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Combined Effects of Persistent Organic Pollutants and Biological Variables on Vitamin D in Polar Bears

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Abstract

Because of long-range transport, the Arctic is chronically exposed to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), pesticides and brominated flame retardants, such as polybrominated flame retardants (PBDEs). Because of POPs are persistent and lipofilic, they are bioaccumulated in lipids and biomagnified in the food chains. The structures of some POPs resemble endogenous hormones, and have been shown to disrupt the TH homeostasis in animals. It has also been reported that POPs may affect the levels of vitamin D in seals, and thereby possibly disturb the calcium homeostasis and bone metabolism. The polar bear (*Ursus maritimus*) is a top predator in the Arctic and is exposed to high levels of POPs through its diet. However, the polar bear has a very good capacity to biotransform (metabolize) POPs, and because of the restrictions in the use of industrial chemicals and pesticides, the levels of POPs in polar bears from Svalbard have decreased during the last ten years. Still, polar bears have the potential to accumulate high levels of the most persistent congeners of PCBs.

The aim of this study was to investigate the combined interactive influences of POPs and thyroid hormones (THs: free T4 [FT4], total T4 [TT4], free T3 [FT3], total T3 [TT3]) on 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D$) in two groups of female polar bears in different physiologic status, (females with cubs of the year [FWCOY] and females without cubs of the year [FWOCOY]) and in males. Blood samples from eight FWCOY, 15 FWOCOY and 20 males were obtained from the population at Svalbard, Norway, in April and May of 2008. Plasma concentrations of $1,25(OH)_2D$ and THs were analysed by radioimmunoassay. Plasma concentrations of POPs were analysed using gas chromatography-mass spectrophotometry (GC-MS). Levels of $1,25(OH)_2D$, was examined in relation to contaminant load, biological and environmental factors by the use of principle component analysis (PCA), orthogonal projections to latent structures (OPLS) analysis, analysis of variance (ANOVA) and Pearson´s and Spearman´s rank correlation tests.

Levels of $1,25(OH)_2D$ did not differ significantly between the three groups of adult polar bears. In FWCOY, both multivariate data analysis and bivariate correlation tests indicated positive relationships between plasma levels of $1,25(OH)_2D$ and plasma levels of THs (FT3, FT3:TT3, FT4 and TT4), age and biometric variables (zygomatic width). Negative relationships were indicated between levels of $1,25(OH)_2D$ and two POPs (HCB and BDE-153). For FWOCOY, there were indicated positive relationships between $1,25(OH)_2D$ and mainly OH-PCBs, but also PCBs, PBDEs and pesticides seemed to be positively related to $1,25(OH)_2D$ levels. In contrast, one PBDE was negatively related to $1,25(OH)_2D$, but this compund was suspected to have high background levels. Thus, this particular relationship should be considered weak/semi-quantitative. None of the THs or the biometric variables were related to $1,25(OH)_2D$ levels in FWOCOY. However, it should be noted that in FWOCOY, the OPLS model was relatively weak, with fairly low explained variance. In males, both multivariate data analysis and bivariate correlation tests indicated positive relationships between $1,25(OH)_2D$ levels and THs (TT3 and TT4). Although in males the multivariate data analysis indicated that several of the contaminant compounds and some of the biometric variables were related to $1,25(OH)_2D$ levels, the bivariate correlation tests did not support that. It should also be noted that the OPLS model for males was weak, with low explained variance and low predictability. Thus, for males, no final conclusion can be drawn based on the model.

The results suggests possible vitamin D endocrine disrupting effects of mainly OH-PCBs on the most active metabolite of vitamin D3, $1,25(OH)_2D$. The results also indicate that there may be combined (interactive) effects of POPs and THs on plasma levels of $1,25(OH)_2D$ in polar bears. Because of the dominance of OH-PCBs in the plasma of both female and male polar bears, and their known thyroid hormone disrupting effects, the potential disrupting effects of OH-PCBs on the vitamin D endocrine system indicated in this thesis, suggest the need for further research.

Abbreviations

AG	Axillary girth
ANOVA	Analysis of variance
BCI	Body condition index
BM	Body mass
CV	Coefficient of Variation
CV-ANOVA	Cross validated analysis of variance
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
df	Degrees of freedom
EDC	Endocrine disrupting chemicals
FT3	Free triiodothyronine
FT4	Free thyroxine
FWCOY	Females with cubs of the year
FWOCOY	Females without cubs of the year
GC	Gas chromatograph
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HL	Head length
HPT	Hypothalamic-pituitary-thyroid axis
ID	Identification number
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
MS	Mass spectrometer
N	Number of observations
NTNU	Norwegian University of Science and Technology
OH-PCBs	Hydroxylated polychlorinated biphenyls
OPLS	Orthogonal projection to latent structures

p	Probability of rejecting the hypothesis
PBDEs	Polybrominated diphenyl ethers
PC	Principal component
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PLS	Projection to latent structures
POPs	Persistent organic pollutants
Q^2	Goodness of prediction coefficient
r	Correlation coefficient
RIA	Radioimmunoassay
R^2X	Explained variance
R^2Y	Goodness of fit, correlation coefficient
RPM	Rounds per minute
SD	Standard deviation
SL	Straight length
TBM	Total body mass
TH	Thyroid hormone
TT3	Total triiodothyronine
TT4	Total thyroxine
TSH	Thyroid stimulating hormone
UV	Unit variance
VIP	Variables importance in projections
w.w	Wet weight
ZW	Zygomatic width
$1,25(OH)_2D$	1,25-dihydroxyvitamin D3 (calcitriol)
$25(OH)D_3$	25-hydroxyvitamin D3 (calcifediol)

Contents

1. Introduction

Due to long-range transportation with air and ocean currents, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and others, biomagnify in the Arctic marine foodweb (Bustnes et al., 2010). As a result of their physiochemical properties, the POPs are resistant to biodegradation and will accumulate in wildlife (AMAP, 1998, Wania & Mackay, 1995). The Arctic wildlife is exposed to varying levels of natural stress due to the fluctuating external conditions (e.g. temperature, food-availability, sea-ice contidions). There has been detected high levels of POPs in the polar bear (*Ursus maritimus*) at Svalbard (Bernhoft et al., 1997). Thus, in addition to natural stress, polar bears are affected by anthropogenic stressors, such as POPs (Bustnes et al., 2008, Jenssen, 2006).

1.1 Persistent organic pollutants

POPs are mainly manufactured chemicals (e.g. PCBs), byproducts of industrial processes (e.g. hexachlorobenzene (HCB)), or pesticides (e.g. dichlorodiphenyltrichloroethanes (DDT), chlordane) (de March et al., 1998). There have been a decreasing trend in levels of legacy POPs in the Arctic biota during the last decade, a result of restrictions in production and use of industrial chemicals and pesticides from the 1970s up to now (Braune et al., 2005, Bustnes et al., 2010). However, it should be noted that several novel classes of POPs, such as brominated flame retardants (BFRs) and perflouralkyl substances (PFASs), are detected in increasing levels in Arctic biota (de Wit et al., 2010). Since POPs have toxic and endocrine disruptive effects (Jones & De Voogt, 1999, Letcher et al., 2010), there is an increasing concern about the effects of these compounds on the health of Arctic wildlife.

