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

*RANDI GRØNNESTAD*[†#], *GRO D. VILLANGER*[‡#], *ANUSCHKA POLDER*[§], *KIT M. KOVACS*[//], *CHRISTIAN LYDERSEN*[//], *BJØRN M. JENSSEN*[#ж], *KATRINE BORGÅ*[†]*

[†]Department of Biosciences, University of Oslo, Oslo, Norway.

[‡]Department of Child Health, Norwegian Institute of Public Health, Oslo, Norway.

[§]Norwegian University of Life Sciences, Oslo, Norway.

[//]Norwegian Polar Institute, Fram Centre, Tromsø, Norway.

[#]Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway.

[ж]Department of Arctic Biology, The University Centre in Svalbard, Longyearbyen, Norway

Department of Biosciences, Aarhus University, Campus Roskilde, Denmark

*Address correspondence to katrine.borga@ibv.uio.no

## 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 have yet to be investigated. Previously, correlations were found between plasma thyroid hormone (TH) concentrations in hooded seals (*Cystophora cristata*), 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; FT3 levels and TT3:FT3 ratios increased with increasing PFCA levels. 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 ratio 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.



**KEYWORDS:** PFASs, , OHCs, , Arctic, Marine mammals, *Cystophora cristata*

**CAPSULE:** In a complex contaminant mixture including chlorinated and fluorinated organic compounds, perfluoroalkyl substances (PFASs) contribute to thyroid disruption in hooded seal mothers and pups

## 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 [1]. Many organohalogen contaminants (OHCs) and their metabolites affect multiple targets in the hypothalamus-pituitary-thyroid (HPT) axis (Figure 1) [2, 3].

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 [4, 5]. 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 [5].

Perfluoroalkyl substances (PFASs) have been shown to have endocrine disruptive effects and to disrupt the thyroid homeostasis in both experimental, human and wildlife studies [6-8]. The hooded seal (*Cystophora cristata*) is a predator that feeds at a high trophic level in the Arctic marine food web [9]. This results in high levels of persistent organic contaminants (POPs) [10, 11] due to biomagnification and with 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 [12].

Previous studies of contaminants in hooded seal mother-pup pairs found associations between various chlorinated and brominated contaminants and TH [10, 11]. 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 (Figure 1) that create a potential for combined effects of individual OHCs acting through similar or different modes of action [3, 13]. However, few studies have included PFASs when investigating such combined effects of OHCs on the thyroid system in wildlife [14-18].

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 [10-12].

## MATERIALS AND METHODS

### *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 (days) of the pups was estimated based on the developmental stage, and the body mass of both mothers and pups was measured to the nearest half kg. See Gabrielsen et al. [11] 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.

### *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), 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 c-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 perfluoroalky 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. [10] for OHCs, Gabrielsen et al. [11] for OH-metabolites, and Grønnestad et al. [12] for PFASs. Lipid content was determined gravimetrically [11], and protein content was determined using a modified Lowry's method [19].

*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. [11])

*Data analyses*

The following contaminants had concentrations below the limit of detection (LOD) in more than 40% of the samples from pups and mothers, and were excluded from statistical analysis: PCB-28, -31, -47, -56, -66, -87, -105, -114, -128, -136, -151, -157, -196, -199, c-HCH, trans-chlordane, o,p'-DDT, o,p'-DDD, PBDE-28, -99, -183, -206, 207, 208, -209, PBT, PBEB, DPTE, HBB, PTBPE, HBCD, PCP, TBP and all OH-PBDEs. In addition, the concentrations of PCB-74, -189, and PBDE-100 were below the detection limits in the mothers but not in the pups, and were thus excluded in the mothers. Contaminants with concentrations above LOD in more than 60% of pups or mothers were included in the statistical analyses, and missing values (i.e. below LOD) were assigned a random value between the LOD and zero. For the PFASs all samples were above LOD in both mothers and pups. Thus, the following compounds were included in the statistical analysis: α- and β- HCH, HCB, oxychlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, *p,p*'-DDE, *p,p*'-DDD, *p,p*'-DDT, Mirex, PCB congeners 52, 74, 99, 101, 110, 118, 137, 138, 141, 149, 153, 156, 170, 180, 183, 187, 189 (only pups), 194, 206 and 209. The PBDE congeners 47, -99, 100 (only pups), 153 and 154, the phenolic metabolites 4-OH-CB107, 3'-OH-CB138, 4-OHCB146, 4'-OH-CB172, 4-OH-CB187 and all PFASs mentioned above. The different contaminants were summarized in their respective groups, i.e. ΣPCBs, ΣHCHs, ΣHCLs, ΣDDTs, ΣPBDEs, ΣOH-PCBs, ΣPFSAs and ΣPFCAs, in the statistical analysis because of the large number of contaminants. We assumed that the mode of action (MoA) within each group were additive because of similar properties. Since the chlorinated and the brominated compounds have lipophilic properties, whereas the PFASs have

