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# Halogenated flame retardant exposure pathways in urban-adapted gulls: Are atmospheric routes underestimated?



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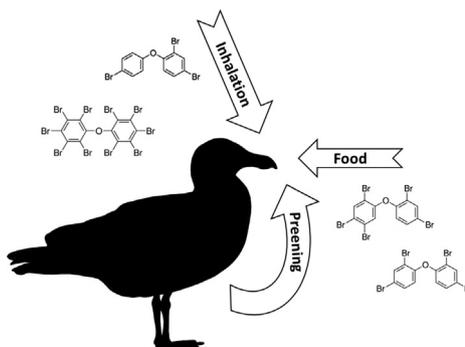
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## HIGHLIGHTS

- We studied several exposure pathways for flame retardants in an urban-adapted gull.
- Levels of BDE-28 in passive air samplers carried by gulls influenced those in lungs.
- Inhalation, feather maintenance (preening), and diet influenced liver PBDE levels.
- Atmospheric exposure to PBDEs should be considered along with diet in urban gulls.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Urban-adapted gulls can be exposed to flame retardants while foraging in landfills where elevated concentrations of polybrominated diphenyl ethers (PBDEs) and other halogenated flame retardants (HFRs) have frequently been measured in air. However, the contribution of atmospheric exposure has largely been overlooked compared to dietary exposure in birds and other wildlife. The overall objective of this study was to investigate the contribution of atmospheric exposure pathways relative to diet for PBDEs and other HFRs in ring-billed gulls (*Larus delawarensis*) nesting in the densely populated Montreal area (QC, Canada). Miniature passive air samplers (PASs) were deployed on the back of wild-caught ring-billed gulls for ten days. Concentrations of PBDEs and other HFRs were determined in PASs carried by ring-billed gulls as well as their lungs, stomach content, liver, preen oil, and onto the surface of their feathers. We evaluated the atmospheric and dietary exposure routes for the most abundant HFRs in samples using a structural equation model implemented in a Bayesian framework. Results indicated that lung concentrations of BDE-28 increased with its levels in air determined using bird-borne PASs. No association was found between BDE-28 concentrations in lungs and liver, whereas BDE-209 concentrations in liver increased with those in lungs. Moreover, BDE-28 and -47 concentrations in liver increased with those on feather surface, while liver BDE-47 concentrations were also positively related with those in stomach content. These findings suggested that, in addition to dietary exposure, atmospheric exposure pathways through inhalation and co-ingestion during feather maintenance (preening) significantly contribute to the accumulation of PBDEs in liver of ring-billed gulls. Atmospheric exposure to HFRs should therefore be considered in future landfill-foraging wildlife species as a potential exposure route compared to the traditional dietary exposure pathway.

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## 1. Introduction

Despite the well-established relationship between air pollution and human exposure, atmospheric contaminant exposure in wildlife, particularly in birds, has received exceedingly limited attention (Smith et al., 2007). Birds may be more susceptible to inhalation of airborne gaseous and particulate-phase contaminants compared to mammals due to their specialized respiratory system (Brown et al., 1997; Sanderfoot and Holloway, 2017). The avian respiratory system is unidirectional and maintains a continuous flow of oxygenated air through the lungs during both inhalation and exhalation, thus improving the efficiency of respiration. Birds possess highly effective parabronchial ventilation compared to mammals while having half the thickness of their pulmonary gas exchange tissues, resulting in a higher uptake of gas from the surrounding air into their bloodstream (Brown et al., 1997). As a result, inhalation represents a direct exposure pathway for airborne pollutants, which can efficiently enter the bloodstream via air capillaries in the parabronchial system. However, little is known about the exposure to pollutants through inhalation in avian species as diet has largely been assumed to be the main exposure route for organic contaminants (Smith et al., 2007).

Among atmospheric pollutants, halogenated flame retardants (HFRs) including the ubiquitous polybrominated diphenyl ethers (PBDEs) have been added to a large array of consumer products (e.g., textiles, upholstered furniture, electrical and electronic equipment, vehicles, and building insulation materials) to limit their flammability. Despite the international ban of all three technical PBDE mixtures due to their high bioaccumulation propensity, environmental persistency and toxicity, these legacy chemicals are still being detected today in the environment and biota worldwide at occasionally elevated levels (Abbasi et al., 2019; Tongue et al., 2019). These semi-volatile organic chemicals (SVOCs) can be released into the air from polymeric materials through volatilization, direct transfer to dust and particles or abrasion, resulting in the formation of small particles (Alaee et al., 2003; Rauert et al., 2015). At their end of life, products containing PBDEs and other HFRs (e.g., pentabromoethylbenzene [PBEB], hexabromobenzene [HBB], and Dechlorane Plus [DP]) enter the waste stream and accumulate in waste management facilities (e.g., landfills). Waste management facilities therefore represent important local hotspots for PBDEs and other HFRs as reported in soil, dust, leachate, and air samples (Cristale et al., 2019; Kerric et al., 2021; Morin et al., 2017; Weinberg et al., 2011).