Although POPs are diverse with respect to their chemical structures, most of them have some elements in common, such as halogenated aromatic or aliphatic rings. They also share some typical physiochemical properties, like that they are highly lipophile, have relatively low

vapor pressure, that means they are semi-volatile, and they are resistant to biodegradation (AMAP, 1998, Borgå et al., 2004, Diamanti-Kandarakis et al., 2009). This explains why POPs are found in the Arctic, far away from the production or emission sources. Their lipophilicity and resistance to biodegradation make them bioaccumulate in Arctic animals, that generally have high lipid contents, such as seals, and POPs follow the lipid transfer from prey to consumer, a process called biomagnification (Borgå et al., 2004, de March et al., 1998). This, in addition to several biological factors such as trophic position, age, condition, body size, and seasonality, which also may affect the bioaccumulation and trophic transfer of POPs in the Arctic marine food web, leads to high concentrations in long-lived animals on top of the food chain, such as polar bears and glaucous gulls (*Larus hyperboreus*) (Borgå et al., 2004, Hop et al., 2002, Kelly et al., 2007, Letcher et al., 2010).

Polar bears are particularly vulnerable because their diet consist mainly of seal blubber, and especially the highly chlorinated PCBs are found to accumulate in polar bears and particularly in males (Bernhoft et al., 1997, Letcher et al., 1998). However, food web magnification depends on the individual species ability to biotransform POPs to more hydrophilic compounds, which are more readily eliminated (Hop et al., 2002, Kelly et al., 2007). Adult polar bears have an effective cytochrome P450 system and can therefore metabolize many of the POPs they ingest from their diet, such as most of the PCB congeners and DDT and its metabolites. The most persistent congeners of PCB can nevertheless reach very high concentrations (Bernhoft et al., 1997, Letcher et al., 1998).

1.2 Endocrine disruption

The ability that some POPs have to interfere with the endogenous hormone system in organisms is a process called endocrine disruption. Especially the conflict between POPs and the sex hormones or the thyroid hormones (THs) has received considerable attention the recent years (Diamanti-Kandarakis et al., 2009). Endocrine disrupting chemicals (EDCs) are chemicals that may bind to, or block, hormone receptors, alter synthesis, transport, or the metabolism of hormones, or interfere with signalling pathways of the hypothalamus-pituitary-endocrine gland axis (Dawson, 2000). The result of EDC activity is a change in the hormonal system that may disrupt the organisms ability to communicate with, and respond to its environment (Diamanti-Kandarakis et al., 2009).

It is also suggested that POPs can cause adverse effects on vitamin status, immune system, organ morphology and behaviour (Haave et al., 2003, Murvoll et al., 2005, Olsen et al., 2003, Routti et al., 2008, Skaare et al., 2001, Wiig et al., 1998).

1.2.1 Vitamin D

The vitamin D endocrine system plays an essential role in calcium homeostasis and bone metabolism. Cholecalciferol, or vitamin D3, which is categorized as a hormone rather than a vitamin (Horst et al., 2005), is either absorbed in the intestine from dietary uptake, or produced in the skin from 7-dehydrocholesterol (Figure 1). First it is metabolized in the liver, to calcifediol (also called 25-hydroxyvitamin D3 (25(OH)D)) an then further in the kidney to calcitriol (also called 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D$)), the most active metabolite of vitamin D3.

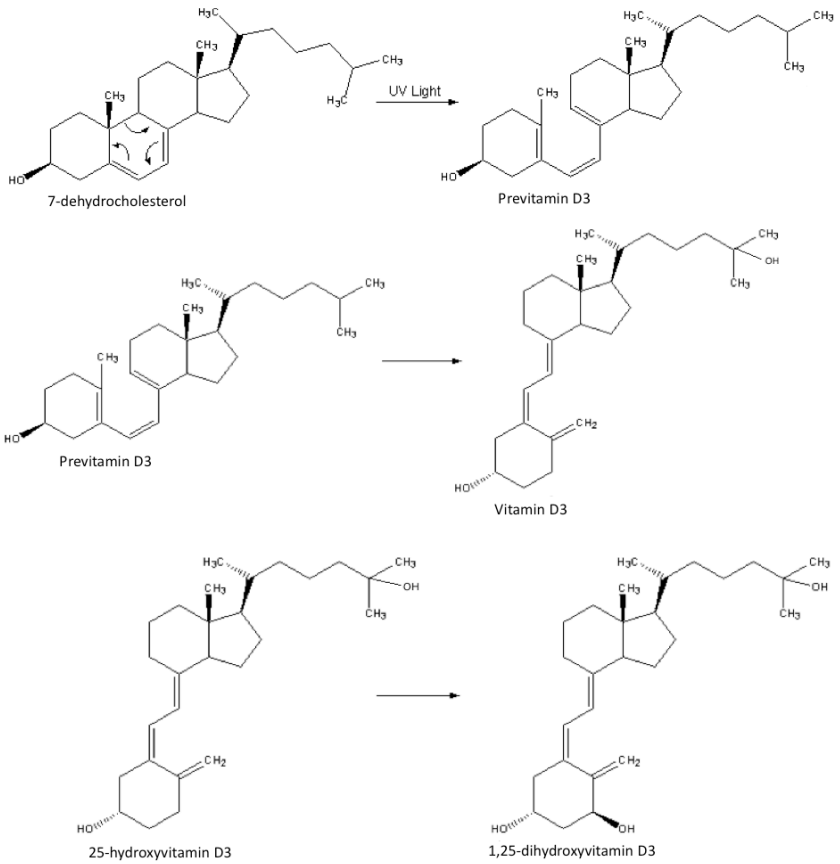

Figure 1: Vitamin D3 is synthesized from 7-dehydrocholesterol, a derivative of cholesterol, which is then photolyzed by ultraviolet light. The product is Previtamin D3, which spontaneously isomerizes to Vitamin D3 (cholecalciferol). Vitamin D3 is then hydroxylated in the liver to 25-hydroxycholecalciferol (calcifediol) and stored until it is needed. 25-hydroxycholecalciferol is further hydroxylated in the kidneys to the main biologically active form 1,25-dihydroxycholecalciferol (calcitriol) (Vieth, 2005)

Together with parathyroid hormone and calcitonin, $1,25(OH)_2D$ plays a major role in regulating serum calcium homeostasis (Vieth, 2005). A physiological level of $1,25(OH)_2D$ stimulates intestinal absorption and renal reabsorption of calcium and phosphate, which further stimulates bone mineralization (Yasuda et al., 2005). Low levels of $1,25(OH)_2D$ may therefore cause disruption of bone mineralization (Baylink et al., 1970, Faibish & Boskey, 2005). In a study conducted on Baltic grey seals (*Halichoerus grypus*), high levels of POPs were associated with deficiencies in bone structure, such as scull lesions (Bergman et al., 1992) and decreased bone density (Lind et al., 2003). Sonne et al (2006) also reported negative relationships between POPs and testis lenght and baculum bone mineral densities in male polar bears from East Greenland, and also between POPs and ovary lenght/weight and uterine horn lenght in females, which might pose a risk to the population regarding reproduction. Few studies have investigated contaminant effects on the vitamin D status, but in one study conducted with PCB-exposed rodents (Lilienthal et al., 2000), decreased levels of vitamin D3 metabolites were detected. Therefore it is possible that the bone disorders observed in seals and polar bears may be related to POP-induced effects on vitamin D homeostasis. According to another study by Routti et al. (2008), bone-related parameters and thyroid homeostasis may be disturbed by contaminants, because they might depress circulating $1,25(OH)_2D$ levels; however $1,25(OH)_2D$ deficiency could be compensated for by elevated TH levels to maintain serum calcium concentration (Mohan et al., 2004, Routti et al., 2008).

