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Interaction between deca-BDE and hepatic deiodinase in a highly PBDE-exposed bird

Anthony François, Jonathan Verreault*

Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montreal, QC, Canada H3C 3P8

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ABSTRACT

Studies have shown that debromination of the major component in the deca-brominated diphenyl ether mixture (deca-BDE), BDE-209, occurs *in vivo* in birds. Recent work from our laboratory on breeding ring-billed gulls (*Larus delawarensis*) exposed to elevated PBDE concentrations in the densely-populated metropolis of Montreal (Canada) further suggests that BDE-209 debromination is potentially catalyzed by deiodinases in liver microsomes. The first objective of this study was to determine if type 1 deiodinase (D1) was involved in the *in vitro* debromination of BDE-209 in liver microsomes of ring-billed gulls. The second objective was to determine if there was an interaction between D1 and BDE-209 using an *in vitro* D1 activity assay. No depletion of BDE-209 was observed in gull liver microsomes. A significant 42% increase in total D1 activity was found in gull liver microsomes at the medium BDE-209 concentration (1.0 nM), although not at the low (0.5 nM) or high (2.5 nM) concentrations, suggesting potential non-dose related interaction with D1. Moreover, no correlation was found between total D1 activity in liver microsomes and plasma thyroid hormone levels, although there was a negative relationship between plasma BDE-209 concentrations and FT_3 levels. Results from this study suggest that debromination of BDE-209 did not occur using present *in vitro* assay conditions, although indicated potential interaction with D1 that may have implication on circulating thyroid hormone status.

1. Introduction

Halogenated flame retardants (HFRs) have been used for decades to comply with flammability standards. In the late 1970s, technical polybrominated diphenyl ether (PBDE) mixtures were among the main HFRs used because of their low cost and efficiency. However, growing concerns on their persistence, bioaccumulation propensity and toxicity resulted in the addition in 2009 for penta- and octa-BDE mixtures and 2017 for deca-BDE mixture to the Annex A of the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2017). The production and use of deca-BDE was also regulated through the Canadian Environmental Protection Act (Canada Gazette, 2016). However, despite international efforts to regulate deca-BDE, up to 60% of this mixture will remain in the use phase in 2020, thus representing an ongoing source of exposure to its major congener, BDE-209 (> 97% of deca-BDE) (Abbasi et al., 2015). This is also a concern for the known or putative BDE-209 debromination products (e.g., nona- and octa-BDE congeners), which were found to be more persistent, bioaccumulative and toxic to the environment (Eriksson et al., 2002; Söderström et al., 2004).

BDE-209 debromination was shown to occur via enzyme-mediated transformation in fish, birds, and mammals leading to the formation of lower-brominated congeners including mainly octa- and nona-BDEs (Huwe and Smith, 2007; Letcher et al., 2014; Stapleton et al., 2006; Van den Steen et al., 2007; Wang et al., 2011). *In vivo* debromination of BDE-209 was observed in two avian studies. The first study on European starlings (*Sturnus vulgaris*) exposed to BDE-209 via silastic implants showed bioaccumulation of BDE-209 during the first days of exposure, followed by depletion of BDE-209 concentrations associated with increasing concentrations of octa- and nona-BDEs (BDE-196, -197, -206, -207, and -208) (Van den Steen et al., 2007). Another study by Letcher et al. (2014) using American Kestrels (*Falco sparverius*) that were fed day-old cockerels spiked with BDE-209 showed consistent results pertaining to BDE-209 depletion and accumulation of octa- and nona-BDEs (BDE-196, -197, -203, -206, -207, and -208) in plasma and liver, suggesting that *in vivo* debromination of BDE-209 occurred in those avian species. Moreover, studies on common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) (Stapleton et al., 2006) as well as cows (*Bos taurus*) (Kierkegaard et al., 2007) reported debromination of BDE-209 associated with tissue accumulation of nona- to

* Correspondence to: Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montréal, QC, Canada H3C 3P8.
E-mail address: verreault.jonathan@uqam.ca (J. Verreault).