Large quantities of food wastes are deposited in landfills and attract wildlife, particularly bird species such as corvids and gulls (Belant et al., 1993; Malekian et al., 2021; Patenaude-Monette et al., 2014). During their foraging activities in landfills, birds can be significantly exposed to PBDEs and other HFRs (Gentes et al., 2015; Sorais et al., 2021; Tongue et al., 2019). As such, in pan-Canadian studies on several gull species and European starlings (*Sturnus vulgaris*), the highest concentrations of HFRs were reported in eggs collected from colonies in or nearby landfills compared to those from rural areas (Chen et al., 2012, 2013). Similarly, tissue concentrations of PBDEs in ring-billed gulls (*Larus delawarensis*) breeding in the densely-populated Montreal area (QC, Canada) were greater in individuals spending more time foraging in landfills compared to birds that preferred other habitats to forage such as agricultural fields, residential areas, lakes, or rivers (Gentes et al., 2015; Sorais et al., 2021).

Although HFR accumulation in bird tissues is well documented, studies exploring the role of atmospheric exposure and the resulting tissue accumulation in birds remain scarce. For instance, polycyclic aromatic hydrocarbon concentrations (i.e., C2-naphthalenes and C1-fluorenes) in muscle tissues of tree swallows (*Tachycineta bicolor*) were found to increase with concentrations in air collected within the colony site in Canada's Athabasca oil sands region (Ferne et al., 2018). Moreover, using GPS dataloggers combined with miniature passive air samplers (PASs) attached to free-ranging ring-billed gulls from the Montreal area, atmospheric exposure to PBDEs, especially BDE-209, was found to be highest in individuals foraging in or nearby landfills (Sorais et al., 2020). In a follow up study, liver PBDE concentrations in these same ring-billed gulls were shown to increase with their presence probability in landfills (Sorais et al., 2021). Collectively,

these findings suggest that the elevated liver concentrations of BDE-209 in Montreal-breeding ring-billed gulls may be partly explained by atmospheric exposure, through inhalation and ingestion during feather maintenance (preening) of airborne HFRs that are deposited onto their feathers. Exposure of gulls and other birds to HFRs may occur through multiple routes including ingestion (food, water, and feather maintenance), inhalation (air), and dermal exposure (air and water). However, determining the relative importance of single or combined exposure pathways in wild birds remains a daunting task, especially for omnivorous species foraging in heterogeneous habitats. To our knowledge, there is no information on the relative importance of these pathways and how they are related in an individual's total HFR exposure for any free-ranging bird or mammal species.

The objective of this study was to investigate the relative contributions of different exposure pathways, namely ingestion (stomach content and feather surface extract samples) and inhalation (air samples), for PBDEs and other HFRs (e.g., DP, PBEB, and HBB) in tissues (liver, lung, and preen oil samples) of ring-billed gulls nesting in the highly urbanized Montreal area. Because ring-billed gulls can be exposed to HFRs via air while foraging in landfills (Gentes et al., 2015; Kerric et al., 2021; Sorais et al., 2021), we hypothesized that gulls are exposed predominantly to HFRs via inhalation (e.g., more volatile PBDEs) and ingestion of HFR-laden particles (less volatile PBDEs) sorbed onto their feathers. Therefore, we further predicted that inhalation and feather maintenance primarily contribute to HFR concentrations in gull liver and lungs. This study is the first to assess the comparative role of different exposure pathways for airborne pollutants in wild birds, thus improving exposure risk assessments for birds and other wildlife species using highly urbanized environments for breeding and/or foraging.

## 2. Materials and methods

### 2.1. Study area and sample collection

Breeding ring-billed gulls (17 males and 9 females) were sampled from May through June 2018 on Deslauriers Island (45.717°N, 73.433°W) in the St. Lawrence River, east of Montreal (QC, Canada). Deslauriers Island supports one of the largest ring-billed gull colonies in North America, where approximately 65,000 individuals breed annually (2016; Canadian Wildlife Service, unpublished data). Once clutches were completed (i.e., three eggs laid), birds were randomly selected and captured while incubating their eggs (one individual per nest) using a radio-controlled noose trap or a dip net. Miniature PASs that collected both gas- and particle-phase HFRs were attached on the middle of the bird's back to avoid compromising its mobility using a protective neoprene patch and customized harness made of Teflon (Bally Ribbon Mills, Bally, PA, USA; Sorais et al., 2017) (Fig. S1). The same birds were also equipped with a GPS datalogger (AxyTrek, TechnoSmArt, Guidonia, Rome, Italy) affixed at the base of central tail feathers using waterproof tape (TESA, Charlotte, NC, USA). GPS data were collected for a companion study (Kerric et al., 2021) and are not presented herein. The total weight of equipment carried by ring-billed gulls including the GPS datalogger, PAS and harness was approximately 14 g, which represented  $3.1 \pm 0.4 \%$  (mean  $\pm$  SD) of their body mass ( $463 \pm 51$  g; mean  $\pm$  SD).

