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Transfer of hexabromocyclododecane flame retardant isomers from captive American kestrel eggs to feathers and their association with thyroid hormones and growth☆

Sarah C. Marteinson [a], Igor Eulaers [b], Veerle L.B. Jaspers [c], Adrian Covaci [d], Marcel Eens [e], Robert J. Letcher [f], Kim J. Fernie [a, *]

[a] Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Science Directorate, Environment and Climate Change Canada, Burlington, Ontario, Canada
[b] Department of Bioscience, Aarhus University, Frederiksborgvej 399, PO Box 358, 4000 Roskilde, Denmark
[c] Environmental Toxicology, Department of Biology, Norwegian University of Science and Technology (NTNU), Høgskoleringen 5, 7491 Trondheim, Norway
[d] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[e] Behavioural Ecology and Ecophysiology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[f] National Wildlife Research Centre, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Science Directorate, Environment and Climate Change Canada, Carleton University, Ottawa, Ontario, Canada

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ABSTRACT

Feathers are useful for monitoring contaminants in wild birds and are increasingly used to determine persistent organic pollutants. However, few studies have been conducted on birds with known exposure levels. We aimed to determine how well nestling feather concentrations reflect *in ovo* exposure to hexabromocyclododecane (α-, β- and γ-HBCDD), and to determine if feather concentrations are related to physiological biomarkers. Captive kestrels (n = 11) were exposed *in ovo* to maternally transferred HBCDD-isomers at concentrations of 127, 12 and 2 ng/g wet weight of α-, β- and γ-HBCDD (measured in sibling eggs), respectively, and compared to controls (n = 6). Nestling growth was monitored at 5 d intervals and circulating thyroid hormone concentrations assessed at d 20. Tail feathers were collected prior to the first molt and analyzed for HBCDD isomers. The mean ΣHBCDD concentration in feathers was 2405 pg/g dry weight (in exposed birds) and α-, β- and γ-HBCDD made up 32%, 13%, and 55%, respectively of the ΣHBCDD concentrations. This isomer distribution deviated from the typical dominance of α-HBCDD reported in vertebrate samples. Exposed chicks had significantly higher feather concentrations of β- and γ-HBCDD compared with controls ($p = 0.007$ and $p = 0.001$ respectively), while α-HBCDD concentrations did not differ between the two groups. Feather concentrations of α-HBCDD were best explained by egg concentrations of β- or γ-HBCDD concentrations ($w_i = 0.50$, 0.30 respectively), while feather concentrations of β- and γ-HBCDD were influenced by growth parameters (rectrix length: $w_i = 0.61$; tibiotarsus length: $w_i = 0.28$). These results suggest that feather α-HBCDD concentrations may reflect internal body burdens, whereas β- and γ-HBCDD may be subject to selective uptake. The α-HBCDD concentrations in the feathers were negatively associated with the ratio of plasma free triiodothyronine to free thyroxine (T_3:T_4; $p = 0.020$), demonstrating for the first time that feather concentrations may be used to model the effect of body burdens on physiological endpoints.

1. Introduction

Birds of prey are valuable as indicators for the impact of organohalogen compounds on wildlife and human health (Rattner, 2009). However, employing them as environmental sentinels is accompanied by ethical and logistic sampling challenges. As feathers grow they are connected to the blood supply, and as a result, contaminants are deposited during this time. Consequently, feathers can act as an archive of contaminant exposure for the individual bird during the time of feather growth (Braune, 1987; Furness et al., 1986; Goede and de Bruin, 1984). Feathers are thus an attractive body compartment for examining and monitoring

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* Corresponding author.
E-mail address: kim.fernie@canada.ca (K.J. Fernie).

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contaminant exposure in raptors for several reasons. Perhaps most importantly, feathers allow for non-destructive sampling, which is useful for wildlife, particularly protected species. They are also easy to collect and transport, can be collected from carcasses, do not have special storage requirements (Burger, 1993), and only one flight feather is required (for larger birds such as raptors (Jaspers et al., 2006a, 2007a)), which reduces many of the logistical restraints of fieldwork.

Feathers have been used to monitor contamination in birds for decades, particularly heavy metals (Braune, 1987; Furness et al., 1986; Goede and de Bruin, 1984). The use of feathers for monitoring anthropogenic persistent organic pollutants (POPs) is more recent, but is also a confirmed strategy that is becoming more widely used. Feathers have been successfully analyzed for several organochlorines (OCs), including polychlorinated biphenyls (PCBs) and other organochlorine pesticides (OCPs) (Jaspers et al., 2006a, 2007a; Van den Steen et al., 2007), and more recent contaminants such as poly- and perfluoroalkyl substances (PFASs) (Jaspers et al., 2013), and the brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs) (Jaspers et al., 2006a, 2007a), hexabromocyclododecane (HBCDD) (Eulaers et al., 2014) and others (Eulaers et al., 2014). For current-use contaminants, which are generally present at considerably lower concentrations than for OCs (e.g. Jaspers et al., 2007a), this is particularly interesting because these small concentrations can still be detected in feather samples.

