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Is the bone tissue of ring-billed gulls breeding in a pollution hotspot in the St. Lawrence River, Canada, impacted by halogenated flame retardant exposure?

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HIGHLIGHTS

- We examined bone tissue metabolism and structure in a high flame retardant (FR)-exposed gull.
- Ring-billed gull males accumulated high liver and plasma levels of PBDEs and current use non-PBDE FRs.
- Lower bone mineral density was determined in most FR-contaminated males.
- This study suggests that bone tissue (structural integrity) may be a target for FR exposure in birds.

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ABSTRACT

Bone metabolism is a tightly regulated process that controls bone remodeling and repair in addition to maintaining circulating calcium and phosphate levels. It has been shown that certain organohalogen contaminants may adversely impact bone tissue metabolism and structure in wildlife species. However, exceedingly few studies have addressed the bone-related effects of organohalogen exposure in birds. The objective of the present study was to investigate the associations between markers of bone metabolism and structural integrity, and concentrations of established and current-use halogenated flame retardants (FRs) in ring-billed gulls (*Larus delawarensis*) nesting in a known FR hotspot area in the St. Lawrence River (Montreal, Canada). Bone metabolism was assessed using plasma calcium and inorganic phosphate levels, and alkaline phophatase activity, while bone (tarsus; trabecular and cortical sections) structure quality was examined using the percentage of bone tissue comprised in the total bone volume (Bv/Tv) and bone mineral density (BMD). Bv/Tv and BMD of the tarsus tended (not significant) to be positively associated with circulating calcium levels in male ring-billed gulls. Moreover, concentrations of FRs in male bird liver (brominated diphenyl ether (BDE)-154, -183, -201, and -209) and plasma (BDE-209) were negatively correlated with trabecular and cortical BMD of the tarsus. These correlative associations may suggest light demineralization of bone tissue associated with FR exposure in male ring-billed gulls. Present findings provide some evidence that bone (tarsus) metabolism and mineral composition may be impacted in high FR-exposed (mainly to PBDEs) ring-billed gulls breeding in the highly urbanized Montreal region.

1. Introduction

Halogenated flame retardants (FRs) have been and are still widely being used in consumer and industrial products for their inhibitory effect on the ignition of organic material combustion. Among FRs, the production volumes of polybrominated diphenyl ether (PBDE) additives have been some of the highest during the last three decades. As a consequence, a large suite of PBDE congeners are ubiquitously found in ecosystems worldwide, including the remote Arctic (de Wit et al., 2010; Covaci et al., 2011). A growing number of studies also have reported the bioaccumulation of PBDEs as well as other established FRs (e.g., polybrominated biphenyls (PBBs) and hexabromocyclododecane (HBCD) isomers) in eggs and tissues of avian species from around the globe (Covaci et al., 2006; Chen and Hale, 2010).

The environmental concern on PBDEs has led to the worldwide ban of two commercial mixtures, penta- and octa-BDE, which started in Europe (2004) followed by the U.S. and Canada (2008) (European Parliament, 2003; UNEP, 2010). Deca-BDE (composed

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of >97% of BDE-209), the last PBDE mixture still in use today, is scheduled to be phased out in the U.S. and Canada by the end of 2013 (USEPA, 2009; Environment Canada, 2011). As a result of the strict fire safety legislation requirements, the restrictions on PBDE mixtures have resulted in the increased usage of alternative FRs in commercial and industrial products. Some of these replacement FRs have been commercialized for some time (e.g., Dechlorane Plus (DP)), while others are considered as emerging FRs of potential environmental concern (e.g., bis-(2-etylhexyl)-tetrabromophthalate (BEHTBP), decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromohenoxy)ethane (BTBPE) and 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (TBECH)) (Betts, 2008). A number of these current-use PBDE alternatives are now increasingly being detected in free-ranging avian species (mainly eggs) (Verreault et al., 2007a; Gauthier et al., 2008; Guerra et al., 2011; Chen et al., 2012a, 2012b). Moreover, a recent study conducted by our research group reported high concentrations of PBDE congeners, including BDE-209, and emerging FRs (BEHTBP and *syn*- and *anti*-DP) in liver of ring-billed gulls (*Larus delawarensis*) nesting on one island in the St. Lawrence River (Montreal, Canada)- a known FR hotspot area (Gentes et al., 2012; Chen et al., 2012a). In the study by Gentes et al. (2012), the contribution of BDE-209 represented 26% of $\sum$PBDE concentrations in liver, and surpassed that of BDE-47 and -99, which generally represent the most abundant congeners in fish-eating birds such as gulls (Laridae). Based on these findings, Gentes et al. (2012) suggested that the diet of urban-breeding ring-billed gulls, characterized by high intake of anthropogenic food sources (Caron-Beaudoin et al., 2013), resulted in high exposure to PBDEs and current-use FRs (e.g., BDE-209, BEHTBP, and DP isomers) in sites where these food items are more frequently found (e.g., city center, wastewater treatment plant basins, landfills, etc.).

