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Effects of a complex contaminant mixture on thyroid hormones in breeding hooded seal mothers and their pups☆

Randi Grønnestad [a, e], Gro D. Villanger [b, e], Anuschka Polder [c], Kit M. Kovacs [d], Christian Lydersen [d], Bjørn M. Jenssen [e, f], Katrine Borgå [a, *]

[a] Department of Biosciences, University of Oslo, Oslo, Norway
[b] Department of Child Health, Norwegian Institute of Public Health, Oslo, Norway
[c] Norwegian University of Life Sciences, Oslo, Norway
[d] Norwegian Polar Institute, Fram Centre, Tromsø, Norway
[e] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[f] Department of Arctic Biology, The University Centre in Svalbard, Longyearbyen, Norway

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ABSTRACT

There is a general lack of information on the possible effects of perfluoroalkyl substances (PFASs) on thyroid hormones (THs) in wildlife species. The effects of PFASs, which are known endocrine disruptors, on the TH homeostasis in hooded seals (*Cystophora cristata*) have yet to be investigated. Previously, correlations were found between plasma thyroid hormone (TH) concentrations in hooded seals, and organohalogen contaminants (OHCs) and hydroxyl (OH)-metabolites. Because animals are exposed to multiple contaminants simultaneously in nature, the effects of the complex contaminant mixtures that they accumulate should be assessed. Herein, we analyse relationships between plasma concentrations of multiple contaminants including protein-associated PFASs, hydroxylated metabolites of polychlorinated biphenyls (OH-PCBs) and lipid soluble OHCs and plasma concentrations of free and total THs, i.e. triiodothyronine (FT3, TT3) and thyroxine (FT4, TT4) in hooded seal mothers and their pups. The perfluoroalkyl carboxylates (PFCAs) were the most important predictors for FT3 concentrations and TT3:FT3 ratios in the mothers. The FT3 levels decreased with increasing PFCA levels, while the TT3:FT3 ratios increased. In the pups, hexachlorocyclohexanes (HCHs) were the most important predictors for TT3:FT3 ratios, increasing with increasing HCHs levels. Additionally, perfluoroalkyl sulfonates (PFSAs) and PFCAs were important predictors for FT4:FT3 ratios in hooded seal pups, and the ratio increased with increasing concentrations. The study suggests that PFASs contribute to thyroid disruption in hooded seals exposed to complex contaminant mixtures that include chlorinated and fluorinated organic compounds.

1. Introduction

Many environmental contaminants cause endocrine disruption, and there is increasing concern that exposure to environmental chemicals during the embryonal and foetal stages can disrupt hormone signalling during early development, thereby causing irreversible, negative effects on health, reproduction and survival in later postnatal life-stages (Zoeller and Crofton, 2000). Many organohalogen contaminants (OHCs) and their metabolites affect multiple targets in the hypothalamus-pituitary-thyroid (HPT) axis (Fig. 1) (Colborn et al., 1994; Crofton, 2008).

Thyroid hormones (TH), mainly thyroxine (T4) and triiodothyronine (T3), are essential for normal development and maintenance of physiological functions. These hormones play important roles in regulating metabolism and growth, and are key hormones for the development of the central nervous system and brain function in mammals (Porterfield and Hendrich, 1993; Zoeller et al., 2007). Exposure to xenobiotic chemicals with thyroid disrupting properties can result in changes in circulating TH levels, the ratio between free and protein bound TH, and the conversion of T4 to T3 (Zoeller et al., 2007).

Perfluoroalkyl substances (PFASs) have been shown to have endocrine disruptive effects and to disrupt the thyroid homeostasis in both experimental, human and wildlife studies (Lau et al., 2003;

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* Corresponding author.
E-mail address: katrine.borga@ibv.uio.no (K. Borgå).

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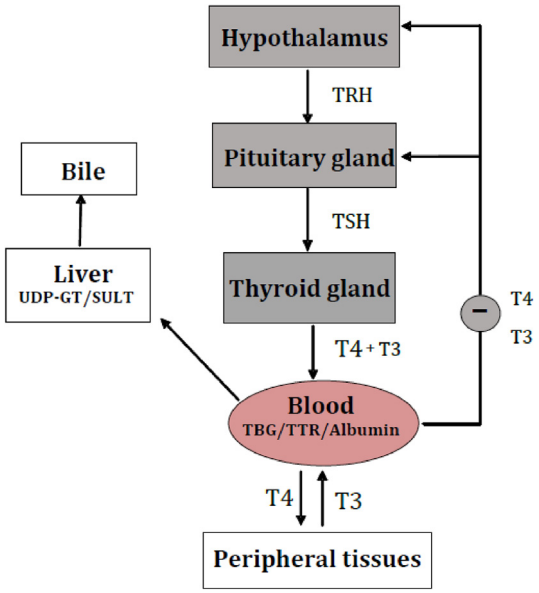

Fig. 1. The mammalian HPT axis. TRH: tripeptide thyrotropin-releasing hormone, TSH: thyroid-stimulating hormone, T4 and T3: Thyroid hormones, TBG: thyroxine-binding globulin, TTR: transthyretin, UDP-GT: UDP-glucuronosyl transferase, SULT: sulfotransferases. Environmental contaminants could interact with all the different steps of the HPT-axis. The results from the present study indicate that PFASs could interact with the binding to transport proteins, or the SULT in the liver.

