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Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds

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ABSTRACT

Thyroid hormones are essential for normal growth and development and disruption of thyroid homeostasis can be critical to young developing individuals. The aim of the present study was to assess plasma concentrations of halogenated organic contaminants (HOCs) in chicks of two seabird species and to investigate possible correlations of HOCs with circulating thyroid hormone (TH) concentrations. Plasma from black-legged kittiwake (*Rissa tridactyla*) and northern fulmar (*Fulmarus glacialis*) chicks were sampled in Kongsfjorden, Svalbard in 2006. The samples were analyzed for thyroid hormones and a wide range of HOCs (polychlorinated biphenyls (PCBs), hydroxylated (OH−) and methylsulphoned (MeSO−) PCB metabolites, organochlorine pesticides (OCPs), brominated flame retardants (BFRs), and perfluorinated compounds (PFCs)).
Concentrations of HOCs were generally low in kittiwake and fulmar chicks compared to previous reports. HOC concentrations were five times higher in fulmar chicks compared to in kittiwake chicks. PFCs dominated the summed HOCs concentrations in both species (77% in kittiwakes and 69% in fulmars).
Positive associations between total thyroxin (TT4) and PFCs (PFHpS, PFOS, PFNA) were found in both species. Although correlations do not implicate causal relationships per se, the correlations are of concern as disruption of TH homeostasis may cause developmental effects in young birds.

1. Introduction

The use of anthropogenic contaminants in Arctic regions has been minimal. However, long-range atmospheric and oceanic currents transport contaminants from industrialized areas at lower latitudes to the Arctic where they are bioaccumulated up the food chain (Burkow and Kallenborn, 2000; Wania and Mackay, 1993). Arctic animals are exposed to a complex mixture of halogenated organic contaminants (HOCs) (Letcher et al., 2010). High concentrations of so-called "novel" HOCs, such as brominated flame retardants (BFRs) and perfluorinated compounds (PFCs) in arctic biota (Butt et al., 2010; Haukås et al., 2007; Jenssen et al., 2007; Sørmo et al., 2006) have raised concerns about their impact on arctic wildlife. Studies of arctic animals have shown several negative health effects of HOCs, including effects on reproduction, immune function, hormone and vitamin homeostasis, biotransformation enzymes and cancer risks (Gabrielsen, 2007; Letcher et al., 2010). The thyroid system is one of the endocrine systems that have been shown to be particularly affected by HOCs (Jenssen, 2006; Rolland, 2000).

Thyroid hormones (THs) are essential for normal animal development and function by controlling growth, cell differentiation, reproduction, behavior and immune system function (McNabb, 1992).

Abbreviations: 4-OH-HpCS, 4-OH-heptachlorostyrene; ANOVA, Analysis of variance; BC, Body condition; BFRs, Brominated flame retardants; BL, Body length; BM, Body mass; CHLs, Chlordane group; DDTs, DDT group; *o,p′*-DDE, 1-chloro-4-[2,2-dichloro-1-(2-chlorophenyl)ethenyl]benzene; *p,p′*-DDE, 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl) ethenyl]benzene; *o,p′*-DDD, 1,1-dicholoro-2-(2-chlorophenyl)-2-4-(chlorophenyl)ethane; *p,p′*-DDD, 1,1-dichloro-2,2-di(4-chlorophenyl)ethane; *o,p′*-DDT, 1,1,1-tricholoro-2-(2-chlorophenyl)-2-4-(chlorophenyl)ethane; *p,p′*-DDT, 1,1,1-trichloro-2,2-di(4-chlorophenyl) ethane; GC–MS, Gas chromatography mass spectrometry; HCB, Hexachlorobenzene; HCHs, HCH group; HCH, Hexachlorocyclohexane; HOCs, Halogenated organic contaminants; HPTH, hypothalamic–pituitary–thyroid axis; LC–Q-TOF MS, Liquid chromatography quadrupole time-of-flight mass spectrometry; LOD, Limit of detection; MeSO, Methylsulfonated compounds; OCPs, Organochlorine pesticides; OH, Hydroxylated compounds; PBB, Polybrominated biphenyls; PBDEs, Polybrominated diphenyl ethers; PCA, Principal component analysis; PCB, Polychlorinated biphenyls (congeners numbered by the IUPAC numbering system); PCP, Pentachlorophenol; PCR, Polymerase chain reaction; PeCB, Pentachlorobenzene; PFCs, Perfluorinated compounds; PFHxS, Perfluorohexane sulphonate; PFHpS, Perfluoroheptane sulphonate; PFOS, Perfluorooctane sulphonate; PFOA, Perfluorooctanoate; PFNA, Perfluorononanoate; PFDcA, Perfluorodecanoate; PFUnA, Perfluoroundecanoate; PFDoA, Perfluorododecanoate; PFTriA, Perfluorotridecanoate; PFTeA, Perfluorotetradecanoate; PFSA, Perfluoroalkyl sulfonates; PFCA, Perfluoroalkyl carboxylates; SRM, Standard reference material; T3, Triiodothyronine (total: TT3, free: FT3); T4, Thyroxine (total: TT4, free FT4); TBA, Tribromoanisole; TBG, Thyroid binding globulin; TH, Thyroid hormones; TSH, Thyroid stimulating hormone; TTR, Transthyretin; UDP-GT, Uridine diphosphate glucuronyltransferase; w.w., Wet weight.