Potential FR (mainly PBDE) exposure-related biological effects reported in birds include, among others, delayed egg laying, thinner egg shell and altered thyroid hormone levels (Fernie et al., 2005, 2009; Verreault et al., 2007b), competitive interactions with thyroid hormone carrier proteins (Ucán-Marín et al., 2010), and smaller egg volume (van den Steen et al., 2009). However, despite that a few avian studies have addressed the endocrine disruptive properties of PBDEs, there has been to our knowledge no investigation on bone tissue effect in birds exposed to these chemicals and any other environmentally-relevant FRs. Bone metabolism is tightly regulated by hormones (i.e., parathormone, calcitonin, thyroid hormones, and estrogen), vitamins (e.g., D) and other signals (e.g., macrophage colony stimulating factor (M-CSF) and interleukines), and involves two main cell types that control bone formation and resorption (osteoblasts and osteoclasts, respectively) (Roodman, 1999; Duncan Bassett and Williams, 2003; Franceschi et al., 2003; Guyton and Hall, 2006). A number of *in vivo* studies have investigated the impact of organochlorine (e.g., polychlorinated biphenyl (PCB), dichlorodiphenyltrichloroethane (DDT), and dioxin) and PBDE dosage on bone tissue and its metabolism in a few mammalian and amphibian species. For example, effects on bone tissue have been observed in rats dosed with PCBs and dioxins (Alvarez-Lloret et al., 2009; Lind et al., 2009a), in sheep (*Ovis aries*) exposed to compounds present in sewage sludge (e.g., PCBs, PBDEs, alkyl phenols, phthalates) (Lind et al., 2009b), in goats (*Capra hircus*) dosed with PCB congeners (Lundberg et al., 2006), and in common frogs (*Rana temporaria*) dosed with *p,p′*-DDE (Lundberg et al., 2007). In these experimental studies, the commonly observed chemically-induced effects on bone tissue were altered biochemical composition (mineral content), weakened mechanical strength, and lower mineral density (BMD). Corroborative evidence of contaminant-mediated bone alteration has also been reported in wildlife species exposed to organochlorines and PBDEs. More specifically, investigations on grey seals (*Halichoerus grypus*) (Lind et al., 2003), polar bears (*Ursus maritimus*) (Sonne et al., 2004), herring gulls (*Larus argentatus*) (Fox et al., 2008) and alligators (*Alligator mississippiensis*) (Lind et al., 2004) naturally exposed to PCBs, PBDEs, dioxins and pesticides have suggested potential chemically-induced impact on bone tissue, mainly increased or reduced BMD.

The high body burden of FRs, and especially PBDEs, in ring-billed gulls breeding in the Montreal region (Gentes et al., 2012; Chen et al., 2012a) can potentially elicit adverse health effects, including those that have previously been described in PBDE-exposed wildlife species such as bone tissue alteration. However, to our knowledge, no study has investigated the impact of PBDE and current-use non-PBDE FR exposure in any free-ranging bird species. The aim of the present study was to investigate the associations between concentrations of major PBDE congeners, HBCD and two emerging FRs (BEHTBP and DP isomers), and bone structure variables (BMD and bone tissue percentage to total bone volume) as well as biochemical markers of bone metabolism (plasma calcium and inorganic phosphate levels, and alkaline phophatase activity) in ring-billed gulls breeding in the greater Montreal area. Because PBDEs and a number of other organohalogens (mainly organochlorines) were demonstrated to exhibit endocrine disruptive (mainly thyroid and gonadal axes) properties in birds (Sanderson and van den Berg, 2003; McNabb, 2005; Darnerud, 2008), and because bone metabolism is partly controlled by thyroid hormones, estrogens and other hormones (Christenson, 1997; Duncan Bassett and Williams, 2003), we hypothesized that the highly FR-exposed ring-billed gulls exhibit altered bone metabolism and structure (mineral volume and density).

2. Materials and methods

2.1. Sample collection

Sampling of ring-billed gulls (21 males and 6 females) was conducted between April 15th and June 1st, 2010 on Deslauriers Island in the St. Lawrence River, east of Montreal (QC, Canada). This time window corresponds to the incubation period of ring-billed gulls. At the beginning of field season, >200 nests with one egg were randomly selected within the different sections of the colony, and identified with a unique number. Once the nests had complete clutches (i.e., three eggs), one of the partner was randomly live-captured using a nest trap that was triggered from a distance by a radio transmitter. A 5–6 mL blood sample was collected from the brachial vein using a heparinized 10 mL syringe (25 g needle), and immediately transferred into an amber centrifuge tube that was kept in a cooler while in the field. The birds were then euthanized by cervical dislocation for tissue collection. The liver (right lobe) and complete left and right leg bones (femur, tibiotarsus and tarsometatarsus; referred to along the text as femur, tibia and tarsus, respectively) were retrieved and cleaned, that is, all flesh was removed, and stored into the cooler. In the laboratory (within 10 h of sample collection), blood samples were centrifuged (7 min; 2500 $\times g$) and the plasma was stored at -20 °C for further analyses (Sections 2.2 and 2.3). The liver samples were also transferred into a -20 °C freezer until chemical analyses (Section 2.2). Capture and handling methods were approved (permit No. 768) by the Université du Québec à Montréal's Committee on Animal Care (CIPA), and comply with guidelines of the Canadian Council on Animal Care (CCAC).

2.2. Chemical analysis

Sample extraction and cleanup procedures as well as instrument analysis for the determination of a suite of FRs (e.g., 47 PBDE