1.2.2 Thyroid hormones

All vertebrates are dependent on an appropriate production and function of thyroid hormones (THs) to achieve a normal development and physiological function (Yen & Chin, 1994). In mammals the THs control the thermoregulation, body mass, growth, lipid metabolism, reproduction, and secondary sex characteristics (Merryman & Buckles, 1998). The structure and mechanism by which THs are synthesized are the same among vertebrate species. The thyroid gland which is part of the hypothalamic-pituitary-thyroid axis (HPT), produces and releases thyroxine (T4), when stimulated by thyroid stimulating hormone (TSH), released from the pituitary. Further T4 is deiodinized mainly in the liver to triiodothyronine (T3), the most active TH (McNabb, 1992). Most of the circulating THs are associated with binding proteins. Several environmental contaminants have a high degree of structural similarity to the THs, and may interfere with the binding of THs to receptors or transport proteins, the

metabolism of THs, or affect the biosynthesis (Boas et al., 2006, Diamanti-Kandarakis et al., 2009, Routti et al., 2008, Zoeller, 2007). Several interactions have been discovered among toxic chemicals and the thyroid hormone system. The reported effects have been abnormal thyroid gland structure and altered levels of THs after exposure to POPs or their metabolites. The effects have been observed in a number of species (e.g. glaucous gulls (Verreault et al., 2004, Verreault et al., 2007), polar bears (Braathen et al., 2004, Skaare et al., 2001), gray seal pups (Sørmo et al., 2005) and rodents (Kato et al., 2010)).

1.3 The polar bears

The polar bear is the top predator in the Arctic food web. Their diet consist mainly of ringed seal (*Phoca hispida*), but also of bearded seal (*Erignathus barbatus*) and harp seal (*Phoca groenlandicus*) (Derocher et al., 2002). As their diet is lipid rich because of their large consumption of seal blubber, concern exist of potential health effects due to long-life exposure to high concentrations of POPs (Braathen et al., 2004, Bytingsvik et al., 2012, Oskam et al., 2003). Possible disturbances in thyroid hormone homeostasis have been reported in polar bears from Svalbard (Braathen et al., 2004). There is also concern about the possible reproductive effects in polar bears, due to reported negative correlations between environmental contaminants and sex hormones found in both male (Oskam et al., 2003) and female polar bears (Haave et al., 2003) at Svalbard. Furthermore possible effects on polar bears immune and vitamin system have been reported (Lie et al., 2004, Skaare et al., 2001). It is reasonable to believe that male polar bears will achieve higher levels of persistent contaminants than females with age, due to maternal transfer of lipophilic compounds from mother to offspring during lactation (Bernhoft et al., 1997, Bytingsvik et al., 2012).

1.4 Objectives

The main objectives of the study was to examine if the levels and prevalence of POPs (PCBs, hydroxylated PCB metabolites, pesticides and PBDEs), biometric variables, environmental (capture location) variables and TH variables were associated with levels of $1,25(OH)_2D$ in three groups of adult polar bears; females with cubs of the year (FWCOY), females without cubs of the year (FWOCOY) and males. It is hypothesized that there are associations between plasma concentrations of POPs and that there are interactions between plasma concentrations

of POPs, biometric variables, environmental variables and THs, and $1,25(OH)_2D$. Thus, the aim was to examine effects on $1,25(OH)_2D$, caused by either single POP compounds, or by their combined effects, or by interactions between POPs, biometric and environmental factors and THs.

2. Materials and methods

2.1 Sampling

In April 2008, blood samples were collected from 43 polar bears at Spitsbergen and Edgeøya (76.72 – 80.62° N, 12.10 – 23.70° E) at Svalbard, Norway. Blood were sampled from the femoral vein of eight females with cubs of the year (FWCOY), 15 females without cubs of the year (FWOCOY) and 20 male polar bears.

The polar bears were sedated by the remote injection of a dart (Palmer Cap-Chur Equipment, Douglasville, Georgia) filled with Zoletil® (200 mg/mL; Virbac Laboratories, Carros, France), fired from a helicopter. The individual amount of drug was determined based on an estimation of the bears bodyweight observed from the helicopter (5-10 mg/kg body mass). The blood was collected into heparinised Venoject® tubes (10 mL, Thermo Electron Corporation, Belgium) and separated into plasma and blood cells by centrifugation (3500 rpm, 10 min) within 8 h after sampling. Plasma samples were transferred to cryogenic vials and stored at – 20 °C in the field and then (after approximately four weeks) at – 70 °C in the lab freezer until analysis. Capture and handling procedures followed standard protocols (Derocher & Wiig, 2002, Stirling et al., 1989) and were approved by the National Animal Research Authority (The National Animal Research Authority (NARA), Oslo, Norway). After sedation, a selection of morphometric variabes, representing the bears body size and head size were collected. Straight lenght (SL), head lenght (HL), zygomatic width (ZW) and axillary girth (AG) were measured in all bears. Body mass (BM) was estimated based on SL and AG using a morphometric equation (Derocher & Wiig, 2002) before further recalculation into body condition index (BCI) using a BCI equation developed for polar bears (Cattet et al., 2002). For some of the bears, age was known because they had been caught previously. For the remaining ones, age was estimated by counting annual growth layers in the cementum of an extracted vestigial premolar (Calvert & Ramsay, 1998, Christensen-Dalsgaard et al., 2010).

Detailed information on sampling coordinates and age for the three groups are listed in Table A.1 (Appendix A).

2.2 Contaminant analysis

The analysis of organochlorinated and brominated compounds: chlorinated pesticides, PCBs, OH-PCBs and PBDEs, were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science (Oslo, Norway) using gas chromatography-mass spectrophotometry (GC-MS).

The polar bear plasma samples were quantified for the compounds presented in Table 1. The method used for extraction, clean up, analyses and information on quality assurance of PCBs and OH-PCBs is described by Bytingsvik et al. (2012) based on the method originally described by Brevik (1978). It should be noted that 4´OH-CB130 and 4´OH-CB-172 were detected in > 60 and 70 % of the individuals, respectively, but they both co-eluted with other OH-PCB isomers. Nevertheless, the results on these two compounds were included in the analysis. Methods used for extraction, clean up, analyses and quality control of PBDEs and chlorinated pesticides are described elsewere (Murvoll et al., 2005, Murvoll et al., 2006, Sørmo et al., 2006, Villanger, 2011). Briefly, the extracted samples were analysed for five PBDEs (*i.e. BDE-47, -153, -154, -183 and -209*) using a GC-MS. Limits of detection (LOD) for individual compounds were determined as three times the noise level. The detection limit for PBDEs were 0.02 ng/g wet weight (w.w). The concentrations of BDE-153, -183 and -209 were suspected to have high background levels, but results on these were nevertheless included in the analysis. Methods used for extraction, clean up, analyses and quality control of chlorinated pesticides are described elsewere (Miljeteig et al., 2009). Briefly, the extract samples were analysed for eight chlorinated pesticides (*i.e. oxychlordane, trans-nonachlor, mirex, HCB, α-HCH, β-HCH, p,p,´-DDT and p,p,´-DDE*) using a GC-MS. Limit of detection for individual compounds were determined as three times the baseline noise level. The detection limit for chlorinated pesticides ranged from 0.12-0.68 ng/g w.w. Plasma levels of contaminants are expressed as nmol/L.

Table 1: The organochlorinated and brominated contaminants and chlorinated pesticides analysed in plasma from polar bear (*Ursus maritimus*) at Svalbard. The abbreviation and IUPAC nomenclature are given.