Gulls were recaptured after approximately 10 days ( $10.5 \pm 2.1$  days; mean  $\pm$  SD) using the same capture methods as described above to retrieve the GPS dataloggers and PASs. Gulls were then euthanized by cervical dislocation and their lungs, liver, proventriculus, and gizzard were collected immediately. Gull tissues and PASs were wrapped in individual pre-cleaned (i.e., double-rinsed with acetone and hexane) aluminum foil and placed in hermetic bags (Reloc Zippit, Lima, OH, USA). Preen oil was collected directly from the uropygial gland using a pre-cleaned scalpel and tweezer and transferred into a pre-cleaned cryotube. Care was taken to ensure that no feathers or other tissues came in contact with preen oil to prevent cross-contamination. The sex of the birds was confirmed by gonad examination. The bird carcasses were then placed in separate hermetic

bags for subsequent feather collection in the laboratory (see below). All capture and handling procedures were approved by the Institutional Committee on Animal Care (CIPA) of the Université du Québec à Montréal (permit no. 885), which complies with guidelines outlined by the Canadian Council on Animal Care (Ottawa, ON, Canada).

All PASs and tissue samples were kept in a cooler while in the field. In the laboratory, sorbent materials (i.e., polyurethane foam [PUF]) were retrieved from all PAS housings, wrapped in individual pre-cleaned aluminum foil, and stored in hermetic bags. PAS sorbents and gull tissues were kept at  $-30\text{ }^{\circ}\text{C}$  until chemical analysis (Section 2.2). The proventriculus and gizzard of each gull were thawed for 1 h at room temperature and their entire content was collected. It was then homogenized using a pre-cleaned mortar, transferred into pre-cleaned glass tube, and stored in the refrigerator during approximately 15 days until chemical analysis (Section 2.2). Finally, the entire plumage from each gull carcass was collected by cutting feathers at the base of the skin. Feathers were weighed and immersed for 3 min in a pre-cleaned beaker filled with 200 mL of acetone followed by 3-min of sonication. This washing procedure was performed three times. The feather surface extracts were combined, concentrated to 2 mL using a rotary evaporator (Rotavapor R-215, Büchi Labortechnik AG, Flawil, Switzerland) set at  $40\text{ }^{\circ}\text{C}$ , and transferred into individual pre-cleaned (acetone and hexane) glass tubes. The feather surface extract (including two hexane rinses) was concentrated to 1 mL using a gentle nitrogen flow (N-EVAP 111, Organomation Associates, Berlin, MA, USA), and stored in the refrigerator until chemical analysis (Section 2.2).

## 2.2. Chemical analysis

A suite of 35 PBDE congeners and 15 other HFRs (Tables S1 and S2) were analyzed in PAS sorbents as part of a companion study using the same ring-billed gull individuals (Kerric et al., 2021). The same HFRs were analyzed in these birds' preen oil, lungs, liver, stomach content, and feather surface extract following methods by Sorais et al. (2021) with minor modifications. Briefly, between 0.05 and 1.05 g of sample matrix as well as feather surface extract were homogenized with diatomaceous earth (J.T. Baker, Phillipsburg, NJ, USA), spiked with an internal standard solution (BDE-30, BDE-156,  $^{13}\text{C}$ -BDE-209, and  $^{13}\text{C}$ -*syn*-DP; Wellington Laboratories, Guelph, ON, Canada), and extracted using a pressurized liquid extraction system (Fluid Management Systems, Billerica, MA, USA) using dichloromethane and *n*-hexanes (1:1, volume ratio). Sample extracts were further cleaned-up using an acid-basic-neutral silica column followed by a neutral alumina column (Fluid Management Systems). Identification and quantification of HFRs were conducted using a gas chromatograph (GC) coupled to a single quadrupole mass spectrometer (MS) (Agilent Technologies 7890B GC-5975C MS, Palo Alto, CA, USA) operating in electron capture negative ionization (ECNI) mode. Separation of target analytes was achieved on a DB-5 HT capillary column ( $15\text{ m} \times 0.25\text{ mm i.d.} \times 0.10\text{ }\mu\text{m}$  film thickness; J & W Scientific, Brockville, ON, Canada).

Several measures were taken for quality assurance and control including internal standard recovery and analysis of standard reference materials (SRM 1947; Lake Michigan fish tissue, National Institute of Standards and Technology, Gaithersburg, MD, USA) and procedural blanks. The mean ( $\pm$  SD) recoveries of internal standards in samples were as follows: BDE-30 ( $91.7 \pm 10.9\%$ ), BDE-156 ( $89.7 \pm 11.9\%$ ),  $^{13}\text{C}$ -BDE-209 ( $62.5 \pm 15.3\%$ ), and  $^{13}\text{C}$ -*syn*-DP ( $87.2 \pm 11.7\%$ ). Concentrations of the five PBDE congeners in SRM 1947 samples ( $n = 8$ ) showed an average variation of 9 % from the certified values. PAS field blanks ( $n = 2$ ) were also added and consisted of PUF disks originating from the same lots as those deployed in the field that were transported to the field, taken out of their hermetic bags, and processed similarly as the other PASs. Trace HFR levels were found in both procedural (i.e., BDE-47) and PAS field blanks (i.e., BDE-209 and -47, and *anti*-DP), although these were negligible compared to analyte contributions originating from the samples (Tables S1 and S2), and hence no blank correction was applied. Method limits of detection (MLODs; defined as signal to noise ratio  $S/N = 3$ ) and method limits of quantification (MLOQs; minimum amount of analyte producing a peak

with  $S/N = 10$ ) were based on replicate analyses ( $n = 8$ ) of matrix samples spiked at a concentration of 3–5 times the estimated detection limit (Tables S1 and S2).