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Thus, in wildlife species, disruption of TH homeostasis during early life may have detrimental developmental implications, and effects on health and thus fecundity may be manifested at later age stages.

THs are produced in the thyroid gland and transported to peripheral target tissue aided by thyroxine hormone binding proteins (e.g. transthyretin (TTR), thyroid binding globulin (TBG) and albumin). Most circulatory THs are protein bound (McNabb, 1992) and the dominant blood carrier proteins for THs in avian species are TTR and albumin (Richardson et al., 1994). The main TH, thyroxin (T4) is deiodinated to the biologically active form triiodothyronine (T3) by monodeiodinases in target tissues (McNabb, 1992, 1995).

Considering the extensive control exerted by THs in animals, disruption of THs homeostasis by HOCs is of particular concern in young individuals as it may alter normal development and body function. Modulation of the thyroid parameters can be exerted on different biochemical levels in the organism (Boas et al., 2006) and the relative sensitivity of different TH endpoints to contaminants exposure varies (Wade et al., 2002). When assessing effects of HOCs on the concentrations of circulating TH, the most relevant mechanisms of disruption is synthesis and metabolism of THs and competitive displacement on their binding proteins. TH synthesis is controlled by the thyroid stimulating hormone (TSH) signaling pathway, which has been shown to be interrupted by polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) through receptor binding and inhibited intracellular messaging after TSH stimulus (Chana et al., 2002; Santini et al., 2003). Inhibition of monodeiodinase activity can skew the concentration of the biologically active TH, T3. Indeed, PCB mixtures administered to white Leghorn chick (*Gallus gallus*) embryos showed decreased hepatic deiodinase activities (Gould et al., 1999). Metabolism of THs in Sprague Dawley rats (*Rattus norvegicus*) by hepatic conjugation by uridine diphosphate glucuronyltransferase (UDP-GT) was increased after exposure to a PCB mixture (van Birgelen et al., 1995; Webb and McNabb, 2008) and a complex HOCs mixture (Wade et al., 2002). Furthermore, hydroxylated metabolites of PCBs (OH-PCBs) have been shown to inhibit the activity of other conjugation enzymes involved in THs metabolism, the sulfotransferases (e.g. Schuur et al., 1998). Competitive displacement of THs on TTR, the dominant binding protein in kittiwakes and fulmars, by HOCs have been shown for PCBs (Chauhan et al., 2000; Rickenbacher et al., 2002), OH-PCBs (Lans et al., 1993), hexachlorobenzene (HCB), dichlorodiphenyldichloroethane (*p,p′*- and *o,p′*-DDD) (van den Berg et al., 1991), BFRs (Meerts et al., 2000) and PFCs (Weiss et al., 2009). Studies assessing competitive displacement of HOCs on TBG or albumin have not shown the same interferences as on TTR (Lans et al., 1994; van den Berg, 1990).