Organochlorinated and brominated contaminants	
Abbreviation	**Analyte**
PCB-47	2,2',4,4' - Tetrachlorobiphenyl
PCB-74	2,4,4',5 - Tetrachlorobiphenyl
PCB-99	2,2',4,4',5 - Pentachlorobiphenyl
PCB-101	2,2',4,5,5' - Pentachlorobiphenyl
PCB-105	2,3,3',4,4' - Pentachlorobiphenyl
PCB-114	2,3,4,4',5 - Pentachlorobiphenyl
PCB-118	2,3',4,4',5 - Pentachlorobiphenyl
PCB-128	2,2',3,3',4,4' - Hexachlorobiphenyl
PCB-137	2,2',3,4,4',5 - Hexachlorobiphenyl
PCB-138	2,2',3,4,4',5' - Hexachlorobiphenyl
PCB-153	2,2',4,4',5,5' - Hexachlorobiphenyl
PCB-156	2,3,3',4,4',5 - Hexachlorobiphenyl
PCB-157	2,3,3',4,4',5' - Hexachlorobiphenyl
PCB-167	2,3',4,4',5,5' - Hexachlorobipheny
PCB-170	2,2',3,3',4,4',5 - Heptachlorobiphenyl
PCB-180	2,2',3,4,4',5,5' - Heptachlorobiphenyl
PCB-183	2,2',3,4,4',5',6 - Heptachlorobiphenyl
PCB-187	2,2',3,4',5,5',6 - Heptachlorobiphenyl
PCB-189	2,3,3',4,4',5,5' - Heptachlorobiphenyl
PCB-194	2,2',3,3',4,4',5,5' - Octachlorobiphenyl
PCB-206	2,2',3,3',4,4',5,5',6 - Nonachlorobiphenyl
4-OH-CB107	4-OH-2,3,3´,4´,5 - Pentachlorobiphenyl
4´OH-CB130	4´-OH-2,2´,3,3´,4´,5 - Hexachlorobiphenyl
3´OH-CB138	3´-HO-2,2´,3´,4,4´,5 - Hexachlorobiphenyl
4-OH-CB146	4-OH-2,2´,3,4´5,,5 - Hexachlorobiphenyl
4´OH-CB159	4-OH-2´,3,3´,4´,5,5 - Hexachlorobiphenyl
4´OH-CB172	4-OH-2,2´,3,3´,4´,5,5´ - Heptachlorobiphenyl
3´OH-CB180	3-OH-2,2´,3´,4,4´,5,5´ - Heptachlorobiphenyl
4-OH-CB187	4-OH-2,2´,3,4,5,5´,6´- Heptachlorobiphenyl
*Oxy*chlordane	*oxy*-chlordane
trans-Nonachlor	*trans*-Nonachlor
Mirex	1,1α,2,2,3,3α,4,5,5,5α,5β,6-dodecachloro-octahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene
HCB	Hexachlorobenzene
α-HCH	1α,2α,3β,4α,5β,6β-hexachlorocyclohexane
β-HCH	1α,2β,3α,4β,5α,6β-hexachlorocyclohexane
p,p,'-DDT	p,p,'-dichloro-α,α-diphenyl-β,β,β-trichloroethane
p,p,'-DDE	o,p,'-dichloro-diphenyl-dichloroethylene
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
BDE-153	2,2',4,4',5,5'-Hexabromobiphenyl ether
BDE-154	2,2',4,4',5,6'-Hexabromobiphenyl ether
BDE-183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether
BDE-209	Decabromodiphenyl ether

The Laboratory of Environmental Toxicology at The Norwegian School of Veterinary Science (Oslo, Norway) is accredited for the determination of several POPs in biological material of animal origin according to the requirements of NS-EN ISO/IEC 17025 (TEST 137). Determination of OH-PCBs is not an accredited method, but is validated after the same procedure as the accredited PCB-method. Detailed information on validation and quality assurance on POPs are given elsewere (Bytingsvik et al., 2012, Miljeteig et al., 2009, Murvoll et al., 2005, Murvoll et al., 2006, Sørmo et al., 2006, Villanger, 2011).

2.3 Radioimmunoassay

Radioimmunoassay (RIA) was applied to measure levels of vitamin D (e.g. $1,25(OH)_2D$) and thyroid hormones (e.g. TT4, free T4 [FT4], TT3 and free T3 [FT3]), in polar bear plasma. The analysis was conducted at Department of Biology, at Norwegian University of Science and Technology (NTNU).

The RIA-method is a specific and sensitive method based on the use of antibodies to measure concentrations of antigens (e.g. hormonelevels in the blood). This due to competitive binding to the antibody by unlabeled (analyte) and radioactive-labeled (tracer) antigens (hormones), when there is limited bindingsites available. The unlabelled antigen is the compound to be quantified, on the basis of the counts of the tracer-antibody complex, which are inversely proportional to the antigen concentration. Based on the counts of bound complexes, a standard curve is made. The calibration curve is then used to estimate the concentration of the unknown sample (Berson & Yalow, 1968).

2.3.1 Vitamin D analysis

Analysis of circulating $1,25(OH)_2D$ were conducted using RIA (Diasorin, Stillwater, MN, USA). A gamma-scintillation counter (Cobra Autogamma Counting System, Model 5003; Packhard Instrument, Dowers Grove, IL, USA) was used to detect the radioactivity of the sample. Samples were analysed as duplicates. The variance between parallels was 3.2 %, and the intra-assay variance was 15.5 %. The detection limit was 2.5 pg/ml.

2.3.2 TH analysis

Thyroid hormone kits (Coat-A-Count Total T4, Free T4, Total T3 and Free T3, Siemens Medical Solutions, Diagnostics, Los Angeles, CA, USA) were used to measure the concentrations of total thyroxin (TT4), free thyroxin (FT4), total triiodothyronine (TT3) and free triiodothyronine (FT3), in the polar bear plasma (Bertinussen, 2009). The results from the analyses in some of the females (ID: 23958, 23781, 23966, 23962, 23703, 23909 and 23924) are previously reported by Bertinussen (2009).

The analytical methods are described in detail by Bertinussen (2009). The kits consisted of antibody coated tubes, tracer (^{125}I labeled thyroxin or triiodothyronine) and calibrators. The software for the gammacounter (Spectra Works Spectrum Analysis Software, Meriden, USA) calculated a calibration curve based on the calibrator concentrations and the respective count numbers. All samples were analysed in duplicates (TT4, TT3 and FT3) and triplicates (FT4). The accepted variation between parallels was 15 %. The detection limits were < 1.33 nmol/L for TT4, < 0.31 nmol/L for TT3, < 1.29 pmol/L for FT4 and < 0.06 pmol/L for FT3. For information on quality assurance, see Bertinussen (2009).

2.4 Statistical methods

Simca P12+ (Version 12.0.1, Umetrics, Umeå, Sweden) and SPSS Statistical software (Version 20.0 for Mac, IBM, SPSS Inc., Chicago, IL) were used for the multivariate data analysis. Other calculations were performed using Microsoft Excel for Mac, 2011. Statistical significance was set as $p \leq 0.05$.

The data were analysed for normal distribution using the Shapiro-Wilk´s test ($n \leq 50$) in SPSS 20. Variables not normally distributet were $\log_{10}$ transformed to obtain normality. For female polar bears with cubs of the year (FWCOY), this was applied for the following variables: latitude, lipid %, condition, head length, zygomatic width, estimated total body mass (TBM), body condition index (BCI), oxychlordane, trans-nonachlor, Mirex, HCB, β-HCH, p,p´-DDE, PCB-47, -74, -99, -101, -137, -138, -156, -156, -157, -170, -180, -183, -187, -189, -194 and -206, 4-OH-CB107, 4´OH-CB130, 3´OH-CB138, 4´OH-CB159, 3´OH-

CB180, 4-OH-CB187, BDE-154, -183 and -209, 1,25$(OH)_2$D, cholesterol, TT4, FT4, TT3, FT3 and FT3:TT3 ratio, Σpesticides and ΣPCBs.

