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Blood plasma clinical–chemical parameters as biomarker endpoints for organohalogen contaminant exposure in Norwegian raptor nestlings

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ABSTRACT

Raptors are exposed to biomagnifying and toxic organohalogenated compounds (OHCs) such as organochlorines, brominated flame retardants and perfluorinated compounds. To investigate how OHC exposure may affect biochemical pathways we collected blood plasma from Norwegian northern goshawk ($n=56$), golden eagle ($n=12$) and white-tailed eagle ($n=36$) nestlings during three consecutive breeding seasons. We found that blood plasma concentrations of calcium, sodium, creatinine, cholesterol, albumin, total protein, urea, inorganic phosphate, protein:creatinine, urea:-creatinine and uric acid:creatinine ratios and liver enzymes ALKP and ALAT were positively correlated to PCBs, chlordanes, *p,p′*-DDE, HCB, PFCs and/or PBDEs. Total bilirubin and glucose were negatively correlated to PCBs while magnesium and potassium were negatively correlated to HCB and *p,p′*-DDE. In addition, protein:creatinine and ALAT were also negatively correlated to PCBs and PFCs, respectively. The most significant relationships were found for the highly contaminated northern goshawks and white-tailed eagles. The statistical relationships between OHCs and BCCPs indicate that biochemical pathways could be influenced while it is uncertain if such changes have any health effects. The OHC concentrations were below concentrations causing reproductive toxicity in adults of other raptor species but similar to those of concern for endocrine disruption of thyroid hormones in e.g., bald eagles.

1. Introduction

Xenobiotic pollutants, such as organohalogenated compounds (OHCs) are environmental stressors suspected to have various health impacts, including neuro-endocrine/immune disruption and organ toxicity (AMAP 1998, 2004; Chrousos and Gold, 1992; Johnson et al., 1992; Letcher et al., 2010; Sharit and Salvendy, 1982; Sonne, 2010). It has been shown that OHCs have a potential toxic impact on liver and kidney function in wild bird species as well as in humans and laboratory mammals (de le Court et al., 1995; Dieter et al., 1976, 1977; Fischbein, 1985; Hayes et al., 1984; Kutlu et al., 2007; van Wyk et al., 1998).

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Blood clinical–chemical parameters (BCCPs) can be useful as biomarker endpoints for OHC exposure, but are still rarely analysed for in wildlife studies. BCCPs reflect e.g., health and homeostasis of liver (alkaline phosphatase; alanine aminotransferase; bile acid; total bilirubin; albumin; total protein and cholesterol) while other reflect kidney function (urea, protein, uric acid; creatinine; uric acid:creatinine; protein:creatinine) and bone metabolism (alkaline phosphatase; total protein; protein:creatinine, inorganic phosphate and calcium) (de le Court et al., 1995; Thrall et al., 2006; van Wyk et al., 1998). In addition, the energy metabolism is reflected by the total concentrations of proteins, uric acid, glucose, fructosamine and creatinine, and digestion and pancreatic diseases can be evaluated by amylase levels. Furthermore, magnesium, potassium, sodium, urea, uric acid and proteins are important parameters to reflect electrolytic homeostasis and dehydration (Thrall et al., 2006). In conclusion, pollution studies may benefit from the inclusion of such biomarkers in order to shed light on potential health effects.

In northern Norway, several marine bird and fish species have considerable loads of OHCs, which biomagnify in white-tailed eagles (*Haliaeetus albicilla*) preying upon marine organisms (Bustnes et al., 2008; Eulaers et al., 2011; Gjershaug et al., 2008; Helberg et al., 2005; Julshamn et al., 2004). Terrestrial prey species are relatively less exposed to OHCs than those from the marine environment. Therefore, raptors, such as golden eagles (*Aquila chrysaetos*) and northern goshawks (*Accipiter gentilis*), that have specialised on terrestrial food webs are likely to be less exposed to OHCs than marine predatory species (AMAP, 1998, 2004; Gjershaug et al., 2008; Herzke et al., 2002, 2005; Letcher et al., 2010; Sonne, 2010; Sonne et al., 2010).

A pilot study on OHCs and BCCPs was conducted on golden eagle, white-tailed eagle and northern goshawk nestlings in 2008 (Sonne et al., 2010). We suggested that (some) OHCs may impact organ-systems and homeostasis in northern Norway raptor nestlings. In the present paper we extend our investigation of these potential impacts of OHC exposure based on extensive sampling and analysis of BCCPs in nestlings of white-tailed eagles, golden eagles and northern goshawks from Troms and Finnmark counties of northern Norway. The study species and nest locations were chosen in order to have breeding pairs and nestlings that were supposedly high (white-tailed eagles breeding in the coastal environment) and low (northern goshawks and golden eagles in the terrestrial environment) in their OHC body burdens due to their reliance on the respective food webs in their habitat. Thus, we present an extensive investigation in three raptor species of biochemical parameters previously used as biochemical health parameters for OHC-induced perturbations in organ-systems. Doing so, we investigate species-specific differences and year-to-year variations.

2. Materials and methods

2.1. Study design and sampling

The study was conducted on white-tailed eagles ($n=36$) and northern goshawks ($n=56$) from Troms Province, and golden eagles ($n=12$) from Finnmark Province, northern Norway. The study area ranged from N 69° to 71° and from E 18° to 26° (Fig. 1). During year 2008, 2009 and 2010, nests of the three species were checked for breeding activity from late March to the middle of May using binoculars and telescopes, whilst keeping a distance to avoid disturbing the breeding pairs. The presence of at least one bird lying on the nest was used as a confirmation of breeding activity. A large number of nests of all three species were visited and birds with territorial behaviour were recorded. In late April and early May it was determined if the birds had laid eggs. In May – June nestlings in successful nests were inspected ca. 3 weeks after hatching. The nestlings were lowered from the nest in a nylon bag and brachial vein blood plasma (0.1–4.0 mL; heparin-coated syringe) was sampled from a total of 51 different nests. After each sampling day, the blood was centrifuged at 8000 rpm for 10 min, 1 mL supernatant plasma was transferred to a sterile 1.5 mL Eppendorf® tube and frozen at −20 °C until biochemical and contaminant analyses. The study was approved by the National Animal Research Authority of Norway.

2.2. Analyses for BCCPs

The 19 BCCP analyses were conducted at the Central Laboratory at the Department of Small Animal Clinical Sciences (University of Copenhagen, Denmark) and all samples were analysed within six months after sampling. The analyses included albumin (Alb; g L^{-1}), glucose (Glu; mmol L^{-1}), total protein (TP; g L^{-1}), alkaline phosphatase (ALKP; U L^{-1}), alanine aminotransferase (ALAT; U L^{-1}), total bilirubin (TB; μmol L^{-1}), fructosamine (Fructo; μmol L^{-1}), cholesterol (Cho; mmol L^{-1}), creatinine (Cre; μmol L^{-1}), inorganic phosphate (Iph; mmol L^{-1}), bile acids (BA; μmol L^{-1}), amylase (Amy; U L^{-1}), urea (Urea; mmol L^{-1}), gamma glutamyltransferase (GGT; U L^{-1}), calcium (Ca; mmol L^{-1}), magnesium (Mg; mmol L^{-1}), uric acid (UA; U L^{-1}), sodium (Na; mmol L^{-1}) and potassium (K; mmol L^{-1}). When interpreting BCCPs, they are best categorised into three liver enzymes and a liver function test compound (ALKP, ALAT, GGT and bile acid), one digestive enzyme (Amy), two protein groups (Alb; TP), two erythrocyte metabolism waste products (TB; BA), cholesterol, two carbohydrates (Glu; Fructo), one muscle break-down product (Cre), five electrolytes/minerals (Iph, Ca, Mg, Na, K) and two protein waste products (Urea; UA). In addition protein:creatinine, urea:creatinine and uric acid:creatinine ratios were introduced to represent creatinine clearance and reflect glomerular filtration rates (renal functioning). Further details on BCCP analysis and quality control can be found in Sonne et al. (2010).

2.3. Determination of OHCs

Details on OHC determination can be found in Herzke et al. (2005), Götsch et al. (2004) and Sonne et al. (2010). Briefly, blood plasma samples from nestlings were analysed for a set of organochlorines (OCs), including polychlorinated biphenyls (PCBs: CB 18, 28, 99, 101, 105, 118, 138, 153, 180, 183, 187 and 194), *p,p′*-DDE (dichlorodiphenyldichloroethylene), hexachlorobenzene (HCB), chlordanes (CHLs: t-chlordane, c-chlordane, oxy-chlordane, t-nonachlor, c-nonachlor), polybrominated diphenyl ethers (PBDEs: BDE 28, 47, 99, 100, 153 and 154) and perfluorinated compounds (PFCs: PFHpS, PFOS, PFDcS, PFPA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTriA). The compounds were categorised into ∑PCBs, *p,p′*-DDE, HCB, ∑CHLs, ∑PBDEs, ∑PFCs and all concentrations are given in ng mL^{-1} ww (wet weight) (Table 2). Due to low plasma volumes from nestlings in 2009 and 2010 some values are missing and the sample size is reduced. In order to meet QA/QC requirements, ^{13}C-labelled internal standards were used. A blank sample and a standard reference material (NIST 1958, human serum) were analysed for each 10th sample. No blank contamination was detected with the applied methods. The recovery of the ^{13}C-labelled OCs and PFCs varied between 50 and 110%. The results of the analysed SRM fitted within 75 and 110%. For the PFCs a fit of 85% was achieved.

2.4. Statistical analyses

The statistical analyses were performed R version 2.12.1 (R Development Core Team, 2011). Initially, OHC data were log-transformed in order to meet the assumptions of equal variance and homogeneity. Then, all BCCP data sets were tested for normality and homogeneity (equal variance) by Shapiro-Wilk and

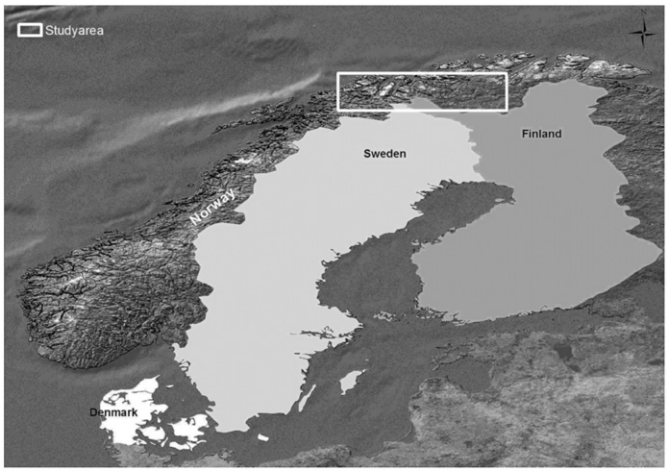

Fig. 1. Map identifying the study area in Troms and Finnmark Provinces of northern Norway.