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Dissimilar effects of organohalogenated compounds on thyroid hormones in glaucous gulls

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ABSTRACT

The glaucous gull (*Larus hyperboreus*) is an arctic top predator and scavenger exposed to high levels of mixtures of organohalogenated contaminants (OHCs) of which many interfere with the thyroid hormone (TH) system. In the present study, we applied statistical modeling to investigate the potential combined influence of the mixture of chlorinated, brominated and perfluorinated organic compounds in plasma of glaucous gulls on their plasma TH concentrations. In females, there were significant negative associations between several organochlorinated compounds (OCs) and free thyroxin (FT4) and triiodothyronine (FT3), indicating additive negative effects on FT4 and FT3. However, in these females there was also a significant positive association between perfluorooctane sulfonate (PFOS) and FT3. The inverse associations between several OCs and FT3 and the contrasting positive association between PFOS and FT3, indicate that these two groups of OHCs may have dissimilar and antagonistic effects on FT3 in female glaucous gulls. In males, there were no associations between any of the OHCs and the THs. That OHCs affect THs in a complex manner involving both additive and antagonistic effects add to the challenge of interpreting the overall functional effect of thyroid disruptive chemicals in wildlife. However, experimental studies are needed to confirm or disprove such effects.

1. Introduction

Arctic wildlife is exposed to complex mixtures of organohalogenated contaminants (OHCs) such as organochlorinated compounds (OCs) which includes polychlorinated biphenyls (PCBs) and organochlorinated pesticides, organobrominated compounds such as brominated flame retardants (BFRs), and poly- and perfluorinated alkylated substances (PFASs) (Letcher et al., 2010). Chlorinated and brominated organic compounds have lipophilic properties and are associated with lipids in organisms, whereas PFASs have amphipathic properties and are associated with proteins in organisms (Jones et al., 2003). The physicochemical properties of many OHCs cause them to be present in biota in pristine arctic areas even decades after their production and use was banned (Letcher et al., 2010). High concentrations of OHCs may cause reproductive, behavioral and developmental stress by having disruptive effects on endocrine systems in arctic animals (Letcher et al., 2010).

The glaucous gull (*Larus hyperboreus*) is a predator and scavenger occupying a top position in the arctic marine food web (Anker-Nilssen et al., 2000). Since the 1970s, high levels of long-transported OHCs have been reported in this species (Bourne and Bogan, 1972; Letcher et al., 2010). Exposure to OHCs through the diet combined with the somewhat restricted capacity for biotransformation of these compounds in glaucous gulls makes the species susceptible for bioaccumulation of high levels of such compounds (Verreault et al., 2005). Previous studies have demonstrated the thyroid disruptive potential of OHCs in glaucous gulls (Ucan-Marin et al., 2010; Ucán-Marín et al., 2009; Verreault et al., 2004, 2007) and a growing body of evidence proposes that arctic wildlife, including glaucous gulls, are being adversely affected by high body burden of various OHC compounds at the population level (Erikstad et al., 2013; Nuijten et al., 2016).

The thyroid hormone (TH) system controls pre- and postnatal development, thermoregulation, lipid metabolism, moulting and several other physiological processes (McNabb, 2000). Thyroxine (T4) is secreted by the thyroid gland and transported by TH-transporting proteins, such as transthyretin (TTR) and albumin, to peripheral tissues where it undergoes enzymatic deiodination to the more biologically active triiodothyronine (T3) (McNabb, 2000). Maintenance of normal

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functioning circulatory concentrations of THs is essential in sustaining good health.

OHCs have been reported to cause lowered plasma concentrations of TH, and concentrations of circulating THs are proposed to be useful biomarkers of effect of these compounds in wildlife studies (Skaare et al., 2002). Thyroid disruption by OHCs may occur by direct toxic effects in the thyroid gland, interference with the TH synthesis, enzymatic deiodination or metabolism, binding to TH receptors in tissues, or transport mechanisms in the plasma. Previous studies have shown that several OHCs may displace T4 and T3 in glaucous gull TTR and albumin (Ucan-Marin et al., 2010; Ucán-Marín et al., 2009). Competitive displacement of THs from TTR have also been demonstrated for PCBs, polybrominated diphenyl ethers (PBDEs), and their hydroxylated metabolites (OH-PCBs and OH-PBDEs), PFASs, hexachlorobenzene (HCB) and metabolites of dichlorodiphenyltrichloroethane (DDT) in other species (Meerts et al., 2000; Van den Berg et al., 1991; Weiss et al., 2009). Such displacement of THs from transport proteins is thought to increase the biliary excretion rates of particularly T4, and has been suggested as a central mechanism of TH disruption by OHCs.

Since thyroid disruptive compounds (TDCs) may act via several different modes of actions, the complex mixtures of OHCs that wildlife are exposed to may cause combined or interactive effects on the plasma concentrations of THs, which are considered as physiological important endpoints for TDC effects. The presence of different compounds with similar and dissimilar modes of actions (Bliss, 1939; Loewe and Muischneck, 1926; Plackett and Hewlett, 1952) with respect to thyroid effects may cause complex combined effects, such as additive, antagonistic, synergistic and potentiation effects. In addition, there may be complex non-monotonous dose-dependent variations in TH-effects caused by exposure to multiple chemicals (Crofton et al., 2005).