amphipathic properties, all concentrations are given in ng/g ww to compare actual plasma concentrations of these groups of chemicals. Levels of contaminant groups and THs are previously published, and are summarized in Table 1.

*Statistical analysis*

The program R (Ver 3.3.1) was used for all statistical analyses. Data were log-transformed prior to data analyses to reduce deviation from normality and homogeneity of variance. Normal distribution tested significant with Shapiro-Wilk's test after transformation. The $\alpha$ level was set to 0.05, and all tests were 2-tailed.

Multivariate analyses (principal component analyses; PCA and redundancy analyses; RDA) were carried out to analyse relationship and variance among the TH variables (response) and the explanatory variables (the contaminants and biometric variables). In the PCAs and RDAs, TH concentrations (TT3, FT3, TT4 and FT4) or TH ratios (TT3:FT3, TT4:FT4, TT4:TT3, FT4:FT3) were response variables. Explanatory variables (percent lipid, protein concentration, lactation period, body mass and contaminant group) were entered as passive variables in the PCA plots. Passive variables do not affect the ordination but are projected onto the unconstrained axes, allowing for visualization of correlations among response and explanatory variables. Variables were standardized to unit variance due to different units. The significance of the explanatory variables in describing the variation in THs among the samples in the multivariate ordination space was analyzed by forward permutation tests.

Based on the results from the PCAs and RDAs, general linear models (GLM) were used to quantify the amount of variance explained ($R^2$) by the respective single explanatory variables for the most important relationships.

## RESULTS AND DISCUSSION

The current paper presents a novel investigation of associations between PFASs and THs in the complex mixture of OHCs (i.e. chlorinated and brominated compounds and their metabolites and PFASs) in the plasma, and investigates the relative importance of these different compounds as possible thyroid disrupters in the seals. Furthermore, protein concentrations in the plasma was used as a predictor variable. Since PFASs, OH-PCBs, OH-PBDEs and THs are proteinophilic, the plasma protein level may affect the toxicokinetics and bioavailability of PFAS and OH-PCB/PBDE, and binding to thyroid transport proteins, such as transthyretin (TTR), thyroxin- binding globulin and albumin. The mean ΣPFSA and ΣPFCA concentrations were much higher than the other OHC contaminant groups in both mothers and pups, when comparing ww. levels (Table 1).

### *Associations in hooded seal mothers*

None of the tested explanatory variables (i.e. neither any of the contaminant groups or biological variables) significantly explained the overall variation in TH concentrations in the plasma of the hooded seal mothers (RDA, $p > 0.05$, Figure 2a). However, of the tested predictor variables, PFCAs were the best predictors in the model for TH concentration, explaining 15% of the variation in TH concentration in maternal plasma (Figure 1a). FT3 levels decreased with increasing PFCAs concentrations (GLM, $R^2 = 0.4$, $p = 0.008$). Furthermore, the TT3 and FT3 levels decreased with increasing PFSA levels (GLM, for TT3: $R^2 = 0.2$, $p = 0.04$; for FT3: $R^2 = 0.2$, $p = 0.04$).

PFASs may interfere with the thyroid hormone homeostasis via several mechanisms. Weiss et al. [20] suggested competitive binding of PFASs to TTR. Thyroid hormones are associated (not covalently) with the transport proteins TTR, thyroxin- binding globulin and albumin. These proteins function as a circulating reservoir to buffer changes in TH levels [21, 22]. The presence of PFASs in the blood would, according to Weiss' hypothesis, lead to

