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# Associations between organohalogen concentrations and transcription of thyroid-related genes in a highly contaminated gull population

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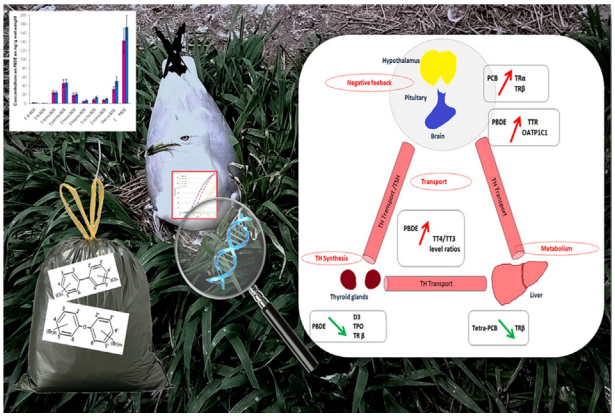
## HIGHLIGHTS

- Gulls from the Montreal area accumulate high liver concentrations of organohalogens
- Associations between organohalogens and variables of the thyroid axis were examined
- Plasma thyroid hormone levels correlated with liver PCBs and PBDEs
- Transcription of many genes in thyroid gland and brain correlated with PCBs/PBDEs
- Compensatory mechanisms of the thyroid axis may have occured in these gulls

## GRAPHICAL ABSTRACT

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## ABSTRACT

A number of studies have reported altered circulating thyroid hormone levels in birds exposed either in controlled settings or in their natural habitat to ubiquitous organohalogen compounds including organochlorines (OCs) and polybrominated diphenyl ether (PBDE) flame retardants. However, limited attention has been paid to underlying homeostatic mechanisms in wild birds such as changes in the expression of genes in the hypothalamic–pituitary–thyroid (HPT) axis. The objective of the present study was to investigate the relationships between hepatic concentrations of major organohalogens (PBDEs and OCs), and circulating thyroid hormone (free and total thyroxine ($T_4$) and triiodothyronine ($T_3$)) levels and transcription of 14 thyroid-related genes in three tissues (thyroid, brain, and liver) of an urban-adapted bird exposed to high organohalogen concentrations in the Montreal area (QC, Canada), the ring-billed gull (*Larus delawarensis*). Positive correlations were found between liver concentrations of several polychlorinated biphenyls (PCBs), PBDEs as well as chlordanes and total plasma $T_4$ levels. Hepatic concentrations of several PBDEs were negatively correlated with mRNA levels of deiodinase type 3, thyroid peroxidase, and thyroid hormone receptor β (*TRβ*) in the thyroid gland. Liver PCB (deca-CB) correlated positively with mRNA levels of sodium–iodide symporter and *TRα*. In brain, concentrations of most PBDEs were positively correlated with mRNA levels of organic anion transporter protein 1C1 and transthyretin, while PCBs positively correlated with expression of *TRα* and *TRβ* as well as deiodinase type 2.

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These multiple correlative linkages suggest that organohalogens operate through several mechanisms (direct or compensatory) involving gene transcription, thus potentially perturbing the HPT axis of this highly organohalogen-contaminated ring-billed gull population.



## 1. Introduction

Among the diverse organohalogen contaminants known to accumulate in wildlife (e.g., polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polybrominated diphenyl ethers (PBDEs)), many possess a chemical structure similar to thyroid hormones and have been associated with thyroid perturbations in birds (reviewed by Dawson, 2000; McNabb and Fox, 2003; McNabb, 2007). The prohormone thyroxine ($T_4$) is synthesized in the thyroid gland and converted primarily into the active triiodothyronine ($T_3$) in peripheral tissues. Perturbations in the hypothalamic–pituitary–thyroid (HPT) axis may have profound consequences as thyroid hormones in birds play a crucial role in the regulation of metabolic rate, thermoregulation, growth, central nervous system development, reproduction, hatching, molt, and behavior such as the onset of migration (Norris, 2007). Evidence for perturbation of thyroid hormone homeostasis in wild birds were the presence of goiter (Moccia et al., 1986) and decreased circulating total $T_4$ ($TT_4$) levels (Fox et al., 2007; McNabb et al., 2001) in adult herring gulls (*Larus argentatus*) exposed to high concentrations of PCBs/OCs in the Great Lakes region. Decreased plasma $TT_4$ levels were also observed in American kestrel (*Falco sparverius*) chicks orally-dosed with a mixture of PBDEs (BDE-47, -99, -100, and -153) (Fernie et al., 2005).

A few studies have investigated potential underlying mechanisms associated with HPT axis perturbations following organohalogen exposure in birds. These include modifications in metabolic activation/inactivation of thyroid hormones by deiodinases (type 1, 2, and 3) and uridine diphosphate glucuronosyltransferase (UDP-GT) (McNabb and Fox, 2003). More specifically, decrease in cerebellum deiodinase type 2 and 3 activity has been observed in chicken embryos following egg injection with PCB-77 (Beck et al., 2006), while chicken embryos exposed to PCB-126 *in ovo* exhibited increased hepatic UDP-GT activity (McCleary, 2001). Another avenue of mechanistic studies involves thyroid hormone transport proteins in blood. PBDEs and/or PCBs as well as their OH- and MeO-containing metabolites were shown to displace thyroid hormones from plasma carrier proteins such as recombinant transthyretin (TTR) and albumin in adult herring gulls (Ucán-Marín et al., 2009; Ucán-Marin et al., 2010).