Median concentrations and standard deviations (SD) of a given compound were calculated to summarize these variables: PASs, preen oil, lungs, liver, stomach content, and feather surface extract (Tables S3 and S4). Concentrations in gull tissues and feather surface extract were reported in ng/g wet weight (ww) and in ng/g, respectively. Concentrations ( $\text{pg}/\text{m}^3$ ) of HFRs in PAS sorbents were calculated using air sampling volumes estimated from the model published online by Harner (2017) and described in details in Kerric et al. (2021). All input parameters used in this model are provided in Table S5.

## 2.3. Bird exposure pathway analysis

We developed a theoretical exposure pathway model for HFR exposure based on a naturally exposed population of ring-billed gulls to HFRs through ingestion and inhalation (Fig. 1). The model was constructed based on the following premises: 1) gulls can inhale HFRs directly from ambient air (Brown et al., 1997); 2) HFRs in ambient air can be deposited onto gull feathers (Eulaers et al., 2011; Jaspers et al., 2008); and 3) exposure to HFRs through inhalation (air) or ingestion (stomach content and feather maintenance) can result in liver accumulation in gulls (Sorais et al., 2021). Factors that may act upon these pathways included air volume passing through the PAS, HFR concentrations in preen oil deposited onto the feathers as well as whole feather weight. Some relationships could not be considered in the structural equation model analysis (Section 2.4.) due to restrictions on the total number of parameters that could be included given our small sample size. However, these relationships (Fig. 1) would be relevant to explore in future studies. For instance, atmospheric HFRs sorbed onto feathers or preen oil co-ingested during preening may enter the proventriculus and gizzard, and HFR concentrations in preen oil may be a function of internal concentrations in liver.

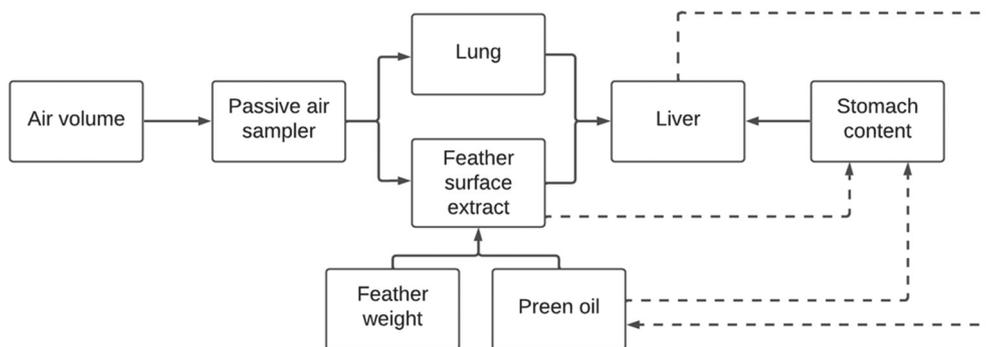
## 2.4. Statistical analysis

To quantify the different exposure pathways in gulls, we developed a single structural equation model that encompassed all parts of the theoretical models for a given HFR compound (Fig. 1; Grace et al., 2010; Lee, 2007). Specifically, this structural equation model enables the simultaneous estimation of all relationships between groups of response variables and explanatory variables for a given HFR compound, where response variables in one part of the model can also act as explanatory variables for another model component (Grace et al., 2010; Shipley, 2004). HFRs were included in the analysis only when their concentrations in at least 50 % of the samples were greater than the compound-specific MLOQ in each PAS, preen oil, stomach content, lung, and liver samples as well as feather surface extract. Following this criterion, only four PBDE congeners (BDE-28, -47, -99, and -209) could be used in the structural equation models. Each component  $i$  (e.g., PAS, feather, lung, or liver) of a given HFR for bird  $j$  was modeled using a log-normal distribution:

$$\text{HFR}_{ij} \sim \log\text{N}(\mu_{ij}, \sigma_i^2)$$

$$\mu_{ij} = X_{ij}\beta_i$$

where  $\mu_{ij}$  denotes the log concentration of component  $i$  of a given HFR for bird  $j$ , and where  $\sigma_i^2$  corresponds to the residual variance (on a log scale) associated with component  $i$ . The regression equation for each component  $i$  is given by the product of the design matrix  $X_{ij}$  and a vector of beta estimates  $\beta_i$  of the relationship between component  $i$  and the explanatory variables. The design matrix  $X_{ij}$  consists of columns including an intercept and the explanatory variables for component  $i$ . Explanatory variables for each component  $i$  are shown in Fig. 1. The  $\beta_i$  estimates for each model component reflect the relationship between one explanatory variable and a response