temporary increased concentrations of circulating free TH (FT4 and FT3) by competitive binding of the compounds to TTR, although the importance of this protein as a TH-carrier in pinniped blood is uncertain. The free fractions of THs would then be subjected to clearance, and subsequently a reduction in free and total TH levels in the blood would occur. This could theoretically explain the unadjusted GLMs (i.e. not adjusted for circulation protein levels) for PFCAs and FT3, and for PFSAs and TT3 and FT3 in the present study. However, as both THs and PFASs are proteinophilic, the levels of PFASs and thyroid hormones in the plasma might be influenced by the protein levels in the blood. Protein could therefore be a confounding variable, where apparent associations between thyroid hormones and PFASs are in reality simply a result of higher plasma protein levels. In the GLM adjusted for protein levels for maternal plasma herein, statistical significance disappeared for PFSAs and TT3 and FT3 levels (TT3: GLM, $p = 0.06$; FT3: GLM, $p = 0.07$). However, the negative relationship between PFCAs and FT3 remained significant (GLM, $R^2 = 0.4$, $p = 0.02$).

In the hooded seal mothers, none of the variables (neither the contaminant groups nor the biological variables) significantly explained the overall variation in TH ratios (RDA, $p > 0.05$). However, the best model included protein concentration, lipid content and PFCA concentration as predictor variables (Figure 2b), and explained 24% of the total variation. Regression analyses with the most important associations observed in the PCA plot, further support that TT3:FT3 ratios significantly increased with PFCAs levels (GLM, $R^2 = 0.4$, $p = 0.009$). This indicates that when PFCA in the plasma of the hooded seal mothers increases, more T3 is bound to proteins, relative to the free T3 fraction. The fact that the relationship between the PFCAs and FT3 remained after correction for plasma protein content (see above), indicates that for plasma FT3, the PFCA content in the blood is more important than the protein content. Furthermore, the lack of relationship between PFCAs and TT3 may indicate that the possible effect of PFCAs on FT3 is not caused by competitive binding of T3 to TTR [20].

The positive correlations between PFCAs and TT3:FT3 demonstrated in this study may be due to PFAS-induced biliary excretion of free T3 that is independent of competitive binding of T3 to plasma proteins. Thyroid hormone imbalance could include PFAS interference with glucuronidation or sulfation of T3, and subsequent excretion of free thyroid hormones [23, 24]. Contaminant-induced increases in glucuronidation has been reported in POP exposed rats [25, 26]. Sulfotransferases (SULT) assists sulfation, which is important for inactivation and excretion of T4 and T3. Studies have shown that OH-PCBs interfere with the sulfation of thyroid hormones in rat liver [26, 27]. Thus, the positive association between the PFAS concentrations and the TT3:FT3 ratio may be due to either direct clearance of FT3 from the plasma due to competitive binding with the PFASs to transport proteins, or an increased "active" SULT sulfation induced by the PFAS and/or other compounds and thus excretion of plasma FT3. It could also be a combination of these two mechanisms. It should be noted that the ability of PFASs to interfere with SULT to has our knowledge yet not been demonstrated.

In the hooded seal mothers, PFCAs was the contaminant group with the highest concentration, and which seemed to be the most important contaminant group when assessing the TH homeostasis in hooded seal mothers. However, HCHs were also important predictors for T3 concentrations and ratios (as shown in previous studies; [10]). While PFCAs and PFSAs were negatively associated with TT3 and FT3 concentrations, and TT3:FT3 ratios, HCHs correlated positively with these TH variables. According to the PCA plot (Figure 2a), PFASs and HCHs have opposite effects on the T3 homeostasis in the hooded seal mothers. This suggests that PFASs and HCHs may have antagonistic effects. However, several physiological steps within the HPT axis could be affected by these contaminants, and through dissimilar modes of action, so predicting potential antagonistic effects is challenging.

*Associations in hooded seal pups*

When investigating the TH concentrations in the pups, HCHs and the temporal point in the lactation period (age) were significant explanatory variables (RDA, $p = 0.007$), explaining 45% of the total variance in TH concentration (Figure 2c). T4 levels decreased with increasing HCH (GLM, TT4: $R^2 = 0.28$, $p = 0.02$ and FT4: $R^2 = 0.3$, $p = 0.02$). Further regression analysis with other apparent associations in the PCA plot showed that PFSAs was a significant predictor for the variation in TT4 and FT4 levels (GLM, TT4: $R^2 = 0.3$, $p = 0.02$; FT4: $R^2 = 0.28$, $p = 0.03$), where positive associations were identified.

