Ecotoxicology and Environmental Safety 73 (2010) 7–17

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Relationships between organohalogen contaminants and blood plasma clinical–chemical parameters in chicks of three raptor species from Northern Norway

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A R T I C L E I N F O

Article history:
Received 3 April 2009
Received in revised form
25 August 2009
Accepted 29 August 2009
Available online 3 October 2009

Keywords:
ALAT
ALKP
Cholesterol
DDT
GGT
Golden eagle
Goshawk
PCBs
Uric acid
White-tailed eagle

A B S T R A C T

Organohalogen contaminants (OHCs) may affect various physiological parameters in birds including blood chemistry. We therefore examined blood plasma clinical–chemical parameters and OHCs in golden eagle, white-tailed eagle and goshawk chicks from Northern Norway. Correlation analyses on pooled data showed that alkaline phosphatase (ALKP), glucose and creatinine were significantly negatively correlated to various OHCs (all: $p<0.05$; r: -0.43 to -0.55; $n=23$), while alanine aminotransferase (ALAT), total protein, cholesterol, uric acid, total bilirubin, ratios protein:creatinine and uric acid:creatinine were significantly positively correlated to various OHCs (all: $p<0.05$; r: 0.43–0.96). Based on these relationships, we suggest that the OHC concentrations found in certain raptor chicks of Northern Scandinavia may impact blood plasma biochemistry in a way that indicates impacts on liver, kidney, bone, endocrinology and metabolism. In order to elaborate further on these relationships and mechanisms, we recommend that a larger study should take place in the near future.

1. Introduction

Homeostasis is important for all vertebrate species in order to maintain the function of organ systems that are critical for survival and reproduction. Infectious diseases, genetic defects and environmental stressors are all factors that induce perturbations in blood biochemistry and in the homeostasis of vertebrate species in general (Braun, 2003; Harr, 2002; Richards and Proszkowiec-Weglarz, 2007; Schulz et al., 2000). Environmental stressors include extreme sound, temperatures, starvation, dehydration and environmental pollutants, such as organohalogen contaminants (OHCs) and heavy metals, that all have an impact on physiology and behaviour (AMAP, 1998, 2004; Chrousos and Gold, 1992; Johnson et al., 1992; Sharit and Salvendy, 1982). OHCs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and

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0147-6513/$ - see front matter
doi:10.1016/j.ecoenv.2009.08.017

perfluorinated compounds (PFCs). Due to transport mechanisms and chemical and biological properties, these compounds are retained within organisms and tend to biomagnify at the top of marine and terrestrial food webs (Muir et al., 1992; Norstrom and Muir, 1994; Rocca and Mantovani, 2006). OHCs are present in the marine environments of the Northern Hemisphere and are particularly high in industrialised areas, such as the Baltic Sea. However, OHCs are also transported by atmospheric and sea current mechanisms from lower latitudes, such as Eurasia and North America, toward the north (AMAP, 1998, 2004; Colborn, 2004; Macdonald et al., 2003, 2005) and consequently lipid soluble organohalogen pollutants biomagnify in the marine food chains of the Arctic. Top predators, such as polar bears (*Ursus maritimus*) and raptors, accumulate high levels of OHC residues that may have adverse health impacts (AMAP, 1998, 2004).

Raptors including white-tailed eagles (*Haliaeetus albicilla*), golden eagles (*Aquila chrysaetos*) and northern goshawks (*Accipiter gentilis*) are top predators of either marine or terrestrial food webs. As a result, significant OHC concentrations are found in specific birds of prey (Colborn, 1991; Donaldson et al., 1999; Herzke et al., 2002, 2005). For example, white-tailed eagles rely mainly on marine fish-eating seabird species, such as great cormorant (*Phalacrocorax carbo*) and several gull species (*Laridae*) and also feed on a variety of marine fish species of which the majority is picked up from gulls or fishing vessels (Willgohs, 1961). Studies have shown that several marine bird species and fish from Northern Norway are heavily polluted with OHCs (Bustnes et al., 2008; Helberg et al., 2005; Julshamn et al., 2004), which subsequently accumulate in eagles and hawks (Gjershaug et al., 2008). Coastal goshawks and golden eagles can also prey on seabirds, but usually rely on terrestrial food sources. The primary main prey species in Northern Norway include amongst others redwing (*Turdus iliacus*), fieldfare (*Turdus pilaris*), carrion crow (*Corvus corone cornix)*, grouse species (*Lagopus* spp.; *Tetrao* spp.) and small mammals such as mountain hare (*Lepus timidus*) and red squirrels (*Sciurus vulgaris*) (Grønnesby and Nygård, 2000). The golden eagle's diet is dominated by grouse, with mountain hares and reindeer (*Rangifer tarandus*) as important secondary sources, the latter often found as carrion (Johnsen et al., 2007; Halley et al., 2007). Terrestrial prey species are all relatively low in OHCs compared with food from the marine environment (AMAP, 1998, 2004). Therefore, raptors that have specialised on the terrestrial food web are likely to be lower in OHCs compared with the marine predator species (Gjershaug et al., 2008; Herzke et al., 2002, 2005).