**Fig. 1.** Theoretical model of exposure pathways through inhalation (air) and ingestion (stomach content and feather maintenance) for wild birds exposed to HFRs. Boxes at the base of arrows denote explanatory variables that potentially influence the response variable at the end point of the arrow. For example, air volume is a potential predictor of the concentrations of a given HFR compound collected in PAS, and the latter is also a potential predictor of the concentrations in lungs and feather surface extract. Dashed arrows indicate potential relationships that could not be examined in the current structural equation model, but that could be investigated in future studies.

variable along the pathway, with the sign of the coefficient indicating the direction of the relationship.

We adopted a Bayesian approach to estimate the parameters of the structural equation model due to our small sample size (Lee, 2007; Lee and Song, 2004). Moreover, the Bayesian approach allowed incorporating censored values (i.e., concentrations < MLODs or < MLOQs). In our case, censored values provide partial information in the form of an interval, where the true values of these observations were between 0 and MLOQ. Instead of substituting censored values with 0 (zero) leading to biased estimators (Helsel, 2006; Hites, 2019), our Bayesian model used data imputation to include censored values as an additional component in the analysis. Specifically, censored observations were incorporated in the model in JAGS with the *dinterval()* function designed specifically to handle censored data (Lunn et al., 2013; Plummer, 2017). We imposed vague priors for all model parameters using a normal distribution  $N(0, \sigma^2 = 1000)$  for  $\beta$  estimates and uniform  $U(0,300)$  prior distributions for residual standard deviations. We used vague priors that did not favor particular values because there was limited information available in the literature on the relationships between the variables under investigation. We ran the model for each HFR compound with five chains based on Markov chain Monte Carlo (MCMC; Gelman et al., 2014a, 2014b). Each chain consisted of 75,000 iterations, using 40,000 iterations as a burn-in period, and a thinning rate of 5. We estimated model parameters using JAGS 4.3.0 (Lunn et al., 2013). The code to implement our model in JAGS is presented in Table S6. We assessed the convergence of MCMC chains with trace plots, posterior density plots, and the Brooks-Gelman-Rubin statistic. We used residuals to check model diagnostics. Model fit was evaluated with posterior predictive checks (Gelman and Hill, 2007; Levy, 2011).

### 3. Results

#### 3.1. Profiles of HFRs

The greatest number of PBDE congeners was detected in feather surface extract (21), followed by liver (19) and lungs (17) of ring-billed gulls, whereas only five PBDEs were detected in stomach content. Among these, BDE-28, -47, -99 and -209 were quantified in most of gull PAS (97 %), preen oil (93 %), stomach content (83 %), lung (100 %), and liver (100 %) samples as well as feather surface extract (100 %). Therefore, only these four PBDE congeners were investigated in structural equation models (Section 3.2.).

PBDE profiles were generally dominated by BDE-209, followed by BDE-47 and -99, except for stomach content and preen oil. As such, BDE-47 (301 ng/g ww) and -99 (274 ng/g ww) concentrations in preen oil were 14- and 13-fold higher than those of BDE-209, respectively, which had median concentration of 21.9 ng/g ww. BDE-47, -209 and -99 in stomach content were found at low levels (0.32–0.37 ng/g ww). Considering only the biological samples, the highest concentrations of BDE-209 occurred in feather surface

extract, with median concentrations (73.7 ng/g) up to 223 times higher than in stomach content (0.33 ng/g ww). In PAS, median BDE-209 concentrations (38 pg/m<sup>3</sup>) were 6–65-fold higher than other PBDE congeners (5.8–6.6 pg/m<sup>3</sup>) (Table S3).

Among other HFRs, only five compounds (Cplus, *anti*-DP, *syn*-DP, HBB, and PBEB) were detected in gull PAS, preen oil, stomach content, lung, and liver samples as well as in feather surface extract. However, none of these HFRs were measured in the majority of PASs and biological samples. We detected four compounds (*anti*-DP, *syn*-DP, HBB, and PBEB) in feather surface extract as well as in liver, while only HBB was measured in stomach content. Therefore, these HFRs were not used in structural equation model analyses (Section 3.2.). The highest concentrations of *anti*-DP (<MLOQ-19.5 ng/g) and HBB (<MLOQ-1.6 ng/g) were found in feather surface extract. In PASs, median concentrations of *anti*-DP (1.1 pg/m<sup>3</sup>) were three times higher than those of HBB (0.42 pg/m<sup>3</sup>) and PBEB (0.43 pg/m<sup>3</sup>) (Table S4).