When investigating the TH ratios in hooded seal pups, the HCHs and the PFSAs also significantly explained the variation in the ratios (RDA, $p = 0.002$), and explained 39% of the total variation (Figure 2d). The TT3:FT3 increased with increasing HCH levels (GLM, $R^2 = 0.73$, $p < 0.001$), opposite to what was found in the mothers. The previous studies on the same hooded seal individuals reported that both α-HCH and β-HCH were positively correlated with TT3:FT3 in hooded seal pups [10], and the same pattern was evident for most of the lipophilic POPs (see papers [10, 11]).

The results from the present study show that PFASs are important predictors for the TH-ratios in the hooded seal pups, as positive associations between PFASs and FT4:FT3 levels were identified (GLM, PFSA: $R^2 = 0.32$, $p = 0.02$; PFCA: $R^2 = 0.21$, $p = 0.04$). This concentration-dependent increase in the FT4:FT3 ratio could indicate that the PFSAs and PFCAs may inhibit the de-iodination of the prohormone, T4 to the active hormone, T3. Experimental and wildlife studies have shown that other POPs can inhibit or decrease deiodinase enzyme activity [28, 29], which would result in increased FT4:FT3 ratios. Another explanation for the positive associations between these compounds and the FT4:FT3 ratio, could, as discussed above for the hooded seal mothers, be due to competitive binding to transport proteins, or induction of SULT. Both these mechanisms would result in decreased plasma concentrations of FT3 and thus increased plasma FT4:FT3 ratios. A previous study on

the same hooded seal pups showed a negative association between 4-OH-CB107 and FT4:FT3 and 3-OH-CB138 and TT3:FT3 ratios [11]. However, these associations were not important in the mixture of contaminants, regarding TH-homeostasis in the hooded seal pups.

For both mother and pups, the observed relationships is probably a combination of the OHC mixture affecting multiple and overlapping target points in the HPT axis (Figure 1) which are difficult to distinguish. Although the HCHs seem to be the most potent TH-disruptors in hooded seal pups, PFASs also seem to affect their TH homeostasis. It is also worthwhile to notice that whereas the associations between the lipophilic HCHs and T4 in the pups were negative, the associations between the proteinophilic PFASs and T4 were positive. Such apparently contradictory, or possible antagonistic effects, of lipophilic chlorinated POPs and proteinophilic PFASs on THs have previously also been reported in glaucous gulls [17], and the present study provides additional indications of such interacting effects.

*Conclusion*

In the present study, we report on effects of a mixture of contaminants (OHCs, OH-metabolites and PFASs) on the thyroid homeostasis in hooded seal mothers and their pups.
In mothers, PFCAs seem to be the most important predictors for the thyroid hormone levels and ratios, while in pups, HCHs seem to be the most important predictors, followed by the PFSAs and PFCAs. Due to the proteinophilic nature of both PFASs and THs, plasma protein levels may be an important factor to consider in these relationships. However, it is important to bear in mind that this study is based on associations and that TH levels may vary with many biological factors, and we cannot draw any cause-effect conclusions. The results from this study add to the emerging evidence that PFASs may act as thyroid disrupting chemicals in Arctic wildlife species, also when assessed in a mixture-approach consisting of different POPs with thyroid disrupting potential.

*Acknowledgements*

The field sampling was conducted by the Norwegian Polar Institute. We thank M. Karimi and K. Løken at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences for assistance with the contaminant analyses.

*Funding*

The field sampling was financed by the Norwegian Polar Institute and the Norwegian Research Council (project 176477/S30). The chemical analyses were financed by the Norwegian University of Science and Technology (project 70115700/N31007) at the Department of Biology.