For females without cubs of the year (FWOCOY), the following variables were log_{10} transformed: latitude, longitude, condition, lipid %, trans-nonachlor, p,p´-DDE, p,p´-DDT, PCB-74, -101, -105, -114, -167, -170, -180, -183, -194, -206, 4-OH-CB107, 4´OH-CB130, 3´OH-CB138, 4-OH-CB187, BDE-153, -183 and -209, 1,25$(OH)_2$D, TT4, FT4, FT4:TT4 and TT3:TT4.

For males, the following variables were log_{10} transformed: latitude, condition, head lenght, oxychlordane, trans-nonachlor, α-HCH, β-HCH, p,p´-DDE, p,p´-DDT, PCB-47, -99, -105, -118, -128, -153, -156, -157, -167, -170, -180, -187, -189, -194, and -206, 4´OH-CB130, 3´OH-CB138, 4-OH-CB146, 4´OH-CB159, 3´OH-CB180, 4-OH-CB187, BDE-47, -153, -154, -183 and -209, testosterone, FT4, FT3, FT3:TT3, FT4:TT4, TT3:TT4, Σpesticides, ΣPCBs and ΣOH-PCBs.

ANOVA was used to test for between-group differences in contaminant compounds and groups, TH, 1,25$(OH)_2$D, cholesterol and lipid content (%). Correlations between 1,25$(OH)_2$D, THs, POPs and biometric variables were tested using Pearson`s correlation test (two-tailed) and Spearman`s rank correlation test (two-tailed), depending on whether the data was normally distributed or not, respectively. In the variables analysed, some of the non-detected values were replaced with random values below the limit of detection (LOD) of the chemical. This was applied for the following variables: α-HCH, p,p´-DDT, PCB-74, -101, -114, -128, -167, TT4 and FT3.

2.4.1 Principal component analysis (PCA)

In Simca P12+ (Umetrics, Umeå, Sweden) intercorrelations between variables were investigated using principle component analysis (PCA). The main goal was to investigate if the plasma 1,25$(OH)_2$D levels in the three groups of polar bears were correlated to biometric variables, THs or POP concentrations. In a PCA plot, the included variables are transformed to a dataset of uncorrelated variables orthogonally projected on each other, termed principle components (PCs), that explains the variance in the dataset. R^2 describes the degree of fit in the model (explained variance) and Q^2 describes the predictive ability of the model (predicted variance) (Eriksson et al., 2006). The variables were unit variance (UV) scaled, so that their

contribution to the final model is equal, independent of their original values. This is important in datasets were the numerical values for variables vary on a great scale. Mean-centering was also performed on the data to increase the models interpretability (the mean-value is substracted) (Eriksson et al., 2006). A critical validation of the PCA is essential. This is performed with respect to explained variance of each of the PCs and the models goodness of prediction. Significant PCs have eigenvalues > 1. Σconcentrations of the contaminant groups were not included in the PCA, this to avoid strong covariations.

2.4.2 Orthogonal projection to latent structures regression (OPLS)

Orthogonal projections to latent structures (OPLS) regression can be performed when the explanatory variables show a high degree of multicolinearity, such as in the present dataset, by using Simca P12+. OPLS is a statistical tool designed to perform multiple regression when somehow the number of observations are limited and when there is a high degree of colinearity.

The OPLS method can assess the relationship (positive or negative) between $1,25(OH)_2D$ and the most important predictor variables, and may thus identify combined effects of different POP compounds and biometric, environmental and TH variables on $1,25(OH)_2D$. Therefore, the OPLS complements the statistical series and may detect relationships when several explanatory variables affect the models, which is not displayed by PCA or a bivariate test. The indications from the PCA and correlation test were used to optimize the OPLS model. This, by removing some of the variables of low importance, i.e., with small regression coefficients and low VIP values, to see if significant OPLS regression models could be obtained for $1,25(OH)_2D$ (Wold et al., 2001).

The OPLS and PLS is a better tool than multiple regression when handling data with a high degree of colinearity and noise in *X* and *Y* matrix, according to Trygg and Wold (2002). The OPLS differs from its precursor PLS, in that an OPLS separates the variation in predictor variable *X* into variation which is correlating and non-correlating (orthogonal[90°]) with the variation in response variable *Y.* An OPLS is critical validated with respect to R^2X (explained variance) and R^2Y (goodness of fit), and a high goodness of prediction (Q^2) is essential. $R^2Y > 0.7$ and a $Q^2 > 0.4$ denote highly significant models when analyzing biological data

(Lundstedt et al., 1998). “Variable importance in projection” (VIP) plots denote the importance of each *X* variable in the predicted model and rank them in their explanatory power of *Y*. Combined with coefficient plots with jack-knifed confidence intervals, one can identify both the important and significant model predictors. VIP values > 1 is the most relevant variables explaining the *Y*. An “Analysis of variance of the cross-validated residuals” (CV-ANOVA) tests the significance of the OPLS regression.

A multiple linear regression was performed between $1,25(OH)_2D$ and some of the most correlated variables. This was performed in the default enter method in SPSS to examine the possible multicolinearity. The aim for the OPLS and multiple regressions was to investigate the combined effects from POPs, biometric, environmental and TH variables on $1,25(OH)_2D$.

3. Results

3.1 Biometric variables

Information on capture locations, estimated age (years) and biometric variables of the polar bears are presented in Table 2 below. The individuals ranged in ages from 4 to 21 years, wich defines them all as being adults. Individual biometric measurements are presented in Table B.1 (Appendix B).

Table 2: Capture location, age and biometric variables in female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY, n=8), females without cubs of the year (FWOCOY, n=15) and males (n=20), sampled in Svalbard (Norway) in 2008. Variables are presented as mean (X) ± standard deviation (SD), median and range (min – max).

	X ± SD	Median	Min - Max	n[c]
FWCOY				
Latitude (°)	78.4 ± 0.9	78.4	77.1 – 79.5	8
Longitude (°)	17.9 ± 2.6	17.8	13.9 – 21.9	8
Age (years)	10.12 ± 3.8	11	5 – 15	8
Condition (1=poor, 5=good)	2 ± 0	2	2 – 2	8
Straight length (cm)	194 ± 7	193	183 – 205	8
Axillary girth (cm)	113 ± 12	112	99 – 136	8
Head length (mm)	342 ± 8	344	329 – 351	8
Zygomatic width (mm)	198 ± 5	199	186 – 203	8
Estimated total body mass (kg)[a]	180 ± 37	174	139 – 260	8
Body Condition Index[b]	155 ± 14	154	138 – 184	8
FWOCOY				
Latitude (°)	78.6 ± 1.1	79.0	76.7 – 79.8	15
Longitude (°)	16.5 ± 3.7	16.1	12.1 – 22.3	15
Age (years)	9 ± 3	8	6 – 16	15
Condition (1=poor, 5=good)	3 ± 1	3	2 – 4	15
Straight length (cm)	195 ± 7	195	181 – 205	15
Axillary girth (mm)	112 ± 7	110	101 – 127	15
Head length (mm)	346 ± 10	345	331 – 366	15
Zygomatic width (mm)	200 ± 10	199	183 – 216	15
Estimated total body mass (kg)[a]	181 ± 26	170	134 – 237	15
Body Condition Index[b]	156 ± 11	152	135 – 177	15

Table 2. Continued

	X ± SD	Median	Min - Max	n[c]
Males				
Latitude (°)	78.9 ± 1.1	79.2	76.7 – 80.6	20
Longitude (°)	17.6 ± 3.1	18.1	12.6 – 23.7	20
Age (years)	13 ± 3	13	4 – 20	20
Condition (1=poor, 5=good)	3 ± 1	3	2 – 4	20
Straight length (cm)	232 ± 11	231	214 – 252	20
Axillary girth (cm)	152 ± 14	154	127 – 176	20
Head length (mm)	400 ± 18	402	358 – 439	20
Zygomatic width (mm)	255 ± 20	258	215 – 288	20
Estimated total body mass (kg)[a]	390 ± 81	396	255 – 539	20
Body Condition Index[b]	213 ± 16	215	183 – 239	20

[a] Estimated total body mass (BM) of the polar bears is based on the following equation: BM = 0.00003377 * axillary girth$^{1.7515}$ * straight length$^{1.3678}$ (Derocher & Wiig, 2002).
[b] Body Condition Index (BCI) of the polar bears were estimated based on the following equation: BCI = (ln body mass – 3.07 * ln straight length + 10.76)/(0.17 + 0.009 * ln straight length= (Cattet et al., 2002). ln = natural logarithm.
[c] Number of observations

3.2 Vitamin D and thyroid hormone levels

The serum levels of 1,25$(OH)_2$D, thyroid hormones, cholesterol and lipid content in the three groups are presented in Table 3 (FWCOY), Table 4 (FWOCOY) and Table 5 (males).