#### 3.2. Exposure pathways for PBDEs

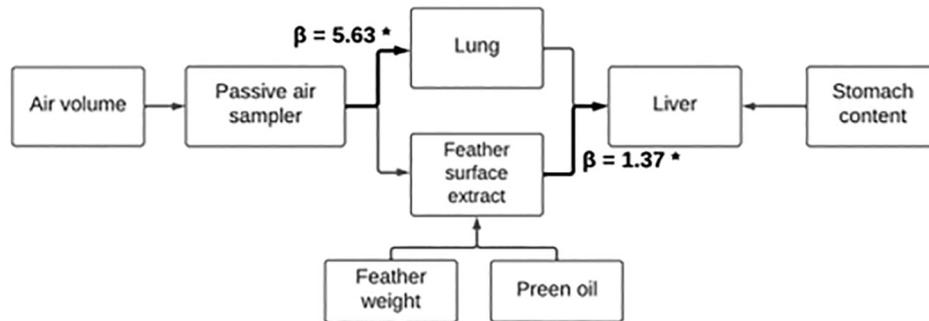
Model diagnostics suggested that the length of chains was sufficient for inferences with Brooks-Gelman-Rubin statistic <1.1, all ratios of MCMC error to posterior standard deviation <5 %, and trace plots suggesting that chains stabilized to similar values (Lunn et al., 2013). Residual diagnostics did not suggest departure from model assumptions of homoscedasticity and log-normality. The results obtained from the structural equation model analysis for each HFR compound are presented in Table S7 as well as in Fig. 2 in which the regression slopes that differed from 0 are written above arrows linking a response and explanatory variable. Thus, arrows without values of regression slopes correspond to the absence of an association between two variables. Concentrations of BDE-28 in lungs increased with those determined in PASs, but we found no such relationship for the other PBDE congeners. None of the PBDE congener concentrations determined in feather surface extract varied with air levels measured using PASs. However, the concentrations of BDE-28 and -47 in liver increased with those in feather surface extract. Nonetheless, feather surface extract concentrations of BDE-47 and -99 increased with those in preen oil. In addition, liver concentrations of BDE-209 and -47 increased with those in lungs and stomach content, respectively. Also, BDE-209 concentrations measured in PASs decreased with increasing air volume passing through the PAS (Fig. 2 and Table S7).

### 4. Discussion

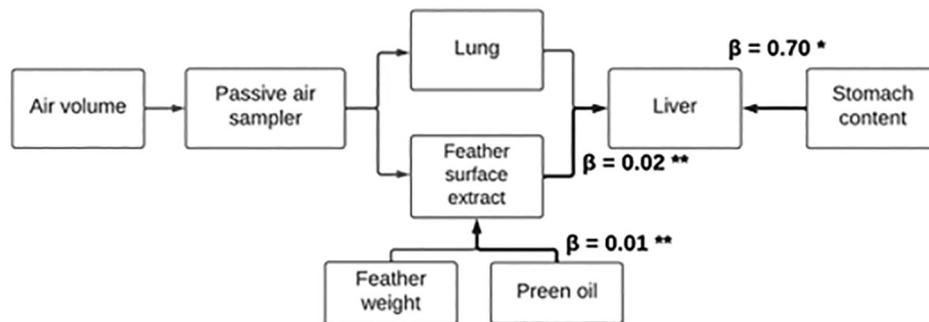
#### 4.1. Exposure of HFRs through inhalation in birds

Lung concentrations of BDE-28 increased with those in air collected in PASs carried by breeding ring-billed gulls for 10 days in the Montreal area. This highly volatile tri-BDE congener is mainly found in the gas