Various laboratory exposure studies involving HOCs have generally shown decreased circulating TH concentrations in mammals (Bastomsky et al., 1976; Brouwer, 1989; Chang et al., 2007; Kato et al., 1998). However, reports of exposure studies in bird species varies (positive, negative or no effects on TH concentrations) (Brouwer et al., 1998; Dawson, 2000; Letcher et al., 2010). Wildlife studies are equally inconsistent; a review of 22 wildlife studies reported increasing, decreasing and unchanged TH concentrations with increasing HOC concentrations in many species, also in avian (Rolland, 2000). A review assessing effects on thyroid status of herring gulls (*Larus argentatus*) from the Great Lakes region proposed that decreased circulating TH concentrations were fairly common during the developmental stages but rare in adults (McNabb and Fox, 2003). Studies investigating effects of HOCs on TH homeostasis in avian species have reported on different biochemical effects and endpoints such as modulated hormone concentrations and ratios, disruption of the hypothalamic–pituitary–thyroid (HPT) axis, thyroid gland lesions, competitive displacement for plasma binding proteins, increased interconversion of hormones, increased or inhibited metabolism of hormones along with observed anatomical effects like developmental deformities. The current study reports correlations between concentrations of thyroid hormones and HOCs and biometric measurements.

There is a great lack of knowledge related to exposure to low HOC concentrations and TH status in developing seabirds. The aim of the present study was to assess plasma concentrations of HOCs and to investigate their correlations with circulating TH concentrations in chicks of two Arctic breeding seabird species; black-legged kittiwakes (*Rissa tridactyla*) and northern fulmars (*Fulmarus glacialis*). These two species differ in migration routes, diet composition, breeding strategies and physiological characteristics (Anker-Nilssen et al., 2000). Arctic studies have reported higher HOC concentrations in fulmars compared to kittiwakes (Braune and Simon, 2003; Buckman et al., 2004; Fisk et al., 2001a). Thus, fulmar chicks may be at greater risk to TH disruption than kittiwake chicks due to higher contaminant burden. Furthermore, few studies have addressed the possible correlations of PFCs and THs in free-living birds. Thus, PFCs were included in a broad array of HOCs analyzed and associations between these compounds and plasma TH concentrations were examined in the two species.

2. Materials and methods

2.1. Study area and sample collection

The seabirds were collected in Kongsfjorden, Spitsbergen, Norway (75.54°N, 12.30°E) in August and September 2006. Fifteen kittiwake chicks and 15 fulmar chicks were captured at the end of the chick rearing period. The fulmar chicks had fledged the nests (~50 days old), and were captured from a boat at sea using a net. The kittiwake chicks were close to fledging (~40 days old) and were captured with a noose-pole on the nest site in the kittiwake colony. The sampling of birds was approved by the Governor of Svalbard, and national guidelines for ethical treatment of experimental animals were followed (NARA, 2005). Analyses for sex and thyroid hormones were performed on all 15 samples, while contaminant analyses were limited to ten random samples of each species.

Blood was drawn from the alar vein located on ventral side of the wing at the humeral–radial–ulnar joint. The blood was kept on ice until the samples were centrifuged in the laboratory at 4000 rpm for 7 min and then frozen. Plasma samples were frozen in aliquots for the different analyses at −20 °C within 5 h of sampling until time of analysis.

The following biometric measurements were obtained from all birds: wing length (±0.1 cm), bill and head length (±0.1 mm), tarsus length (±0.1 mm), total body length (BL; ±1 cm) and body mass (BM; ±10 g) (data not shown).

2.2. Sex determination

Sex determination of birds was performed at the Norwegian University of Science and Technology (NTNU) (Trondheim, Norway) using methods described by Griffiths et al. (1998). This method involved PCR and gel separation of the PCR product. Male birds were expressed as a single band, whereas female birds showed an additional second band.

2.3. Contaminant analyses

Analyses for contaminants in plasma samples were performed at the Norwegian Institute of Air Research (NILU) (Tromsø, Norway). The compounds analyzed included: PCBs (32 congeners), organochlorine pesticides (OCPs) (chlordanes (CHLs), DDT and metabolites, HCHs, HCB and PeCB), BFRs (16 PBDEs and 2 PBBs), tribromoanisole (TBA) and PFCs (20 compounds). Methylsulphoned and hydroxylated metabolites of PCB (MeSO- and OH-PCBs, respectively) were analyzed