There were no significant difference in the 1,25$(OH)_2$D levels between the three groups (Table 6: ANOVA; $p > 0.05$). The average 1,25$(OH)_2$D levels found in the current study was 0.81 nmol/L in FWCOY, 0.68 nmol/L in FWOCOY and 0.56 nmol/L in males, with a standard deviation of 0.43 nmol/L, 0.16 nmol/L and 0.23 nmol/L, respectively (Table 3, 4 and 5). Individual levels of serum 1,25$(OH)_2$D are presented in Table C.1 (Appendix C).

For thyroid hormones, levels of T4 dominated over levels of T3 in all individuals, both for the bound and free fractions (Table 3, 4 and 5). Individual thyroid hormone levels are presented in Table D.1 (Appendix D).

Table 3: Lipid content (%), cholesterol levels (mmol/L), levels of $1,25(OH)_2D$ (nmol/L), total (nmol/L) and free (pmol/L) T3 and T4, and FT3:TT3, FT4:TT4, TT3:TT4 with mean (X) ± standard deviation (SD), median, range (min – max) and number of individuals with detectable levels (n) in plasma/serum samples of female polar bears with cubs of the year (FWCOY) from Svalbard (Norway), sampled in 2008.

	FWCOY (n=8)			
	X ± SD	Median	Min - Max	n[e]
Lipid	1.30 ± 0.23	1.21	1.0 – 1.59	8
Cholesterol	9.24 ± 1.98	9.25	5.4 – 11.5	8
$1,25(OH)_2D$	0.81 ± 0.43	0.61	0.33 – 1.52	8
THs				8
TT4[a]	18.6 ± 6.16	18.1	11.3 – 31.2	8
FT4[b]	7.04 ± 2.21	6.56	5.02 – 11.3	8
TT3[c]	1.10 ± 0.19	1.17	0.69 – 1.27	8
FT3[d]	0.40 ± 0.24	0.52	0.06 – 0.65	7
FT3:TT3	0.03 ± 0.02	0.04	0.01 – 0.06	8
FT4:TT4	0.03 ± 0.01	0.04	0.03 – 0.05	8
TT3:TT4	0.06 ± 0.01	0.06	0.03 – 0.10	8

[a] total thyroxine

[b] free thyroxine

[c] total triiodothyronine

[d] free triiodothyronine

[e] Number of samples with detectable concentrations.

Table 4: Lipid content (%), cholesterol levels (mmol/L), levels of $1,25(OH)_2D$ (nmol/L), total (nmol/L) and free (pmol/L) T3 and T4, and FT3:TT3, FT4:TT4, TT3:TT4 with mean (X) ± standard deviation (SD), median, range (min – max) and number of individuals with detectable levels (n) in plasma/serum samples of female polar bears without cubs of the year (FWOCOY) from Svalbard (Norway), sampled in 2008.

	FWOCOY (n=15)			
	X ± SD	Median	Min - Max	n[c]
Lipid	1.34 ± 0.21	1.36	0.82 – 1.56	15
Cholesterol	9.02 ± 1.61	9.1	5.7 – 13.1	15
$1,25(OH)_2D$	0.68 ± 0.16	0.65	0.41 – 1.05	15
THs				15
TT4[a]	16.7 ± 7.74	15.5	7.78 – 37.8	15
FT4[b]	7.84 ± 2.99	7.23	3.51 – 14.8	15
TT3[c]	1.28 ± 0.24	1.3	0.79 – 1.62	15
FT3[d]	0.60 ± 0.31	0.5	0.24 – 1.49	15
FT3:TT3	0.04 ± 0.01	0.04	0.03 – 0.09	15
FT4:TT4	0.04 ± 0.01	0.05	0.04 – 0.08	15
TT3:TT4	0.08 ± 0.03	0.08	0.04 – 0.18	15

[a] total thyroxine

[b] free thyroxine

[c] total triiodothyronine

[d] free triiodothyronine

[e] Number of samples with detectable concentrations.

Table 5: Lipid content (%), cholesterol levels (mmol/L), levels of $1,25(OH)_2D$ (nmol/L), total (nmol/L) and free (pmol/L) T3 and T4, and FT3:TT3, FT4:TT4, TT3:TT4 with mean (X) ± standard deviation (SD), median, range (min – max) and number of individuals with detectable levels (n) in plasma/serum samples of male polar bears from Svalbard (Norway), sampled in 2008.

	Males (n=20)			
	X ± SD	Median	Min - Max	n[e]
Lipid	0.82 ± 0.17	0.79	0.56 – 1.22	20
Cholesterol	6.29 ± 1.19	6.25	3.9 – 8.30	20
$1,25(OH)_2D$	0.56 ± 0.23	0.59	0.19 – 1.03	20
THs				20
TT4[a]	9.19 ± 4.39	8.31	1.33 – 18.9	20
FT4[b]	4.63 ± 1.67	4.38	1.51 – 8.58	20
TT3[c]	0.83 ± 0.21	0.77	0.52 – 1.34	20
FT3[d]	0.21 ± 0.18	0.17	0.06 – 0.7	20
FT3:TT3	0.02 ± 0.01	0.02	0.01 – 0.05	20
FT4:TT4	0.05 ± 0.02	0.05	0.03 – 0.11	20
TT3:TT4	0.11 ± 0.09	0.09	0.06 – 0.51	20

[a] total thyroxine

[b] free thyroxine

[c] total triiodothyronine

[d] free triiodothyronine

[e] Number of samples with detectable concentrations.

In the present study, plasma concentrations of TT4 and FT4 in males were significantly lower than in FWCOY and in FWOCOY (ANOVA; TT4: df = 2, F = 9.93, $p < 0.001$; FT4: df = 2, F = 8.9, $p = 0.001$). TT3 and FT3 levels were significantly lower in males compared to levels in FWCOY (TT3: ft = 2, F = 17.59, $p < 0.001$; FT3: ft = 2, F = 10.72, $p < 0.001$), but did not differ significantly from levels in FWOCOY. FT3:TT3 levels were significantly higher in FWOCOY compared to levels in males (FT3:TT3: ft = 2, F = 8.26, $p = 0.001$), but did not differ significantly from levels in FWCOY. Levels of FT4:TT4 were significantly higher in males compared to FWCOY (ft = 2, F = 3.74, $p = 0.032$), but not in comparison to levels in FWOCOY. There were no significant differences in TT3:TT4 levels between the three groups. Furthermore, there were no difference in any of the TH variables between FWCOY and FWOCOY (Table 6). Levels of cholesterol, and lipid content were significantly lower in males, in comparison with levels in females.

For more information on group differenses, see Table E.1 (Appendix E).