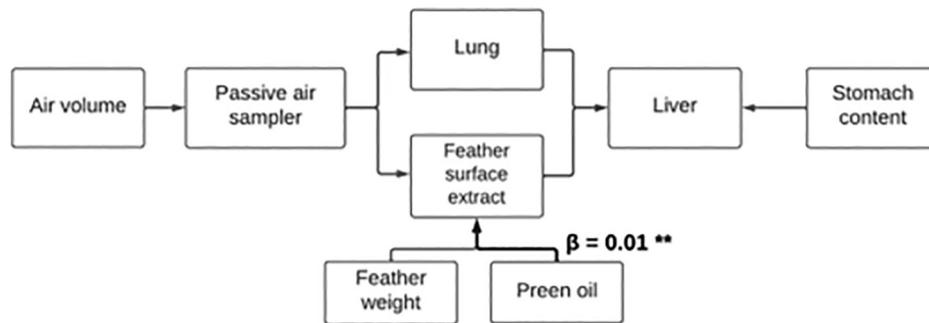
## BDE-28



## BDE-47



## BDE-99



## BDE-209

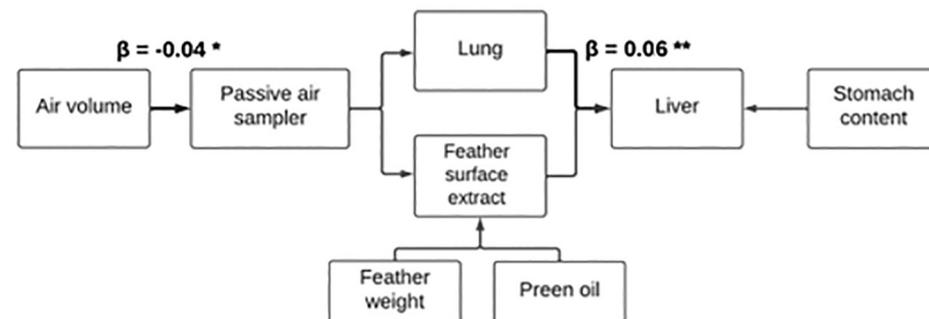


Fig. 2. Exposure pathways inferred from structural equation model path diagram for BDE-28, -47, -99, and -209 in ring-billed gulls breeding in the Montreal area (QC, Canada). Values above the bold arrows represent the regression coefficients ( $\beta$ ) on a log scale linking a response and explanatory variable that differed from 0. Estimates with one asterisk (\*) indicate 90 % credible interval (CI) excluding 0, while estimates with two asterisks (\*\*) denote 95 % CI excluding 0.

phase of air (Chen et al., 2006; de la Torre et al., 2018; He et al., 2014). Specifically, among 11 PBDE congeners detected in outdoor air of Guangzhou (Guangdong, China), Chen et al. (2006) reported that BDE-28 almost exclusively occurred (>96 %) in the gas phase. The respiratory system of birds yields a high unidirectional airflow with a highly efficient cross-flow-mediated gas exchange to meet the high oxygen demand of bird flight. Moreover, to enhance gas uptake in the bloodstream, pulmonary gas exchange tissues in birds are thinner than those of mammals (Brown et al., 1997; Scheid and Piiper, 1972). As a result, contaminants present in air can be directly transferred to the blood of birds via air capillaries, making birds particularly vulnerable to airborne contaminant exposure. To our knowledge, no study has been conducted on the atmospheric exposure of birds to PBDEs. Nevertheless, exposure to SVOCs other than PBDEs through inhalation in birds was found to increase their stress response and detoxification efforts in controlled laboratory settings (Cruz-Martinez et al., 2015; Fernie et al., 2016). For instance, inhalation of SVOCs such as benzene in American kestrels (*Falco sparverius*) elicited an induction of liver 7-ethoxyresorufin-O-deethylase (EROD) activity as well as increased plasma corticosterone levels (Cruz-Martinez et al., 2015). In addition, an *in vivo* study on human exposure to gas-phase diethyl phthalate (DEP) and particle-phase phthalates (e.g., di(2-ethylhexyl)phthalate [DEHP]) reported that average uptake via inhalation of DEP was four times higher than that of DEHP (Andersen et al., 2018). This study suggested that SVOCs in the gas phase of air are more likely to enter the bloodstream through alveoli in human lungs compared to compounds mostly adsorbed onto aerosol particles or dust. These authors further suggested that phthalates deposited in the alveolar region of lungs directly enter the bloodstream, while those deposited in the tracheobronchial and extra thoracic regions through clearance processes move up in the airways before being swallowed and ingested. Despite their different chemical structures, both phthalates and PBDEs are SVOCs. Thus, the absorption of the predominantly gas-phase BDE-28 in ring-billed gulls may occur through inhalation by entering the bloodstream through air capillaries in the lungs.

We were unable to establish a direct relationship for BDE-28 concentrations between lungs and liver. However, we showed that liver concentrations of the fully brominated BDE-209 increased with those in lungs. These findings concurred with observations of Sorais et al. (2021) who reported higher daily sampling rates in PASs and liver concentrations of BDE-209 in ring-billed gulls foraging in landfills compared to individuals predominantly foraging in other habitats. However, little is known about gaseous and particulate phase contaminant fate in avian lungs, especially for PBDEs and other HFRs.

#### 4.2. HFR exposure through ingestion

BDE-28 and -47 concentrations in liver of ring-billed gulls were significantly related to those isolated from the surface of their feathers. The covariation in concentrations of PBDEs between liver and feather surface extract of ring-billed gulls could be explained by co-ingestion during preening of PBDEs adsorbed onto their feathers. However, we were unable to establish a direct association between PBDE concentrations in feather surface extract and stomach content, except for BDE-99 (Fig. S2). To our knowledge, no study has explored the linkages between PBDE and other HFR concentrations on feather surface and tissue concentrations of any species.

Given that a wide range of organic contaminants concentrate in lipid-rich tissues, preen oil may be a good vehicle for depuration from the organism (Gutiérrez et al., 1998; Solheim et al., 2016). We showed that BDE-47 and -99 concentrations in feather surface extract of ring-billed gulls increased with those in preen oil. We also found positive associations between BDE-47 and -99 concentrations in preen oil of ring-billed gulls and their liver (Figs. S3 and S4). Jaspers et al. (2008) indicated that the primary source of external contamination for BDE-47 and -99 on the feather surface of common magpie (*Pica pica*) in Belgium was largely endogenous, that is, originating from the preen oil produced by the uropygial gland. Similar results were reported for BDE-47 concentrations between feathers and preen oil of white-tailed eagle (*Haliaeetus albicilla*) from Norway (Løseth et al.,

2019). Nevertheless, both Jaspers et al. (2008) and Løseth et al. (2019) examined a limited number of PBDE congeners, excluding the highly particle-bound BDE-209. Furthermore, the very volatile BDE-28 was below the detection limit in these two studies. Because concentrations of BDE-28 in feather surface extract were not related with those in preen oil in ring-billed gulls, we suggest that airborne BDE-28 deposited in the preen oil layer on the feathers may represent a significant exposure source, leading to increased liver concentrations.