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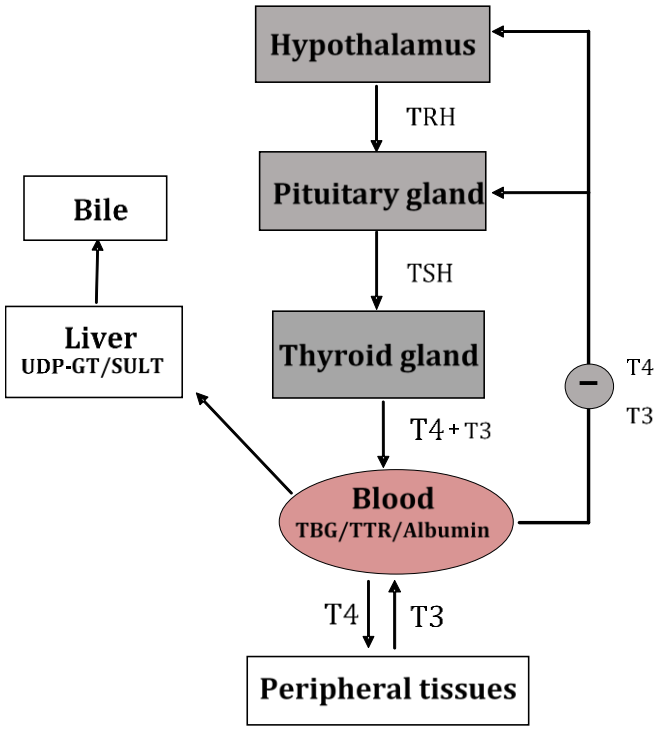
**Table 1.** Mean, median, minimum (Min) andmaximum (Max), levels of the different contaminant groups (ng/g ww) and thyroid hormones (pmol/L) in hooded seal mothers (n = 15) and pups (n = 15). Results have previously been published in Gabrielsen *et al.* 2011[a], Villanger *et al.* 2013[b] and Grønnestad *et al.* 2016[c].

| | Mothers | | | | Pups | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Median** | **Min** | **Max** | **Mean** | **Median** | **Min** | **Max** |
| **ΣPCBs** | 3.8 | 3.6 | 1.3 | 5.8 | 12 | 9.8 | 3.1 | 27 |
| **HCB** | 0.09 | 0.08 | 0.052 | 0.16 | 0.20 | 0.14 | 0.073 | 0.63 |
| **ΣHCHs** | 0.049 | 0.049 | 0.018 | 0.083 | 0.13 | 0.087 | 0.046 | 0.46 |
| **ΣCHLs** | 0.79 | 0.72 | 0.30 | 1.4 | 2.7 | 1.8 | 0.92 | 6.6 |
| **Mirex** | 0.10 | 0.078 | 0.020 | 0.22 | 0.29 | 0.22 | 0.061 | 0.79 |
| **ΣDDTs** | 1.8 | 1.8 | 0.47 | 2.8 | 7.3 | 5.5 | 1.9 | 18 |
| **ΣPBDEs** | 0.091 | 0.085 | 0.024 | 0.26 | 0.35 | 0.21 | 0.060 | 1.1 |
| **ΣOH-PCBs** | 1.4 | 1.3 | 0.34 | 2.0 | 0.68 | 0.71 | 0.14 | 1.1 |
| **ΣPFSAs** | 14 | 13 | 8.8 | 26 | 33 | 31 | 7.5 | 63 |
| **ΣPFCAs** | 22 | 20 | 13 | 41 | 32 | 31 | 11 | 57 |
| **TT4** | 16 | 16 | 7.8 | 21 | 16 | 16 | 8 | 21 |
| **TT3** | 0.78 | 0.79 | 0.59 | 1.1 | 0.78 | 0.79 | 0.59 | 1.1 |
| **FT4** | 3.9 | 3.6 | 1.3 | 5.8 | 3.9 | 3.6 | 1.3 | 5.8 |
| **FT3** | 0.56 | 0.56 | 0.15 | 0.96 | 0.56 | 0.56 | 0.15 | 0.96 |

## FIGURE CAPTIONS

**Figure 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.

**Figure 2.** Biplot of **a)** TH concentrations (TT4, FT4, TT3, FT3) and **b)** TH ratios (TT4:FT4, TT3:FT3, TT4:TT3, FT4:FT3) in plasma of hooded seal mothers (n = 15) and **c)** TH concentrations and **d)** TH ratios in plasma of hooded seal pups (n = 15). Explanatory variables are projected as passive arrows (blue solid line). The % of the total variance explained by each principal component (PCs) is given on each axis. The PCAs were based on logarithmically transformed concentrations. Direction and length of arrows indicate respective strength and increasing variance of loading.