While the usefulness of feathers for assessing contaminant exposure has been well established, few studies have determined how well feathers reflect and model internal body burdens and environmental exposure concentrations of bioaccumulative contaminants. European starlings (*Sturnus vulgaris*) exposed to CB-153 by silastic implants showed significant correlations between feather concentrations of CB-153 in feathers and other body compartments (muscle, liver, brain, and blood) (Van den Steen et al., 2007). Additionally, a few studies on wild birds have demonstrated that some chlorinated and brominated contaminant levels detected in feathers are related to concentrations in tissues used more frequently for biomonitoring including liver and plasma in adult (Jaspers et al., 2006a,b, 2007a; Van den Steen et al., 2007) and nestling birds (Eulaers et al., 2011a,b, 2014). These studies confirm that feathers can be useful indicators of exposure to POPs, but more research would be beneficial.

Studies reporting how feather concentrations relate to standard physiological biomarkers such as hormone concentrations, as well as reproductive and developmental parameters, are fewer still. Reductions in immune and growth biomarkers have been linked to heavy metal concentrations in feathers of two heron species (Barata et al., 2010; Golden et al., 2003), suggesting that they may be as useful for this purpose as conventionally used tissues. Additionally, feather heavy metal concentrations were positively associated with feather corticosterone concentrations in urban dwelling common blackbirds (*Turdus merula*) (Meillère et al., 2016). However, considerably more research is needed particularly for xenobiotics, and information on the associations between feather contaminant levels and other biomarkers is required. Controlled experiments where contaminant exposure concentrations are known are critical to further our understanding of how POPs are taken up or deposited in bird feathers, and how these concentrations may relate to internal health biomarkers. However, to our knowledge, no studies have yet examined these questions.

HBCDD, as a high-production volume additive BFR, is a xenobiotic contaminant that is detectable in bird feathers (Eulaers et al., 2014). It is globally distributed in the environment and wildlife (reviewed in Covaci et al., 2006), including birds of prey (Eulaers et al., 2014), where relative to all other biota, maximal levels were reported in peregrine falcon (*Falco peregrinus*) eggs in Canada (Guerra et al., 2012). HBCDD demonstrates endocrine disrupting potential *in vitro* since it binds to estrogen, androgen and progesterone receptors causing inhibition, and binds to the thyroid receptor causing potentiation, of the ensuing pathways (Hamers et al., 2006). In captive American kestrels (*Falco sparverius*), *in vivo* exposure to the technical mixture of HBCDD (HBCDD-TM) causes changes in reproduction compared to controls including earlier lay dates and lighter eggs (Fernie et al., 2011), and reductions in courtship and parental behaviors (Marteinson et al., 2012). Compared to controls, adult male kestrels exposed to HBCDD demonstrated increased testicular mass and changes in histology as well as moderate increase in circulating testosterone and reductions in thyroxine, supporting the *in vitro* endocrine disrupting potential of HBCDD (Marteinson et al., 2011).

In the present study, we measured the concentrations of the major α-, β- and γ-HBCDD isomers in feathers of captive American kestrel juveniles exposed *in ovo* through direct maternal transfer from females exposed by diet to environmentally relevant levels of the HBCDD-TM (Fernie et al., 2011). The behavioral, reproductive and physiological effects of exposure to HBCDD have been previously reported for the parents of these birds (Fernie et al., 2011; Marteinson et al., 2011, 2012), as has its uptake, distribution and depletion in adult kestrel tissues (Letcher et al., 2015). Our objectives in the current study were twofold: first to determine how well nestling kestrel feather concentrations reflect *in ovo* exposure concentrations of HBCDD isomers, and second to determine if feather concentrations may be related to the growth and thyroid hormones (which are affected by exposure to HBCDD in kestrels (Marteinson et al., 2011)). To the best of our knowledge, this is the first report of feather concentrations in nestling birds experimentally exposed to any xenobiotic, and the first time feather contaminant concentrations have been examined in conjunction with detailed growth and endocrine data collected during the time of feather growth and contaminant deposition.

2. Materials and methods

2.1. Animal treatment and exposure protocol

Captive American kestrels from McGill University (Montreal, QC, Canada) were used for this experiment. Breeding pairs were housed in separate enclosures and the present study subjects were reared naturally by their parents in an attached nest box. Twenty-one of the initial 30 pairs (N = 20 HBCDD-exposed, 10 control) hatched and raised nestlings: 16 pairs from the HBCDD-exposed group (from which 11 were randomly selected for the present study due to logistical restraints) and 6 from the control group (Fernie et al., 2011). All experimental procedures, protocols and care of the birds followed Canadian Counsel on Animal Care guidelines and were approved by the Animal Care Committee of McGill University.

Throughout the study, parent birds were fed day-old frozen-thawed cockerels (*Gallus domesticus*) under *ad libitum* conditions. For the exposure group, the HBCDD-TM was injected into the cockerel feed daily, immediately prior to feeding. The exact exposure procedures are detailed elsewhere (Fernie et al., 2011; Marteinson et al., 2012). Briefly, the kestrel parents were exposed to 0.52 μg HBCDD-TM/g kestrel/d for the four weeks preceding pairing through until 2 days before the chicks hatched (~75 days). Comparisons were made to control pairs exposed to safflower oil vehicle only. Egg HBCDD concentrations resulting from direct maternal transfer from the above exposure regime were determined for the first-laid egg of each brood: means ± standard error about the means (SEM) were 163.5 ± 75.1 ng/g wet weight (ww) for α-HBCDD, 13.9 ± 5.6 ng/g ww for β-HBCDD/g ww and 2.5 ± 3.8 ng/

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