Science of the Total Environment 478 (2014) 48–57

Contents lists available at ScienceDirect

Science of the Total Environment

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

Brominated and phosphorus flame retardants in White-tailed Eagle *Haliaeetus albicilla* nestlings: Bioaccumulation and associations with dietary proxies ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$)

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HIGHLIGHTS

- Non-PBDE BFRs and PFRs were highly detected in feathers, but poorly in plasma.
- PFR levels in feathers were up to 100-fold those of BFRs and selected OCs.
- $\delta^{15}N$ and $\delta^{13}C$ explained intra-specific variation in OC, PBDE and PFR exposure.
- $\delta^{15}N$ and $\delta^{34}S$ were respectively enriched and depleted close to an urbanised centre.

ARTICLE INFO

Article history:
Received 28 November 2013
Received in revised form 15 January 2014
Accepted 15 January 2014
Available online 11 February 2014

Keywords:
BFR
Feather
Haliaeetus albicilla
Stable isotope
PFR

ABSTRACT

Very little is known on the exposure of high trophic level species to current-use brominated (BFRs) and phosphorus flame retardants (PFRs), although observations on their persistence, bioaccumulation potential, and toxicity have been made. We investigated the accumulation of BFRs and PFRs, and their associations with dietary proxies ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$), in plasma and feathers of White-tailed Eagle *Haliaeetus albicilla* nestlings from Trøndelag, Norway. In addition to accumulation of a wide range of polybrominated diphenyl ether (PBDE) congeners in both plasma and feathers, all non-PBDE BFRs and PFRs could be measured in feathers, while in plasma only two of six PFRs, i.e. tris-(2-chloroisopropyl) phosphate (TCIPP) and tris-(2,3-dichloropropyl) phosphate (TDCPP) were detected. PFR concentrations in feathers (0.95–3000 ng g^{-1}) were much higher than selected organochlorines (OCs), such as polychlorinated biphenyl 153 (CB 153; 2.3–15 ng g^{-1}) and dichlorodiphenyldichloroethylene (*p,p′*-DDE; 2.3–21 ng g^{-1}), PBDEs (0.03–2.3 ng g^{-1}) and non-PBDE BFRs (0.03–1.5 ng g^{-1}). Non-significant associations of PFR concentrations in feathers with those in plasma ($P \geq 0.74$), and their similarity to reported atmospheric PFR concentrations, may suggest atmospheric PFR deposition on feathers. Most OCs and PBDEs, as well as tris(chloroethyl) phosphate (TCEP), tris(phenyl) phosphate (TPHP) and tri-(2-butoxyethyl) phosphate (TBOEP) were associated to $\delta^{15}N$ and/or $\delta^{13}C$ (all $P \leq 0.02$). Besides $\delta^{15}N$ enrichment, $\delta^{34}S$ was depleted in nestlings from fjords, inherently close to an urbanised centre. As such, both may have been a spatial proxy for anthropogenic disturbance, possible confounding their use as dietary proxy.

1. Introduction

Since the 1960s, flame retardants (FRs) have been incorporated in consumption products in order to inhibit or minimise their inflammation. From an estimated total of 175 produced FRs (Alaee et al., 2003), polybrominated diphenyl ethers (PBDEs) have been studied most intensively. PBDEs were identified to be persistent, bioaccumulative and toxic (Chen and Hale, 2010; de Wit, 2002; Wiseman et al., 2011), and their use has therefore been legally regulated or voluntarily reduced on largely a worldwide scale (Directive EEC, 2003; European Court of Justice, 2008; Renner, 2004; UNEP Stockholm Convention, 2013). In order to comply with international fire regulations (Betts, 2008), Firemaster 550, containing the brominated flame retardants (BFRs) 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-