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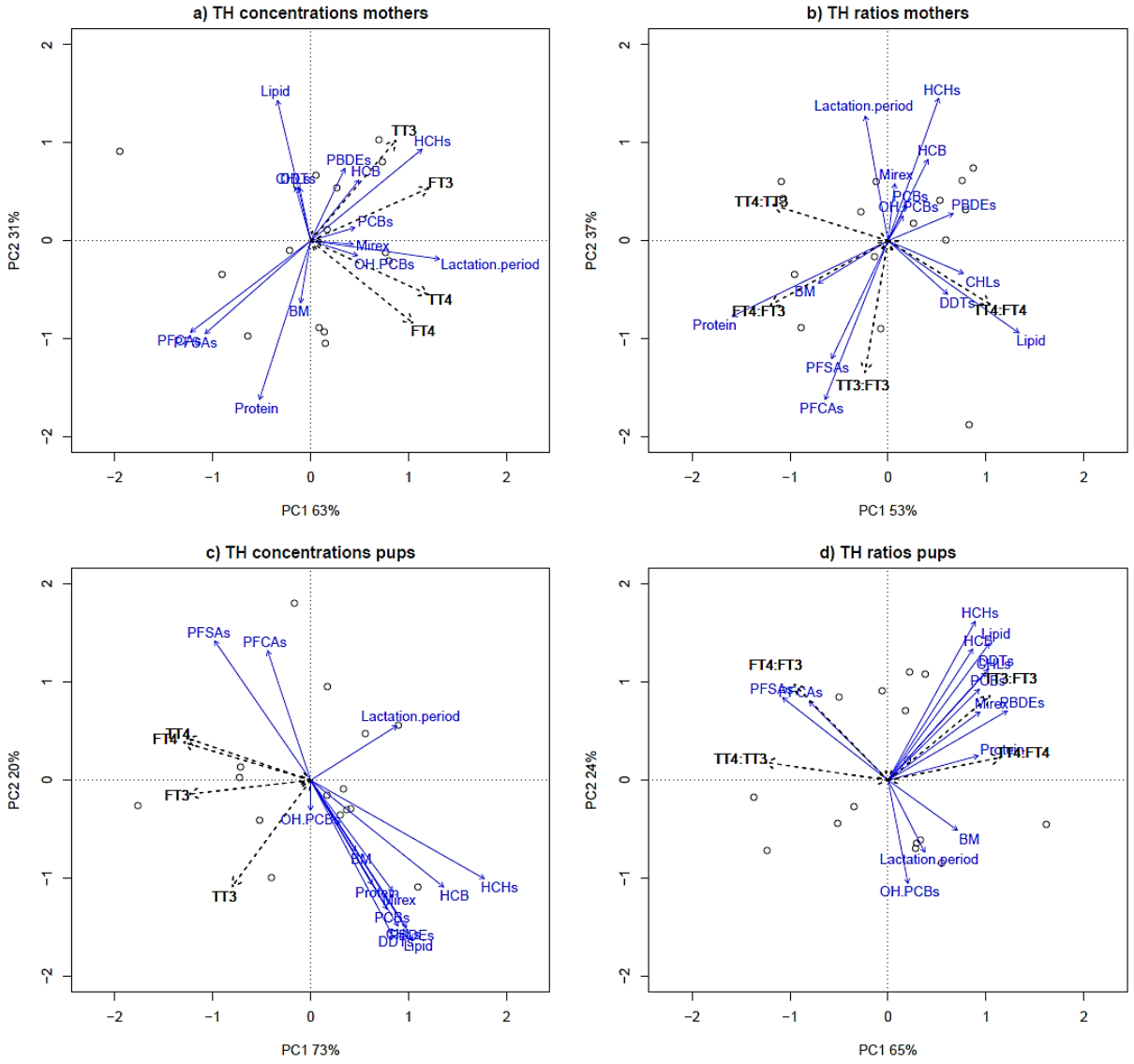


