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Effects of complex organohalogen contaminant mixtures on thyroid homeostasis in hooded seal (*Cystophora cristata*) mother–pup pairs

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HIGHLIGHTS

- Organohalogen contaminants in nursing hooded seal mothers and pups were reported.
- Multivariate associations between thyroid hormones and contaminants were shown.
- Specific contaminants appear to affect thyroid homeostasis in mothers and pups.
- Similar thyroid responses may reflect linked mother–pup exposure or thyroid effects.
- Some contaminants may have higher thyroid disruptive potency in pups (e.g. OH-PCBs).

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ABSTRACT

Many lipid-soluble and phenolic compounds present in the complex mixture of orgaohalogen contaminants (OHCs) that arctic wildlife is exposed to have the ability to interfere with the thyroid hormone (TH) system. The aim of this study was to identify compounds that might interfere with thyroid homeostasis in 14 nursing hooded seal (*Cystophora cristata*) mothers and their pups (1–4 d old) sampled in the West Ice in March 2008. Multivariate modelling was used to assess the potential effects of measured plasma levels of OHCs on circulating TH levels of the measured free (F) and total (T) levels of triidothyrine (T3) and thyroxine (T4). Biological factors were important in all models (e.g. age and sex). In both mothers and pups, TT3:FT3 ratios were associated with α- and β-hexachlorocyclohexane (HCH), *ortho*-PCBs, chlordanes and DDTs. The similarities between the modelled TT3:FT3 responses to OHC levels in hooded seal mothers and pups most probably reflects similar exposure patterns, but could also indicate interconnected TH responses. There were some differences in the modelled TH responses of mothers and pups. Most importantly, the negative relationships between many OH-PCBs (particularly 3'-OH-CB138) and TT3:FT3 ratio and the positive relationships between TT4:FT4 ratios and polybrominated diphenyl ether [PBDE]-99, -100 and 4-OH-CB107 in pups, which was not found in mothers. Although statistical associations are not evidence *per se* of biological cause–effect relationships, the results suggest that thyroid homeostasis is affected in hooded seals, and that the inclusion of the fullest possible OHC mixture is important when assessing TH related effects in wildlife.

1. Introduction

Maternal transfer of environmental contaminants via the placenta or milk in mammals can result in young neonates having high levels of exposure (Polischuk et al., 1995; Wolkers et al., 2006; Polder et al., 2008; Needham et al., 2011). This is of great concern for both wildlife and humans since developing mammals have reduced ability to metabolise and excrete xenobiotics and are generally considered to be more susceptible to toxic effects compared to adults (Milsap and Jusko, 1994; Grandjean and Landrigan, 2006; Wolkers et al., 2009). Many environmental contaminants have been shown to have endocrine disruptive capabilities and pre- and postnatal contaminant exposure might differentially affect endocrine regulation during early developmental stages. Pre- and postnatal exposure to endocrine disruptors can also result in serious fitness-related impairments that become evident during

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adolescence or adulthood (Colborn et al., 1993; Darnerud, 2008; Nichols et al., 2011).

The thyroid hormone (TH) system is an important endocrine target for many organohalogen contaminants (OHCs). These include e.g. polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs, e.g. hexachlorobenzene [HCB]), polybrominated diphenyl ethers (PBDEs) and their phenolic metabolites, which are formed during biotransformation mediated by cytochrome P450 (CYP) enzymes (e.g. hydroxylated [OH]-PCBs, pentachlorophenol [PCP] and OH-PBDEs) (Brouwer et al., 1998; Letcher et al., 2000; Boas et al., 2006; Jugan et al., 2010).

Tetraiodothyronine (T4, thyroxine) is produced and released by the thyroid gland in much larger quantities than triiodothyronine (T3) and deiodination of T4 in extra-thyroidal tissues is the main supplier of the biologically more active hormone, T3 (McNabb, 1992; Hadley, 1996). THs are required in vertebrates to regulate a wide range of physiological processes related to growth, energy metabolism, temperature regulation and general physiological homeostasis (McNabb, 1992; Zoeller et al., 2007). Thyroid hormones are also essential for foetal and postnatal development of the brain and nervous system, and are also important for controlling sexual development (Cooke et al., 2004; Santisteban and Bernal, 2005; Ahmed et al., 2008). Maternal and foetal/neonatal disruption of TH balance can cause permanent developmental neurocognitive and motor deficits, alter behaviour and disturb sexual development in offspring, as shown in experimental animals and indicated in human studies (Brouwer et al., 1998; Pop et al., 1999; Porterfield, 2000; Zoeller et al., 2007; Darras, 2008; Jugan et al., 2010).