Table 6. Statistical differences between levels of $1,25(OH)_2D$ (nmol/L), cholesterol (mmol/L), lipid content (%), and total (nmol/L) and free (pmol/L) T4 and T3 in plasma of polar bears (FWCOY, FWOCOY and males) sampled in Svalbard (Norway) in 2008, given as F-statistics and significance level (*p*) from analysis of variance. For significant differences *p*-values are given in bold.

	ANOVA	
	F	*p*
$1,25(OH)_2D$	2.76	0.076
Cholesterol	**18.53**	**< 0.001**
Lipid (%)	**35.44**	**< 0.001**
THs		
TT4[a]	**9.93**	**< 0.001**
FT4[b]	**8.90**	**0,001**
TT3[c]	**17.59**	**< 0.001**
FT3[d]	**10.72**	**< 0.001**
FT3:TT3	**8.26**	**< 0.001**
FT4:TT4	**3.74**	**0.032**
TT3:TT4	1.83	0.173

[a] total thyroxine

[b] free thyroxine

[c] total triiodothyronine

[d] free triiodothyronine

3.3 Prevalence and levels of contaminants

The results of analysis are given in Table 7 (PCBs, OH-PCBs), Table 8 (chlorinated pesticides) and Table 9 (PBDEs). Forty-two contaminants were analysed, and PCBs and chlorinated pesticides were quantified in 100 % of the individuals. OH-PCBs and PBDEs were quantified in 60 % or more, of the individuals in the three groups of polar bears. 4'-OH-CB130 were not detected in 3 (37.5 %) of FWCOY, and was only detected in 12 (60 %) of the male individuals. For the PBDEs, BDE-47 and BDE-153 were detected in 7 (87.5 %) and 6 (75 %) of FWCOY, respectively. BDE-153 was detected in 14 (93.3 %) of the FWOCOY. For males, BDE-153, BDE-183 and BDE-209 were all detected in 19 (95 %) of the male individuals. The contaminant group that were present in the highest concentration in the plasma samples of female and male polar bears were, in decreasing order, Σ_8OH-PCBs > Σ_{21}PCBs > $\Sigma_8$pesticides > Σ_5PBDEs. Σ_8OH-PCBs was almost 2 times higher than Σ_{21}PCBs, and amost 7 times higher than $\Sigma_8$pesticides (FWCOY and FWOCOY), while Σ_8OH-PCBs was 17 times higher than $\Sigma_8$pesticides in males. The most prevalent congener of Σ_8OH-PCBs in plasma of all three polar bear groups were 4-OH-CB187, which constituted 49.6 %, 49.3 %, and 35.0 % of the total Σ_8OH-PCBs in FWOCOY, FWCOY and males, respectively. The Σ_{21}PCBs was the second largest contaminant group, of which PCB-153 was the most prevalent congener, constituting 39.0 %, 38.8 %, and 37.0 % of the Σ_{21}PCBs in FWCOY, FWOCOY and males, respectively. The plasma concentrations of pesticides and PBDEs (i.e $\Sigma_8$pesticides, Σ_5PBDEs) were low as compared to the concentrations of Σ_8OH-PCBs and Σ_{21}PCBs, and contributed in sum with < 1.6 % of the total contaminant burden, for all three polar bear groups.

Table 7. Concentrations of PCBs and OH-PCB (nmol/L), with mean (X), standard deviation (SD) and number of individuals with detectable levels (n) in plasma samples of polar bears (FWCOY, FWOCOY and males) from Svalbard (Norway), sampled in 2008.

	FWCOY (n = 8)		FWOCOY (n = 15)		Males (n = 20)	
	X ± SD	n[d]	X ± SD	n[d]	X ± SD	n[d]
PCB-47	0.53 ± 0.46	8	0.55 ± 0.34	15	0.54 ± 0.48	20
PCB-74[a]	0.38 ± 0.14	8	0.33 ± 0.08	14	0.31 ± 0.12	17
PCB-99	12.0 ± 12.5	8	11.7 ± 7.20	15	7.93 ± 5.31	20
PCB-101[a]	0.22 ± 0.24	4	0.21 ± 0.11	12	0.17 ± 0.11	11
PCB-105	0.24 ± 0.15	8	0.20± 0.10	15	1.00 ± 3.10	20
PCB-114[a]	0.07 ± 0.03	6	0.07 ± 0.04	15	0.06 ± 0.01	20
PCB-118	1.23 ± 0.66	8	1.19 ± 0.42	15	1.30 ± 0.69	20
PCB-128[a]	0.13 ± 0.11	7	0.14 ± 0.10	11	0.12 ± 0.09	12
PCB-137	1.15 ± 1.16	8	1.13 ± 0.67	15	0.68 ± 0.42	20
PCB-138	9.66 ± 10.1	8	10.7 ± 6.79	15	8.35 ± 5.79	20
PCB-153	60.4 ± 68.7	8	58.2 ± 36.4	15	48.3 ± 36.1	20
PCB-156	2.56 ± 2.80	8	2.21 ± 1.48	15	2.09 ± 1.27	20
PCB-157	1.66 ± 1.94	8	1.57 ± 1.41	15	2.11 ± 1.18	20
PCB-167[a]	0.06 ± 0.03	2	0.05 ± 0.04	5	0.04 ± 0.04	9
PCB-170	14.7 ± 16.3	8	15.0 ± 12.3	15	17.0 ± 12.9	20
PCB-180	36.8 ± 43.5	8	35.0 ± 21.1	15	28.7 ± 21.8	20
PCB-183	0.99 ± 1.13	8	1.00 ± 0.59	15	0.57 ± 0.40	20
PCB-187	0.19 ± 0.14	8	0.22 ± 0.11	15	0.14 ± 0.10	20
PCB-189	0.39 ± 0.37	8	0.38 ± 0.25	15	0.55 ± 0.37	20
PCB-194	9.74 ± 7.99	8	8.45 ± 3.98	15	9.22 ± 5.51	20
PCB-206	1.70 ± 1.12	8	1.51 ± 0.59	15	1.36 ± 0.73	20
4-OH-CB107	15.8 ± 13.6	8	9.41 ± 4.97	12	13.4 ± 8.39	19
4'-OH-CB130	0.51 ± 0.53	5	0.42 ± 0.18	12	0.51 ± 0.27	12
3'-OH-CB138	1.71 ± 1.19	8	2.11 ± 1.08	12	3.24 ± 2.13	19
4-OH-CB146	75.4 ± 41.9	8	66.1 ± 20.2	12	31.1 ± 21.7	19
4'-OH-CB159	2.00 ± 2.63	8	0.59 ± 0.28	12	0.66 ± 0.33	19
4'-OH-CB172	43.9 ± 31.5	8	46.9 ± 14.3	12	60.3 ± 19.8	19
3'-OH-CB180	2.43 ± 1.00	8	2.34 ± 1.02	12	5.58 ± 3.22	19
4-OH-CB187	137 ± 88.2	8	126 ± 52.0	12	78.5 ± 62.2	19
Σ_{21}PCBs[b]	155 ± 167		150 ± 91.2		131 ± 88.7	
Σ_{8}OH-PCBs[c]	279 ± 151		254 ± 82.4		225 ± 104	

[a] Missing values were given a random number between zero and limit of detection (LOD) and included in the following statistics.

[b] Σ_{21}PCBs include PCB-47, -74, -99, -101, -105, -114, -118, -128, -137, -138, -153, -156, -157, -167, -170, -180, -183, -187, -189, -194 and -206.

[c] Σ_{8}OH-PCBs include 4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187.

[d] Number of samples with detectable concentrations.