Mixture effects on THs of multiple compounds and groups of chlorinated and brominated organic compounds have been studied in arctic animals, indicating that both additive and antagonistic effects are present (Villanger et al., 2011). A study on northern fulmar (*Fulmar glacialis*) and black-legged kittiwake (*Rissa tridactyla*) chicks showed positive associations between levels of PFASs and plasma concentrations of free and total T4 (Nøst et al., 2012). This is in contrast to the previously reported negative associations between OCs and plasma T4 in adult male glaucous gulls (Verreault et al., 2004). Since levels of OCs, BFRs and PFASs are high in glaucous gulls from the Norwegian Arctic (Verreault et al., 2005, 2007) there may be mixture effects of these contaminant groups on circulating TH concentrations in this species.

The aim of the present study was to apply statistical modeling to investigate the potential combined effects on thyroid hormones in glaucous gulls caused by the mixture of chlorinated, brominated and fluorinated anthropogenic compounds in their plasma. Thus, plasma concentrations of 28 chlorinated, 10 brominated, 16 per-and polyfluorinated compounds, and total (T) and free (F) T4 and T3 were analyzed in glaucous gulls breeding in Kongsfjorden, Svalbard. Associations between these contaminants and the THs were examined using principle component analysis (PCA) and correlation analyses. The effects of the contaminants on the THs were also modelled using orthogonal partial least-squares (OPLS) regression analysis. The effects of possible confounding factors, such as sex and body condition were also investigated and taken into consideration.

2. Materials and methods

2.1. Sampling

A total of 39 breeding glaucous gulls were collected during the second half of the incubation period at the Kongsfjorden area, Spitsbergen, Norway (78°55′N 11°56′E) in June 2011, 2012 and 2013. All glaucous gulls sampled were breeding, thus the age of the birds were expected to be four years or older (Gaston et al., 2009). Blood was drawn from the branchial veins and kept cool and dark until centrifugation within 8 h, and the plasma was frozen at −20 °C. Biometric measures (body mass, bill length, gonys height, head length and wing length) were recorded. Sex was determined biometrically or by molecular determination. Further details on sampling procedures and sex determination are given in the Supporting Information. The project was approved by the Governor of Svalbard (2010/00053-8, 2011/01095-42 and 2013/00050-28) and the sampling and handling of the birds were in accordance with the regulations of the Norwegian Animal Welfare Act.

2.2. Contaminant and lipid content analysis

Analysis of OHCs in plasma samples were performed at the Norwegian Institute of Air Research (NILU) in Tromsø, Norway. The compounds analyzed included PCBs (12 congeners), chlorinated pesticides (chlordanes [CHLs], DDT and its metabolites, hexachlorocyclohexane [HCH] and its isomers, hexachlorobenzene [HCB]), PBDEs (10 congeners) and PFASs (16 compounds). Quantification was conducted by the internal standard method. Because of the differing properties of the compounds (lipophilic vs amphipilic) and because OHC concentrations in wildlife studies and in ecological wildlife risk assessments of OHCs usually are provided on a mass/mass basis (Letcher et al., 2010; Dietz et al., 2015; Nuijten et al., 2016) contaminant concentrations are given on a wet weight (ng/g ww) basis assuming that the density of the plasma was 1.025 g/mL. In addition, information on plasma lipid content (%) is given in the results, allowing for estimation of the lipid weight concentrations.

The method for analysis of chlorinated and brominated organics is described previously (Herzke et al., 2009). Briefly, plasma samples (500 μL) were extracted with *n*-hexane after denaturation with ethanol and a saturated ammonium sulfate solution in water. Clean-up on Florisil columns, separation on an Agilent Technology 7890 gas chromatograph and detection on an Agilent Technology 5975 C mass spectrometer were performed (Herzke et al., 2009). Limits of detection (LOD) were defined as three times the noise or blank signal if these exceeded the instrumental detection limits. Blanks and standard reference materials (SRM) (1958 human serum, NIST, USA) were analyzed every fifteenth sample for quality assurance of the results. Blank contamination was observed for γ-HCH, HCB, *trans*-chlordane, *cis*-chlordane, *oxy*-chlordane, *trans*-nonachlor, *cis*-nonachlor, *p,p*'-DDE and PCB-28. The SRM analyses were within the given limits of accuracy.

The methods for analysis of PFAS are described in detail previously (Powley et al., 2005). Briefly, extraction of plasma samples (200 μL) was conducted using methanol, before matrix removal by ENVI-Carb graphitized carbon absorbent and glacial acetic acid. Quantification was conducted by an ultra-high pressure liquid chromatography triple-quadrupole mass spectrometry (UPLC-MS/MS). LOD was defined as three times the noise or blank signal. Blanks and SRM (1958 human serum, NIST, USA) were analyzed every fifteenth sample for quality assurance of the results. No contamination of the blanks was observed. The SRM analyses were within the given limits of accuracy.

Enzymatic quantification of lipid content of the blood was performed by Unilab Analysis AS, Tromsø, Norway based on levels of triglycerides, free cholesterol, total cholesterol and phospholipids (Akins et al., 1989). The total lipid concentration was converted to plasma lipid percentage of the wet weight samples.

2.3. Thyroid hormone analysis

TH analysis by commercially available radioimmunoassay (RIA) Coat-A-Count kits (Siemens medical solution, Diagnostics, LA, USA) was performed at NTNU, Trondheim, Norway. The method is a sensitive technique also for analysis in birds and the kits have been verified for glaucous gulls (Verreault et al., 2004, 2007). The procedure was followed as described in the kit descriptions. Plasma samples were analyzed for free and total T3 (FT3 and TT3) and free and total T4 (FT4 and