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hexa-BDEs with higher occurrence of meta-substituted congeners in both *in vivo* and *in vitro* studies. Another study conducted on common carp reported that BDE-99 was biotransformed *in vitro* using liver microsomes (Noyes et al., 2010). In this study, the authors observed an inhibition of BDE-99 biotransformation rate following the addition of reverse 3,3′,5′-triiodothyronine (rT_3) and thyroxine (T_4), which are both substrates for deiodinases (Noyes et al., 2010). These authors further reported disruption of type 3 deiodinase (D3) gene transcription, suggesting that an interaction between PBDEs and deiodinases might have taken place.

Deiodinases are a family of selenocysteine-containing enzymes that are associated with the endoplasmic reticulum. The three main deiodinase isoforms (D1, D2, and D3) are involved in the deiodination of thyroid hormones in mainly peripheral tissues where they exhibit different substrate preference and activity. Deiodinases can elicit outer or inner ring deiodination to form triiodothyronine (T_3), rT_3, and 3,3′-diiodothyronine (T_2). In a study investigating the associations between BDE-209 concentrations and deiodinase activity in liver microsomes of a gull species exposed to elevated concentrations of HFRs in the Montreal area (Canada), the ring-billed gull (*Larus delawarensis*), correlations were observed between D1 activity and BDE-209 to octa- and nona-BDE concentration ratios (Francois et al., 2016). As such, gulls that accumulated greater liver concentrations of octa- and/or nona-BDEs relative to BDE-209 exhibited lower D1 activity in liver microsomes. Based on those results, it was suggested that BDE-209 modulated *in vitro* D1 activity in liver microsomes of ring-billed gulls, thus altering homeostasis of circulating thyroid hormones that has previously been reported in this species (Francois et al., 2016; Techer et al., 2016). Another study on ring-billed gulls investigating the impact of HFR exposure on bone tissue reported that PBDE concentrations (including BDE-209) negatively correlated with bone mineral density (Plourde et al., 2013). These authors also suggested that this effect might be indirectly caused by thyroid hormone disruption since these hormones are involved, at least in part, in bone metabolism.

BDE-209 has been reported to be one of the major PBDE congeners determined in liver of ring-billed gulls nesting near the metropolis of Montreal (mean: 57.2 ng/g wet weight), making up between 10% and 20% of Σ_{45}PBDE concentrations (Gentes et al., 2012). Several nona- and octa-BDE congeners (e.g., BDE-196, -197, -201, -202, -203, -204, -207, and -208) were also reported in liver of these ring-billed gulls (Chabot-Giguère et al., 2013; Gentes et al., 2012). Another study on ring-billed gulls from this same breeding colony showed multiple inter-correlations between PCB and PBDE ($\Sigma_5$tetra- to deca-BDE) concentrations in liver and plasma levels of thyroid hormones and mRNA of several genes involved in the control of the hypothalamic pituitary thyroid (HPT) axis (Techer et al., 2016). For example, concentrations of Σ_{34}PBDE and $\Sigma_5$tetra to $\Sigma_3$nona-BDE in ring-billed gull liver were negatively associated with mRNA levels of D3. Techer et al. (2016) concluded that exposure to PCBs and PBDEs could be involved in the disruption of multiple regulatory pathways of the HPT axis (synthesis, transport, metabolism, and action) in the most exposed gulls. Furthermore, in a study of fathead minnow, the organic anion transporter gene OATCP1C1 was up-regulated following BDE-209 exposure (Noyes et al., 2013). These authors suggested that changes in the transcription of OATCP1C1 were suspected to be caused by compensatory mechanisms to regulate the decrease in circulating T_4 and T_3 levels (Noyes et al., 2013). Lower-brominated PBDEs including BDE-47 and −99 have also been associated with thyroid hormone disruption in birds (Fernie et al., 2005). Recent studies suggested that BDE-209 and other higher-brominated congeners originating from BDE-209 debromination could have similar effects (Guigueno and Fernie, 2017).

The first objective of the present study was to determine if D1 was involved in the *in vitro* debromination of BDE-209 in liver microsomes of ring-billed gulls breeding in the densely-populated Montreal area. The second objective was to investigate potential interaction between T_4 and BDE-209 in an *in vitro* D1 activity assay using liver microsomes isolated from this species. We hypothesized that D1 is involved in the debromination of BDE-209 and that BDE-209 dosage decreases *in vitro* D1 activity in liver microsomes of ring-billed gulls in a dose-dependent manner. Ring-billed gulls nesting near Montreal were selected in this study because of their elevated tissue and egg concentrations of PBDEs (Chen et al., 2012; Gentes et al., 2012), their abundance in this area (Giroux et al., 2016) as well as the potentially contaminant-related thyroid toxicity documented in this species (Francois et al., 2016; Techer et al., 2016; Plourde et al., 2013). This study will provide insights onto the possible implications of BDE-209 exposure on deiodinase functioning in a bird living in a highly contaminated environment to HFRs and other contaminants of high environmental concern.

2. Materials and methods

2.1. Sample collection

Fieldwork was carried out in May and June 2013 (n = 8) and 2015 (n = 15) on Deslauriers Island in the St. Lawrence River, 3 km downstream of Montreal (QC, Canada) (Gentes et al., 2012). This colony hosts approximately 44,000 ring-billed gull pairs annually during the breeding season (Giroux et al., 2016). Gulls were randomly selected in all areas of the colony and live-captured while incubating using a nest trap triggered from a distance by a remote control. Only males were included in the present study to remove bias related to sex-specific differences in tissue PBDE concentrations due to maternal transfer, habitat use preference (Gentes et al., 2015), and liver deiodinase activity and expression (Francois et al., 2016; Techer et al., 2016). Blood samples (8 mL) were collected from the brachial vein of all gulls using a heparinized 25-gauge needle and 10 mL syringe within 10 min after capture. Blood samples were kept in amber centrifuge tubes on ice in a cooler while in the field. Birds were then euthanized by cervical dislocation. The left lobe of the liver was collected within 5 min of euthanasia, and aliquots were stored immediately in liquid nitrogen in the field, and transferred to a −80 °C freezer in the laboratory until microsome preparation (Section 2.2.1). In the laboratory, blood samples were centrifuged (2500 × g; 7 min) and the plasma was stored at −80 °C until thyroid hormone (Section 2.3) and PBDE analyses (Section 2.4).

Permission to collect ring-billed gulls was granted by the Canadian Wildlife Service (permit no. SC-23), while capture and handling methods were approved by the Institutional Committee on Animal Care of the Université du Québec à Montréal (permit no. 885), which complied with the guidelines issued by the Canadian Council on Animal Care (Ottawa, ON, Canada).

2.2. D1 activity determination

2.2.1. Hepatic microsome preparation

Ring-billed gull liver microsomes were prepared by differential ultracentrifugation based on previous work (Francois et al., 2016) without modification. Briefly, 500 mg of liver from the left lobe was thawed on ice and homogenized using a Potter-Elvehjem tissue grinder in a phosphate buffered solution (0.1 M KH_2PO_4, 0.1 M Na_2HPO_4; pH 7.4). The homogenized liver was transferred to a cool (approximately 4 °C) polyallomer tube and centrifuged at 9000 × g for 15 min. The supernatant (S9 fraction) was then centrifuged at 100,000 × g for 60 min, and the resulting pellets were re-suspended in a phosphate-sucrose buffered solution (0.1 M KH_2PO_4, 0.1 M Na_2HPO_4 − 0.25 M sucrose; pH 7.0). The final microsomal suspension was aliquoted in 2 mL cryovials that were kept at −80 °C for the BDE-209/T_4 interaction (Section 2.2.2) and BDE-209 biotransformation assays (Section 2.2.3). The hepatic microsomal protein content was determined using the Pierce Modified Lowry Protein Assay Kit (Thermofisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.