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## REFERENCES


1. Zoeller, R.T. and K.M. Crofton, *Thyroid hormone action in fetal brain development and potential for disruption by environmental chemicals.* Neurotoxicology, 2000. **21**(6): p. 935-946.
2. Colborn, T., F.S. vom Saal, and A.M. Soto, *Developmental effects of endocrine-disrupting chemicals in wildlife and humans.* Environmental Impact Assessment Review, 1994. **14**(5): p. 469-489.
3. Crofton, K.M., *Thyroid disrupting chemicals: mechanisms and mixtures.* International Journal of Andrology, 2008. **31**(2): p. 209-223.
4. Porterfield, S.P. and C.E. Hendrich, *The role of thyroid hormones in prenatal and neonatal neurological development - current perspectives.* Endocrine Reviews, 1993. **14**(1): p. 94-106.
5. Zoeller, R.T., S.W. Tan, and R.W. Tyl, *General background on the hypothalamic-pituitary-thyroid (HPT) axis.* Critical Reviews in Toxicology, 2007. **37**(1-2): p. 11-53.
6. Yu, W.-G., et al., *Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system.* Environmental Science & Technology, 2009. **43**(21): p. 8416-8422.
7. Lau, C., et al., *Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation.* Toxicological Sciences, 2003. **74**(2): p. 382-392.
8. Thibodeaux, J.R., et al., *Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: Maternal and prenatal evaluations.* Toxicological Sciences, 2003. **74**(2): p. 369-381.
9. Houde, M., et al., *Monitoring of perfluorinated compounds in aquatic biota: an updated review.* Environmental Science & Technology, 2011. **45**(19): p. 7962-7973.
10. Villanger, G.D., et al., *Effects of complex organohalogen contaminant mixtures on thyroid homeostasis in hooded seal (Cystophora cristata) mother–pup pairs.* Chemosphere, 2013. **92**(7): p. 828-842.

11. Gabrielsen, K.M., et al., *Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (Cystophora cristata) mother–pup pairs.* Aquatic Toxicology, 2011. **105**(3–4): p. 482-491.
12. Grønnestad, R., et al., *Maternal transfer of perfluoroalkyl substances in hooded seals.* Environmental Toxicology and Chemistry, 2016: p. n/a-n/a.
13. Crofton, K.M., et al., *Thyroid-Hormone–Disrupting Chemicals: Evidence for Dose-Dependent Additivity or Synergism.* Environmental Health Perspectives, 2005. **113**(11): p. 1549-1554.
14. Couderc, M., et al., *Thyroid endocrine status of wild European eels (Anguilla anguilla) in the Loire (France). Relationships with organic contaminant body burdens.* Science of The Total Environment, 2016. **550**(Supplement C): p. 391-405.
15. Bytingsvik, J., et al., *Transthyretin-binding activity of contaminants in blood from polar bear (Ursus maritimus) cubs.* Environmental Science & Technology, 2013. **47**(9): p. 4778-4786.
16. Berg, V., et al., *Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment.* Environmental Health Perspectives, 2017. **125**(1): p. 127-133.
17. Melnes, M., et al., *Dissimilar effects of organohalogenated compounds on thyroid hormones in glaucous gulls.* Environmental Research, 2017. **158**(Supplement C): p. 350-357.
18. Nøst, T.H., et al., *Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds.* Science of The Total Environment, 2012. **414**: p. 248-256.
19. Lowry, O.H., et al., *Protein measurement with the folin phenol reagent.* Journal of Biological Chemistry, 1951. **193**(1): p. 265-275.
20. Weiss, J.M., et al., *Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin.* Toxicological Sciences, 2009. **109**(2): p. 206-216.

21. Van den Berg, K.J., *Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin.* Chemico-Biological Interactions, 1990. **76**(1): p. 63-75.
22. Van den Berg, K.J., et al., *Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo.* Archives of Toxicology, 1991. **65**(1): p. 15-19.
23. Visser, T.J., *Role of sulfation in thyroid hormone metabolism.* Chemico-Biological Interactions, 1994. **92**(1–3): p. 293-303.
24. Brouwer, A., et al., *Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs.* Environmental Health Perspectives, 1999. **107**(Suppl 4): p. 639.
25. van Raaij, J.A.G.M., et al., *Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats.* Biochemical Pharmacology, 1993. **45**(3): p. 627-631.
26. Brouwer, A., et al., *Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health.* Toxicology and Industrial Health, 1998. **14**(1-2): p. 59-84.
27. Schuur, A.G., et al., *Effects of pentachlorophenol and hydroxylated polychlorinated biphenyls on thyroid hormone conjugation in a rat and a human hepatoma cell line.* Toxicology in Vitro, 1999. **13**(3): p. 417-425.
28. Gabrielsen, K.M., et al., *Thyroid hormones and deiodinase activity in plasma and tissues in relation to high levels of organohalogen contaminants in East Greenland polar bears (Ursus maritimus).* Environmental Research, 2015. **136**(Supplement C): p. 413-423.
29. Alvarez, L., et al., *The role of type I and type II 5' deiodinases on hexachlorobenzene-induced alteration of the hormonal thyroid status.* Toxicology, 2005. **207**(3): p. 349-362.