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http://dx.doi.org/10.1016/j.scitotenv.2014.01.051

ethylhexyl) tetrabromophthalate (BEH-TEBP), was introduced as alternative for the Penta-BDE mixture. At the same time, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) was already produced since the mid-1970s and now also replaces the Octa-BDE mixture (Covaci et al., 2011; de Wit et al., 2010). In addition, certain phosphorus flame retardants (PFRs), e.g. tris(phenyl) phosphate (TPHP), tris-(2-chloroisopropyl) phosphate (TCIPP) and tris-(2,3-dichloropropyl)-phosphate (TDCPP), existing already in complementary use, serve nowadays also as BFR alternatives (van der Veen and de Boer, 2012). Recent studies have suggested bioaccumulative properties for the above listed non-PBDE BFRs (Tomy et al., 2007; Wu et al., 2011) and PFRs (Evenset et al., 2009; Leonards et al., 2011), as well as their persistence and long-range transport (de Wit et al., 2010), and toxic properties (van der Veen and de Boer, 2012). Nonetheless, many research gaps regarding their environmental distribution and ecotoxicity remain still to be filled (Covaci et al., 2011; van der Veen and de Boer, 2012), which is of critical importance to further assess possible environmental risks.

Ever since the observed detrimental effects of certain organochlorines (OCs), among which especially dichlorodiphenyltrichloroethane (DDT) and its metabolites (Ratcliffe, 1970), birds of prey have been successful early warning systems for a variety of environmental pollutants, including flame retardants (Chen and Hale, 2010; Eulaers et al., 2014). Among other high trophic level species, the White-tailed Eagle *Haliaeetus albicilla* has been a promising model species to study spatio-temporal variation in pollutant exposure and associated health effects (Gjershaug et al., 2008; Helander et al., 2002, 2008; Korsman et al., 2012; Nordlöf et al., 2010, 2012; Nygård and Polder, 2012). The detection of a wide range of organohalogenated compounds (OHCs) in nestling White-tailed Eagles (Bustnes et al., 2013; Eulaers et al., 2011a,b) and their adverse impact on nestling health (Sonne et al., 2010, 2012) underline the toxicological relevance of sampling at the nestling stage. In addition, sampling nestlings rather than adults offers methodological advantages, such as small-scale geographical accuracy (Elliott et al., 2009; Eulaers et al., 2013; Olsson et al., 2000) and the minimisation of confounding by age-related metabolism and life-time bioaccumulation, reproductive state and migratory activity (Eulaers et al., 2011a,b; Ramos et al., 2013). Nonetheless, no study thus far has been dedicated to assess nestling exposure to current-use non-PBDE BFRs and PFRs. In fact, avian exposure to these compounds is very poorly investigated in general, with only a few studies presenting non-PBDE BFR or PFR concentrations (Table 1).

While nestling exposure is predominantly studied in blood (Bourgeon et al., 2013; Bustnes et al., 2013; Elliott et al., 2009; Eulaers et al., 2011a,b; Olsson et al., 2000), the use of nestling body feathers is a recent and promising alternative strategy that implies minimal sampling invasiveness while providing an integrated picture of dietary input (Hobson and Clark, 1992) and pollutant exposure (Eulaers et al., 2011b, 2013) over the larger part of the nestling stage. Body feathers are connected to the blood circulation upon feather growth, are metabolically inactive (Jardine et al., 2006), and contain concentrations that are highly associated with internal body burdens (Eulaers et al., 2011b). In addition, since exposure of higher trophic level species is expected to stem primarily from diet ingestion (Ruus et al., 2002), the analysis for stable isotope (SI) ratios of carbon (δ^{13}C: ^{13}C/^{12}C) and nitrogen (δ^{15}N: ^{15}N/^{14}N) has been used successfully. The ratio of heavier ^{15}N to lighter ^{14}N SIs increases systematically throughout the food chain (Jardine et al., 2006; Kelly, 2000) due to the preferential deamination of light amine groups during de- and transamination processes (Macko et al., 1986). In addition, δ^{13}C indicates the origin of a particular food chain because different photosynthesis mechanisms, e.g. C3 versus C4, result in typical δ^{13}C signatures that are largely not altered throughout the food chain (Jardine et al., 2006; Kelly, 2000). As such, δ^{15}N and δ^{13}C have become established proxies to discriminate between trophic levels and food chain origins, respectively, both in intra-specific (Bourgeon et al., 2013; Bustnes et al., 2013; Elliott et al., 2009; Eulaers et al., 2011a,b, 2013; Sørmo et al., 2011) as well as inter-specific investigations (Eulaers et al., 2011b, 2013; Guzzo et al., 2014) of exposure in nestling avian predators. Almost no attention has been paid to the suitability of sulphur SIs (δ^{34}S: ^{34}S/^{32}S) in exposure assessment studies although earlier ecological studies have successfully used δ^{34}S to discriminate between food chain components in nestlings of both marine (Moreno et al., 2010; Ramos et al., 2009) and terrestrial avian predators (Resano et al., 2011; Resano-Mayor et al., 2013). Moreover, recently, δ^{34}S was successfully used to explain trophodynamics of mercury and selenium in nestling Yellow-legged Gulls (Ramos et al., 2013) and as a proxy for the degree of urbanisation to explain OC and PBDE exposure (Morrissey et al., 2013a).

The first objective of the present study was to investigate if White-tailed Eagle nestling body feathers and plasma could be used to quantify exposure to legacy and current-use FRs, and if quantification is influenced by matrix-specific differences in metabolic activity and exposure time-frame. Under this aim, we also investigated the possibility to expand the established range of OHCs, for which feathers predict internal body burdens, to current-use FRs. Secondly, we investigated how dietary proxies (δ^{13}C, δ^{15}N and δ^{34}S) can be most informatively employed to investigate intra-specific variation in pollutant exposure. Finally, throughout the objectives, results for the FRs were benchmarked against two recalcitrant and two more volatile OCs, i.e. polychlorinated biphenyl (CB) 153 and dichlorodiphenyldichloroethylene (*p,p′*-DDE), and CB 28 and hexachlorobenzene (HCB), respectively.

2. Material and methods

2.1. Sampling

As part of a reintroduction programme of White-tailed Eagle into Ireland (Nygård et al., 2010), 21 nestlings from separate nests were captured shortly before their anticipated fledging dates during the breeding season of 2011 in Trøndelag, Norway (Fig. 1). Both sex (8 male and 13 female) and age (6 to 9 weeks of age) were estimated based upon morphometric measurements. The sampled nests were located in three distinct habitat types, i.e. fjord ($n = 3$), island/sound ($n = 8$) and skerry/open coast ($n = 10$) (Fig. 1). Prior to their transport to Ireland, all nestlings were sampled for body feathers and blood. Body feathers, gently pulled from the dorsal region, were pooled per individual and stored in low-density Ziploc bags at ambient temperature. Blood was collected in standard cryotubes through brachial venipuncture with a heparinised syringe. It is accepted that drawing a blood volume equal to 1% of the total body weight does not pose any immediate or long-term health effects on the bird (McGuill and Rowan, 1989). Extrapolated to the bird with the lowest body weight (i.e. 2950 g) it was safe to draw 14.75 mL of blood, whereas we never exceeded the maximum of a standard 10 mL syringe. All blood samples were centrifuged within 12 h of sampling. The resulting plasma was transferred into sterile Eppendorf® tubes and stored at −20 °C. All samplings were conducted ethically in accordance with the Norwegian Animal Research Authority's guidelines (http://www.fdu.no).

2.2. Pollutant analysis

The pollutant concentrations were determined in body feathers and plasma at the Toxicological Centre (University of Antwerp, Belgium), according to a modified method reported by Eulaers et al. (2014). Briefly, individual feathers were thoroughly rinsed with distilled water and dried overnight at ambient temperature. After length measurements of pooled feather samples (mean: 370 mm; range: 240–530 mm), a narrow section of one random feather per individual was cut off for SI analysis (Figure SupInfo-1). The remaining feather matrix was cut in ~1 mm pieces, weighed (mean: 0.1865 g; range: 0.0917–0.2633 g), spiked with internal standards: 500 pg μL^{-1} CB 143; 100 pg μL^{-1} BDE 77; 100 pg μL^{-1} BDE 128; 1 ng μL^{-1} triamyl phosphate (TAP); 1 ng μL^{-1} tris(propyl) phosphate (TPP-d15); 1 ng μL^{-1} tris-(2,3-dichloropropyl)-phosphate (TDCPP-d15); 1 ng μL^{-1} tri-(2-