OHCs and their metabolites (e.g. OH-PCBs, OH-PBDEs) can affect many target points in the hypothalamic–pituitary–thyroid (HPT) axis, sometimes as a consequence of their structural resemblance to natural hormones. However, the possible mechanisms of disruption by OHCs and metabolites are multiple and may involve interference with the thyroid gland's production and release of hormones, negative feed-back regulation, binding to TH transport proteins in blood (e.g. transthyretin [TTR], thyroxine binding globulin [TBG] and albumin) and enzymatic metabolism and excretion of hormones. Also, some OHCs can bind to the thyroid hormone receptor (TR) and inhibit or facilitate TR-mediated gene expression and thus interfere with the many biological effects of THs (Lans et al., 1993; Brouwer et al., 1998; Howdeshell, 2002; Zoeller, 2005; Boas et al., 2006; Hamers et al., 2006; Langer et al., 2007; Pearce and Braverman, 2009).

The ice-breeding hooded seal (*Cystophora cristata*) is a good mammalian-model for studying trans-generational effects of maternally transferred environmental contaminants. This pinniped species feeds high in the arctic marine food chain and accumulates high levels of lipid-soluble OHCs in its blubber lipid-reservoirs, which are readily transferred from mother to pup via the placenta and the extremely lipid-rich milk (>60%) that the pup consumes during an intensive 3–4 day nursing period (Bowen et al., 1985; Kovacs and Lavigne, 1992; Espeland et al., 1997; Lydersen et al., 1997; Lydersen and Kovacs, 1999; Wolkers et al., 2006). This species also has the enzymatic ability to biotransform contaminants (Wolkers et al., 2009); the recently reported OH-PCBs in plasma of hooded seals are thought to originate from endogenous biotransformation of PCBs (Gabrielsen et al., 2011).

Studies of wild seal populations and exposure studies with seals given naturally contaminated fish-diets in captivity have demonstrated the potential for TH disruption due to OHC exposure (e.g. Brouwer et al., 1989; Jenssen et al., 1995; Hall et al., 2003; Debier et al., 2005; Sørmo et al., 2005; Routti et al., 2010). These studies have generally focused on the associative relationships between TH levels and total levels of OHC groups measured in blood or blubber. However, it is increasingly acknowledged that environmental OHC mixtures may act on the HPT axis via additive or even synergistic effects among the individual contaminants (e.g. Hallgren and Darnerud, 2002; Crofton et al., 2005; Villanger et al., 2011a). In seals and other wildlife species there is a lack of knowledge regarding the effects of individual compounds in OHC mixtures and their potential combined effects. There is a particular need for more knowledge regarding early-stage thyroid disruptive effects through maternally transferred contaminants in wildlife species. In a recent study of the same hooded seal mother–pup pairs investigated herein, some OH-PCBs appeared to be negatively associated with plasma TH ratios of pups but not in their mothers (Gabrielsen et al., 2011). Thus, the thyroid-related effects of OH-PCBs could be dependent on stage of development.

The aim of the present study was to examine the composition of the complex OHC mixture (including previously reported OH-PCBs) and identify the most potent contaminants influencing circulating thyroid hormone levels or ratios in hooded seal mothers and their pups. This study builds upon that of Gabrielsen et al. (2011) where principal component analysis (PCA) was used to demonstrate associative relationships between OH-PCBs, thyroid hormones and biological factors. Only a few of the strongest associations identified by PCA were confirmed by subsequent univariate tests (Gabrielsen et al., 2011). Recent multivariate regression modelling of TH levels of polar bears (*Ursus maritimus*) and white whales (*Delphinapterus leucas*) showed that they were associated with levels of lipid-soluble OHCs as well as biological factors (Villanger et al., 2011a,b). The models also identified the specific contaminants that were most important in explaining TH levels. In the present study, plasma from hooded seal mothers and pups was analysed for OCPs, PCBs, PBDEs and other brominated flame retardants (BFRs). Together with OH-PCBs and biological data from Gabrielsen et al. (2011), these new contaminant data were used in multivariate models to explore associations and thus potential effects on TH levels and ratios. The inclusion of a larger range of contaminants was expected to increase explanatory power regarding impacts of these contaminants on thyroid status and to provide a more complete picture of the role of OH-PCBs as thyroid disruptors, relative to other compounds in the measured OHC mixture. Also, by investigating hooded seal mother–pup pairs during the nursing period knowledge was gained regarding potential thyroid disruptive effects of maternally transferred OHCs in the pups relative to their mothers.

2. Methods

2.1. Sampling

Lactating hooded seal mothers and their recently born pups (1–4 d old) were live-captured in March 2008 in the West Ice, East of Greenland (approximately 73.30°N, 14.50°W). The pups had fed only on milk. Blood was collected and spun to prepare plasma and serum samples. Estimated pup age (d) based on developmental stage (Kovacs and Lavigne, 1992), sex of the pups and body masses (BMs) of pups and mothers were recorded. Procedures for capturing and sampling are described in more detail in Gabrielsen et al. (2011).

Blood was used for measurements of OHCs in the present study because it reflects the on-going mobilisation and transfer of phenolic and lipid-soluble OHCs from mother to pup via the milk. Circulating OHCs may have the potential to affect important TH target points in blood and reach other targets in the HPT axis.

2.2. Analyses of organohalogen contaminants

Chemical analyses of OHCs were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary