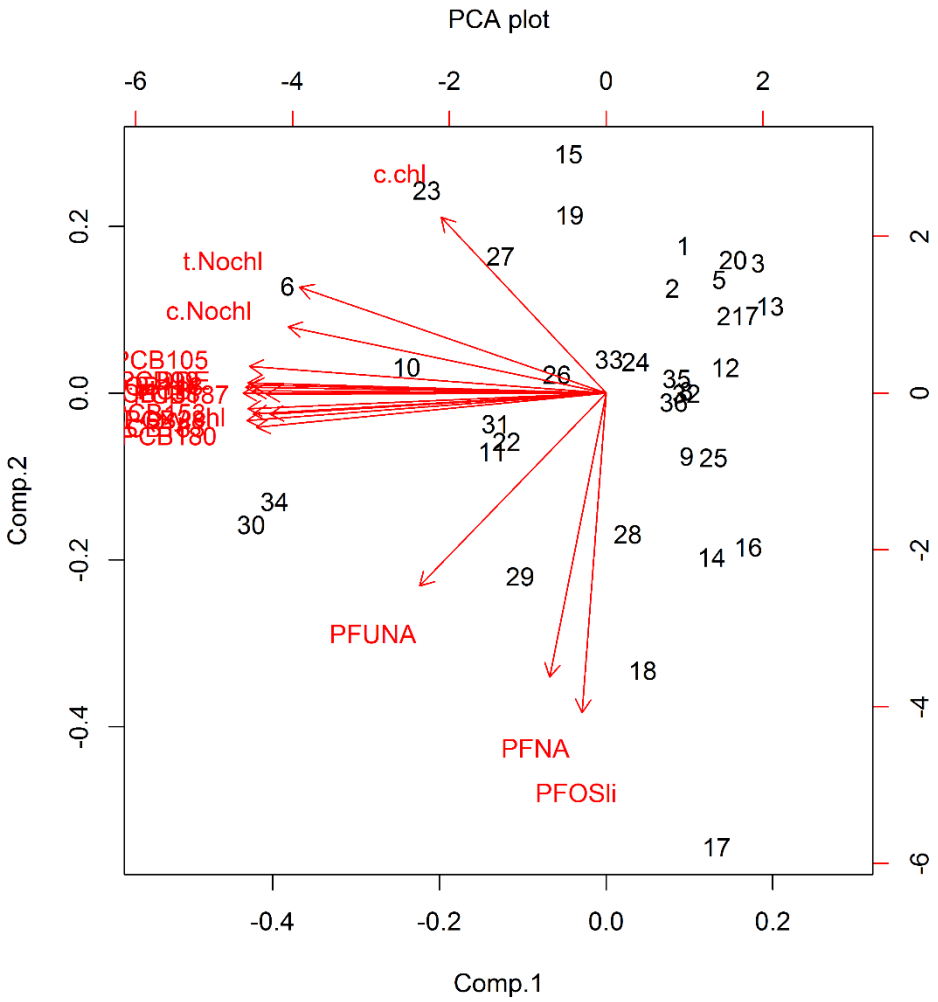

Figure A. A biplot of OHC concentrations in plasma from white-tailed eagle chicks (*Haliaeetus albicilla*) in Troms, Norway. The first and the second principal components (PC1 and PC2) combined explain 82% of the total variation in the data.