The mode of toxic action of OHCs on vertebrate organisms is variable and includes responses of neuro-endocrine pathways such as the hypothalamic–hypophysis–target organ axis and up-regulation of liver cytochrome (CYP) metabolism (Bandiera et al., 1997; Grasman et al., 2000; Henriksen et al., 2000; Kennedy et al., 2003; Letcher et al., 1996; Østby et al., 2005). Briefly, these changes lead to disruptions of specific endogenous peptide and steroid hormones like thyroid hormones T3/T4, estrogens and testosterone with fatal consequences for development, reproductive success and immune competence (Colborn, 2004; Darnerud et al., 2001; Erikstad et al., 2009; Guvenius et al., 2002; Hamers et al., 2006; Harju et al., 2007; Letcher et al., 2000; Verreault et al., 2004, 2006, 2007). A fatal impact on raptor species is a thinning of egg-shells that subsequently results in low reproductive rates due to fragile/porous eggs although the exposure that caused this toxic response seems to have decreased over the last 10 years (Elliott and Martin, 1994; Falk et al., 2006; Helander et al., 2002; Scharenberg and Looft, 2004). Moreover, studies of birds and mammals have shown that OHCs have a toxic impact on liver and kidney function and the general homeostasis measured by blood clinical–chemical parameters (Dieter et al., 1977; Fischbein, 1985; Hayes et al., 1984; Kutlu et al., 2007). Due to the low endocrine plasticity and low homeostatic buffering capacity at the foetal and neonatal stages, these effects are probably most severe at these developmental stages (Damstra et al., 2002; Grandjean and Landrigan, 2006).

To further investigate the potential OHC impacts on the health of raptors, we conducted a study on blood plasma clinical–chemical parameters in golden eagle, white-tailed eagle and goshawk chicks in the Troms and Finnmark counties of Northern Norway (Fig. 1). The study species and nest locations were chosen in order to have breeding pairs and chicks that were supposedly high (white-tailed eagles breeding in the coastal environment) and low (terrestrial goshawks and golden eagles) in their OHC body burdens due to their reliance on the marine versus terrestrial food webs in this area (Cramp and Simmons, 1980). The present study reports on the relationship between blood plasma OHCs and biochemical parameters and thereby the potential health impacts on the chicks of these three raptor species.

2. Materials and methods

The study was conducted on white-tailed eagles and northern goshawks from Troms County, and golden eagles from Finnmark County, Northern Norway, from April to the end of June 2008. The study area ranged from 69° to 71°N and from 18° to 26°E (Fig. 1). 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.

2.1. Study design and sampling

In winter 2008 (January–April), 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 for the *first* time, and a *second* time at beginning of July shortly before fledging, approximately 2 weeks later. Due to variation in hatching date, the first visit took place at a nestling age of between 1 and 3 weeks. The nestlings (2 golden eagles, 16 goshawks and 5 white-tailed eagles) were lowered from the nest in a nylon bag and blood (0.1–4.0 mL) was sampled from the brachial vein during the second visit after which the chicks were returned to their nests. Blood was centrifuged at 8000 rpm for 10 min and 1 mL supernatant plasma was transferred to a sterile 1.5 mL Eppendorf® tube and frozen at −20 °C the same day prior to analyses. The study was approved by the National Animal Research Authority of Norway.

2.2. Analyses of blood plasma clinical–chemical parameters

The analyses were conducted at the Central Clinical Laboratory at the Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen and included the following 19 components: albumin (Alb; g/L), glucose (Glu; mmol/L), total protein (TP; g/L), alkaline phosphatase (ALKP; U/L), alanine aminotransferase (ALAT; U/L), total bilirubin (TB; μmol/L), fructosamine (Fructo; μmol/L), cholesterol (Cho; mmol/L), creatinine (Cre; μmol/L), inorganic phosphate (Iph; mmol/L), bile acids (BA; μmol/L), amylase (Amy; U/L), urea (Urea; mmol/L), gamma glutamyl transferase (GGF; U/L), calcium (Ca; mmol/L), magnesium (Mg; mmol/L), uric acid (UA; U/L), sodium (Na; mmol/L) and potassium (K; mmol/L). The analyses were routinely conducted at the laboratory using an automated spectrophotometrical analyser also containing ion-selective electrodes (ADVIA 1800, Siemens). All assays were subjected to daily internal and quarterly external quality control. Only results from accepted analytical runs are reported here. Information on methods can be found at the Department of Small Animal Clinical Sciences (http://www.life.ku.dk).

2.3. OHC analyses

Details regarding the analyses can be found in Herzke et al. (2005) and Götsch et al. (2004). Briefly, plasma samples from nestlings were analyzed for a set of OCs (organochlorines: PCBs [polychlorinated biphenyls: CB-18, CB-28, CB-99, CB-101, CB-105, CB-118, CB-138, CB-153, CB-180, CB-183, CB-187, CB-194], p,p′-DDE [dichlorodiphenyldichloroethylene], β-HCH [hexachlorohexane], HCB [hexachlorobenzene], heptachlor epoxide, CHLs [chlordanes: t-chlordane, c-chlordane,