PBDE concentrations in liver of ring-billed gulls were not associated with those in stomach content, except for BDE-47. Similarly, Hakk et al. (2010) found that for the lower brominated BDE-47, >60 % of a single oral dose of <sup>14</sup>C-labelled BDE-47 was bioavailable in male broiler chickens after 72 h, with higher concentrations retained in adipose tissue. In addition, Hakk et al. (2021) reported that for laying hens orally exposed to <sup>14</sup>C-labelled PBDEs (BDE-99, -153, and -209), the recovery from BDE-209 in internal tissues was 20 times lower than BDE-99 and -153. Moreover, these authors observed only trace amounts of BDE-209 in lungs (0.006 % of the original dose), with 93 % of the dose readily excreted in feces. These findings support the idea that exposure through inhalation (Section 4.1.) may be an important factor explaining lung and liver concentrations of PBDEs in ring-billed gulls, especially for BDE-209.

Ring-billed gulls breeding in the densely populated Montreal area frequently forage in landfills (Gentes et al., 2015; Patenaude-Monette et al., 2014; Sorais et al., 2020), where HFRs have been detected in surrounding air (Kerric et al., 2021). As such, we previously found greater BDE-209 concentrations (mean: 14.3 pg/m<sup>3</sup>) in static PASs deployed in different areas of a major landfill in the Montreal area that is commonly used by gulls for foraging (Kerric et al., 2021). In this population, it was reported that >40 % of the birds' regurgitations and stomach content are composed of edible human refuse (Patenaude-Monette et al., 2014). Thus, HFR-laden particles and dust may adsorb onto human refuse or be released in water bodies (e.g., ponds) around landfills and be ingested by gulls. Nonetheless, ingestion of human-derived food items and water in landfills may only partially account for the overall exposure to these chemicals. Gulls foraging in landfills ingest not only edible human refuse, but also plastic debris, foam, metal, glass, and building materials (Caron-Beaudoin et al., 2013; Seif et al., 2018). Among these, the inadvertent co-ingestion of plastics containing PBDEs could influence their stomach content concentrations, and thus may have an impact on their overall exposure to PBDEs. However, studies on plastic ingestion-related PBDE exposure are sometimes contradictory and require additional research (Guo et al., 2020; Tanaka et al., 2015; Thaysen et al., 2020). For example, Thaysen et al. (2020) reported a bidirectional transfer of PBDEs between plastics (>0.5 mm) and the gastrointestinal tract of ring-billed gulls from the Montreal area, with a dominance of transfer from bird to ingested plastics.

## 5. Conclusions

To our knowledge, this study is the first to investigate the linkages between atmospheric exposure routes for PBDEs and other HFRs, and their concentrations in tissues for any bird or mammal species. Consistent with our prediction, lung concentrations of the highly volatile BDE-28 were directly related to air levels determined in bird-borne PASs, suggesting that ring-billed gulls may be exposed to atmospheric PBDEs through direct inhalation. However, we could not find any direct effect of air levels (PAS) for PBDEs on the feather surface extract concentrations. Our results suggest that atmospheric concentrations of PBDEs cannot be directly predicted using PBDEs isolated from the feather surface. However, feathers may reveal atmospheric exposure levels in omnivorous birds and as such could be a potential non-destructive biomonitoring tool for organic pollutants. Additional research should also focus on distinguishing endogenous (i.e., preen oil from the uropygial gland) from exogenous sources (i.e., adsorbed airborne contaminants) on feather surface as well as inputs from other sources (e.g., plastic ingestion). We demonstrated that in addition to dietary exposure, atmospheric exposure pathways by inhalation and co-ingestion during feather maintenance contribute to the

accumulation of PBDEs and other HFRs in the liver of ring-billed gulls. Our results emphasized that atmospheric exposure should not be underestimated compared to the traditional dietary exposure pathway and should be included in further studies on organic pollutant exposure in urban-adapted birds. We also showed that the importance of these different routes of exposure is congener-specific and may be related to their physicochemical properties. Future studies would be necessary to understand the dynamics of uptake and distribution of PBDEs and other airborne HFRs in tissues following each exposure route. Our study further highlights that structural equation models in a Bayesian framework represent a potentially powerful tool to explore multiple pathways of contaminant exposure in wildlife, particularly with censored observations (non-detects).

### CRedit authorship contribution statement

**Anais Kerric:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Marc J. Mazerolle:** Formal analysis, Methodology, Writing – review & editing. **Jean-François Giroux:** Funding acquisition, Supervision, Writing – review & editing. **Jonathan Verreault:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

### Data availability

Data will be made available on request.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.160526>.

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