Thibodeaux et al., 2003; Yu et al., 2009). The hooded seal (*Cystophora cristata*) is a predator that feeds at a high trophic level in the Arctic marine food web (Houde et al., 2011). This results in high levels of persistent organic contaminants (POPs) (Gabrielsen et al., 2011; Villanger et al., 2013) due to biomagnification and to potential for maternal transfer of these compounds to their offspring. Indeed, maternal transfer of PFASs to pups via milk and placenta has been documented in hooded seals, resulting in generally higher circulating PFAS levels in pups compared to their mothers (Grønnestad et al., 2016).

The hooded seals have a remarkable reproduction strategy, with a very short and intense lactation period, and are dependent on a large weight gain during this period (Bowen et al., 1985) with growth rates exceeding 7 kg a day (Kovacs and Lavigne, 1992). This makes them vulnerable to changes in hormone balance. Previous studies of contaminants in hooded seal mother-pup pairs found associations between various chlorinated and brominated contaminants and TH (Gabrielsen et al., 2011; Villanger et al., 2013). These studies demonstrated the importance of considering the effects of the mixture of multiple contaminants that are present in wildlife when assessing the potential effects on TH homeostasis. This includes lipid soluble parent compounds; polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs), as well as proteinophilic metabolites; hydroxyl (OH)-PCB and OH-PBDE. The HPT axis is very complex and has multiple receptors and many feed-back loops (Fig. 1) that create a potential for combined effects of individual OHCs acting through similar or different modes of action (Crofton et al., 2005; Crofton, 2008). However, few studies have included PFASs when investigating such combined effects of OHCs on the thyroid system in wildlife (Nøst et al., 2012; Bytingsvik et al., 2013; Couderc et al., 2016; Berg et al., 2017; Melnes et al., 2017).

The aim of the present study was to investigate associations between circulating concentrations of THs and PFASs in adult female hooded seals and their nursing pups, and to investigate the relative importance of PFASs compared to the chlorinated and brominated OHCs and their metabolites with respect to their influence on TH levels. The data were compiled from three previous studies related to levels and effects of OHCs in fifteen mother-pup pairs of hooded seals from the West-Ice off the coast of East-Greenland (Gabrielsen et al., 2011; Villanger et al., 2013; Grønnestad et al., 2016).

2. Materials and methods

2.1. Sampling

Hooded seal mother pup pairs (n = 15) were live-captured in March 2008 in the West Ice, east of Greenland (approximately 73.38N, 14.58W). Blood was collected and centrifuged in the field to separate plasma. The sex of the pups was noted, the age (1–4 days) of the pups was estimated based on the developmental stage (the pups are weaned after 4 days), and the body mass of both mothers and pups was measured to the nearest half kg. See Gabrielsen et al. (2011) for more capturing and sampling details. All animal handling was performed following the principles and guidelines and by permit from the Norwegian Animal Research Authority.

2.2. Contaminant analysis

The contaminant analysis for OHCs, OH-metabolites and PFASs were conducted at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences. The plasma samples were analysed for α-, β- and γ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (*p,p*'-DDE), 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (*p,p*'-DDD), 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (*p,p*'-DDT), 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)- ethane (*o,p*'-DDT), 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane (*o,p*'-DDD), Mirex, PCB congeners IUPAC nos. 28, 31, 47, 52, 56, 66, 74, 87, 99, 101, 105, 110, 114, 118, 128, 137, 136, 138, 141, 149, 151, 153, 156, 157, 170, 180, 183, 187, 189, 194, 196, 199, 206 and 209, and the BFRs pentabromotoluene (PBT), 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE), hexabromocyclododecane (HBCD; sum of a-, b- and g-HBCD), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE), PBDE congeners IUPAC nos. 28, 47, 99, 100, 153, 154, 183, 206, 207, 208 and 209, the phenolic metabolites or compounds 4-OH-CB106, 4-OH-CB107, 4′-OH-CB108, 3-OH-CB118, 4′-OH-CB130, 3′-OH-CB138, 4-OHCB146, 4′-OH-CB159, 4′-OH-CB172, 3′-OH-CB180, 4-OH-CB187, 4-OH-BDE42, 3-OH-BDE47, 6-OH-BDE47, 4′-OH-BDE49, 2′-OHBDE68, PCP, and 2,4,6-tribromophenol (TBP). The same plasma samples were also analysed for the perfluoroalkyl sulfonates (PFSA): perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS), and the perfluoroalkyl carboxylates (PFCAs): perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), and perfluorotridecanoic acid (PFTrDA). For details on the chemical analyses, see Villanger et al. (2013) for OHCs, Gabrielsen et al. (2011) for OH-metabolites, and Grønnestad et al. (2016) for PFASs. Lipid content was determined gravimetrically (Gabrielsen et al., 2011), and protein content was determined using a modified Lowry's method (Lowry et al., 1951).

2.3. Thyroid hormone analysis

Hooded seal plasma samples were analysed for TH (total T4 (TT4), free T4 (FT4), total T3 (TT3) and free T3 (FT3)) using commercially available solid-phase radioimmunoassay (RIA) kits (for details, see Gabrielsen et al., (2011))