HPT axis investigations at the genomic level may represent a relevant and sensitive tool to assess organohalogen-mediated effects on thyroid hormone homeostasis. A limited number of in vitro studies of birds have investigated changes in the expression of genes involved in HPT axis regulation in relation to selected organohalogens. For instance, changes in the transcription of genes involved in the first step of thyroid hormone synthesis (e.g., sodium–iodide symporter (*NIS*), thyroglobulin (*TG*), and thyroid peroxidase (*TPO*)) were associated with a decrease in levels of $T_4$ and $T_3$ secreted from chicken thyroid gland explants exposed to PCB-126 (Katarzyńska et al., 2015). Moreover, down-regulation of *TTR* (majorly expressed in the brain) and thyroid hormone responsive gene spot 14-α (involved in fat deposition) was observed in cultured chicken hepatocytes exposed to the Penta-BDE commercial mixture DE-71 for 24 h (Crump et al., 2008a), but no change was observed in the transcription of *TTR* and thyroid hormone receptors alpha and beta (*TRα* and *TRβ*) in adult herring gull neuronal cells exposed to DE-71 for 48 h (Crump et al., 2008b). Furthermore, although transcription of thyroid-related genes can be tissue- and species-specific, in vivo studies using fish have provided valuable insight into the effects of organohalogens on other pathways such as the negative feedback exerted by thyroid hormones on the HPT axis. More specifically, alterations in thyroid hormone signaling of the HPT axis were demonstrated through up-regulation of *TSH* in zebrafish (*Danio rerio*) embryos exposed to low doses of DE-71 during 14 days (Yu et al., 2010), and up-regulation of *TG* and *TRH* as well as down-regulation of *TRα* and *TRβ*, *TSH-R*, *TTR* and *NIS* in zebrafish embryos exposed to the PBDE metabolite 6-OH-BDE-47 at low to high doses from 4 to 120 h post-fertilization (Zheng et al., 2012).

The PBDE commercial mixtures Penta- and Octa-BDE have been listed as persistent organic pollutants (POPs) (Stockholm Convention, UNEP, 2009), while Deca-BDE (consisting of >97% of BDE-209) was phased-out in North America in 2014. A large reservoir of Deca-BDE has built up in North America and has shown the slowest decline in the environment compared to other PBDE mixtures (Salamova and Hites, 2011) with 70,000 tonnes of this mixture that will remain in the use phase by 2020 (Abbasi et al., 2015). As a result, concentrations of BDE-209 and its debrominated congeners (e.g., BDE-207 and -187) were shown to increase in Great Lakes herring gull eggs between 1982 and 2006, whereas BDE-47, -99, and -100 levels plateaued after 2000 (Gauthier et al., 2008). On the other hand, the global ban of PCBs and several OC pesticides (Stockholm Convention, UNEP, 2001) has led to a substantial decrease in their concentrations in free-ranging birds. For instance, in eggs of herring gulls from the Great Lakes, ΣPCB concentrations have continued to decline by 60% between 2002 and 2012 (Fuentes et al., 2014). Despite these actions, high levels of PCBs and other OCs (e.g., chlordanes, mirex, DDTs, hexachlorobenzene, dieldrin, and oxychlordane) as well as flame retardants (e.g., PBDEs) are still being reported in herring gull eggs across the Great Lakes region (Gauthier et al., 2007, 2009; Chen et al., 2012a; Fuentes et al., 2014). Moreover, high tissue and egg concentrations of PCBs/OCs and PBDEs (and a series of emerging flame retardants) have been reported in ring-billed gulls (*Larus delawarensis*) breeding in the St. Lawrence River near the densely-populated city of Montreal (QC, Canada) (Chen et al., 2012a; Gentes et al., 2012; Marteinson et al., 2015). The unusually high plasma concentrations of BDE-209 and its high relative contributions (25%) to ΣPBDE reported in ring-billed gulls have been explained by the time individuals (males in particular) spent in waste management facilities in the Montreal area (Gentes et al., 2015). Moreover, high PBDE (including BDE-209) exposure in male ring-billed gulls from this same breeding colony has been suggested to negatively impact bone (tarsus) mineral density (i.e., bone tissue demineralization) (Plourde Pellerin et al., 2013), which could be linked, among other factors, to thyroid hormone disruption. In this context, ring-billed gulls breeding in the Montreal area may represent a useful model species to investigate the linkages between exposure to ubiquitous organohalogens and disruption of the HPT axis. The objective of the present study was to investigate in Montreal-breeding ring-billed gulls the relationships between hepatic concentrations of major organohalogens (PBDEs, PCBs and other OCs), and circulating thyroid hormone levels and transcription of thyroid-related genes in selected tissues (thyroid gland, brain, and liver).

## 2. Materials and methods

### 2.1. Field sampling

Male ($n = 9$) and female ($n = 12$) ring-billed gulls were randomly live-captured while incubating (i.e., mid- to late-incubation period) using a nest trap in May–June 2011 on Deslauriers Island (45°42′45″N, 73°26′25″W) in the St. Lawrence River, 3.25 km downstream of the city of Montreal. Immediately upon capture, blood samples (8 mL) were obtained using a heparinized 25-gauge needle and 10 mL-syringe,