Table 8. Concentrations of chlorinated pesticides (nmol/L), with mean (X), standard deviation (SD) and number of individuals with detectable levels (n) in plasma samples of polar bears (FWCOY, FWOCOY and males) from Svalbard (Norway), sampled in 2008.

	FWCOY (n = 8)		FWOCOY (n = 15)		Males (n = 20)	
	X ± SD	n[c]	X ± SD	n[c]	X ± SD	n[c]
HCB	3.23 ± 2.62	8	3.56 ± 3.00	15	5.57 ± 4.77	20
α-HCH[a]	0.08 ± 0.04	6	0.12 ± 0.04	14	0.07 ± 0.02	17
β-HCH	0.72 ± 0.42	8	0.81 ± 0.46	15	1.07 ± 0.70	20
Oxychlordane	34.4 ± 35.7	8	30.4 ± 20.8	15	3.89 ± 2.65	20
Trans-nonachlor	1.23 ± 0.96	8	1.49 ± 0.99	15	1.08 ± 0.77	20
Mirex	0.23 ± 0.14	8	0.18 ± 0.07	15	0.13 ± 0.04	20
p,p'-DDE	1.05 ± 2.01	8	0.81 ± 0.43	15	0.80 ± 0.67	20
p,p'-DDT[a]	0.32 ± 0.31	4	0.32 ± 0.44	8	0.30 ± 0.48	7
Σ_8 pesticides[b]						

[a] Pesticides with missing values were given a random number between zero and limit of detection (LOD) and included in the following statistics.

[b] Σ_8 pesticides include HCB, α-HCH, β-HCH, oxychlordane, *trans*-nonachlor, mirex, p,p'-DDE and p,p'-DDT.

[c] Number of samples with detectable concentrations.

Table 9. Concentrations of polybrominated diphenyl ethers (PBDEs) (nmol/L), with mean (X), standard deviation (SD) and number of individuals with detectable levels (n) in plasma samples of polar bears (FWCOY, FWOCOY and males) from Svalbard (Norway), sampled in 2008.

	FWCOY (n = 8)		FWOCOY (n = 15)		Males (n = 20)	
	Mean ± SD	n[c]	Mean ± SD	n[c]	Mean ± SD	n[c]
BDE-47	0.49 ± 0.31	7	0.43 ± 0.25	15	0.18 ± 0.12	20
BDE-153[a]	0.11 ± 0.05	6	0.13 ± 0.06	14	0.10 ± 0.04	19
BDE-154	0.23 ± 0.23	8	0.21 ± 0.13	15	0.17 ± 0.12	20
BDE-183	0.16 ± 0.19	8	0.40 ± 0.37	15	0.30 ± 0.27	19
BDE-209[a]	0.04 ± 0.01	8	0.04 ± 0.03	15	0.06 ± 0.05	19
Σ_5PBDEs[b]						

[a] Detected concentrations are close to the detection limit.

[b] Σ_5PBDEs include BDE-47, BDE-153, BDE-154, BDE-183 and BDE-209.

[c] Number of samples with detectable concentrations.

Plasma concentrations of oxychlordane, 4-OH-CB146 and BDE-47 in males were significantly lower than in FWCOY and FWOCOY (ANOVA; oxychlordane: df = 2, F = 53.84, $p < 0.001$; 4-OH-CB146: df = 2, F = 11.37, $p < 0.001$; BDE-47: ft = 2, F = 11.07, $p < 0.001$). PCB-183 levels were significantly lower in males, compared to FWOCOY (ft = 2, F = 3.48, $p = 0.041$) but did not differ significantly in comparison to levels in FWCOY. Levels of 4'-OH-CB159 and mirex were significantly lower in males compared to FWCOY (4'-OH-CB159: ft = 2, F = 5.03, $p = 0.012$; mirex: ft = 2, F = 3.74, $p = 0.032$) but were not significantly lower compared to levels in FWOCOY. Levels of 3'-OH-CB138 were significantly higher in males compared to FWCOY (ft = 2, F = 4.72, $p = 0.015$), but did not differ significantly from levels in FWOCOY. There were no significant differences in the other compound levels between the three groups (Table 10).

Table 10. Statistical differences between levels of PCBs (nmol/L), OH-PCBs (nmol/L), pesticides (nmol/L), PBDEs (nmol/L), Σ_{21}PCBs, Σ_{8}OH-PCBs, Σ_{8}pesticides and Σ_{5}PBDEs in plasma of polar bears (FWCOY, FWOCOY and males) sampled in Svalbard (Norway) in 2008, given as F-statistics and significance level (*p*) from analysis of variance. For significant correlations *p*-values are given in bold.

	ANOVA	
	F	*p*
PCB-47	0.32	0.726
PCB-74	0.89	0.417
PCB-99	2.01	0.148
PCB-101[a]	0.90	0.413
PCB-105	2.88	0.068
PCB-114[a]	0.62	0.542
PCB-118	0.25	0.782
PCB-128[a]	0.31	0.738
PCB-137	2.91	0.066
PCB-138	0.96	0.392
PCB-153	0.70	0.503
PCB-156	0.03	0.973
PCB-157	2.56	0.090
PCB-167[a]	1.26	0.295
PCB-170	0.66	0.521
PCB-180	0.67	0.516
PCB-183	3.48	**0.041**
PCB-187	2.53	0.093
PCB-189	1.74	0.189
PCB-194	0.04	0.958
PCB-206	0.32	0.731
4-OH-CB107	0.93	0.403
4'-OH-CB130	0.27	0.764
3'-OH-CB138	4.72	**0.015**
4-OH-CB146	11.4	**< 0.001**
4'-OH-CB159	5.03	**0.012**
4'-OH-CB172	2.95	0.065
3'-OH-CB180	13.2	**< 0.001**
4-OH-CB187	3.57	**0.038**
HCB	1.31	0.281
α-HCH[a]	1.51	0.233
β-HCH	1.72	0.193
Oxychlordane	53.8	**< 0.001**
Trans-nonachlor	0.69	0.509
Mirex	3.74	**0.032**
p,p'-DDE	1.03	0.367
p,p'-DDT	0.09	0.912
BDE-47	11.1	**< 0.001**
BDE-153[b]	1.28	0.291
BDE-154	0.71	0.497
BDE-183	1.78	0.181
BDE-209[b]	1.48	0.240
Σ_{21}PCBs		
Σ_{8}OH-PCBs		
Σ_{8}pesticides		
Σ_{5}PBDEs		

[a] Missing values were given a random number between zero and limit of detection (LOD) and included in the following statistics.

[b] Detected concentrations are close to the detection limit.

3.4 Relationships between vitamin D levels, biometric variables, THs and POPs

3.4.1 Females with cubs of the year, FWCOY

Principal component analysis

The analysis resulted in a PCA model (Figure 2) with 2 significant principal components. PC1 and PC2 explained 49.1 and 15.5 % of the variation, respectively (R^2X = 0.647, Q^2 = 0.144). Since all observations were within the Hotellings T2 range, no outliers were present. The loadings of PCA indicated that several of the contaminants were clustered together along PC1 (PC1 = 0.145 – 0.185), and were distinctly separated from $1,25(OH)_2D$. The plasma levels of $1,25(OH)_2D$ were positively associated with thyroid hormones (FT3, FT3:TT3, TT4, FT4), longitude and age. In addition, $1,25(OH)_2D$ was negatively associated with HCB, BDE-153, PCB-128, straight length, 4-OH-CB187 and lipid%. FT4:TT4 was oriented towards the center of the plot, indicating that it was less important to the model. TT3:TT4 were located along PC2 and thus negatively associated with p,p´-DDE. The levels of TT3 was oriented along PC1 and thus negatively associated with several PCBs (PCB-167, -187, -194 and -206), BDE-47, β-HCH and mirex.

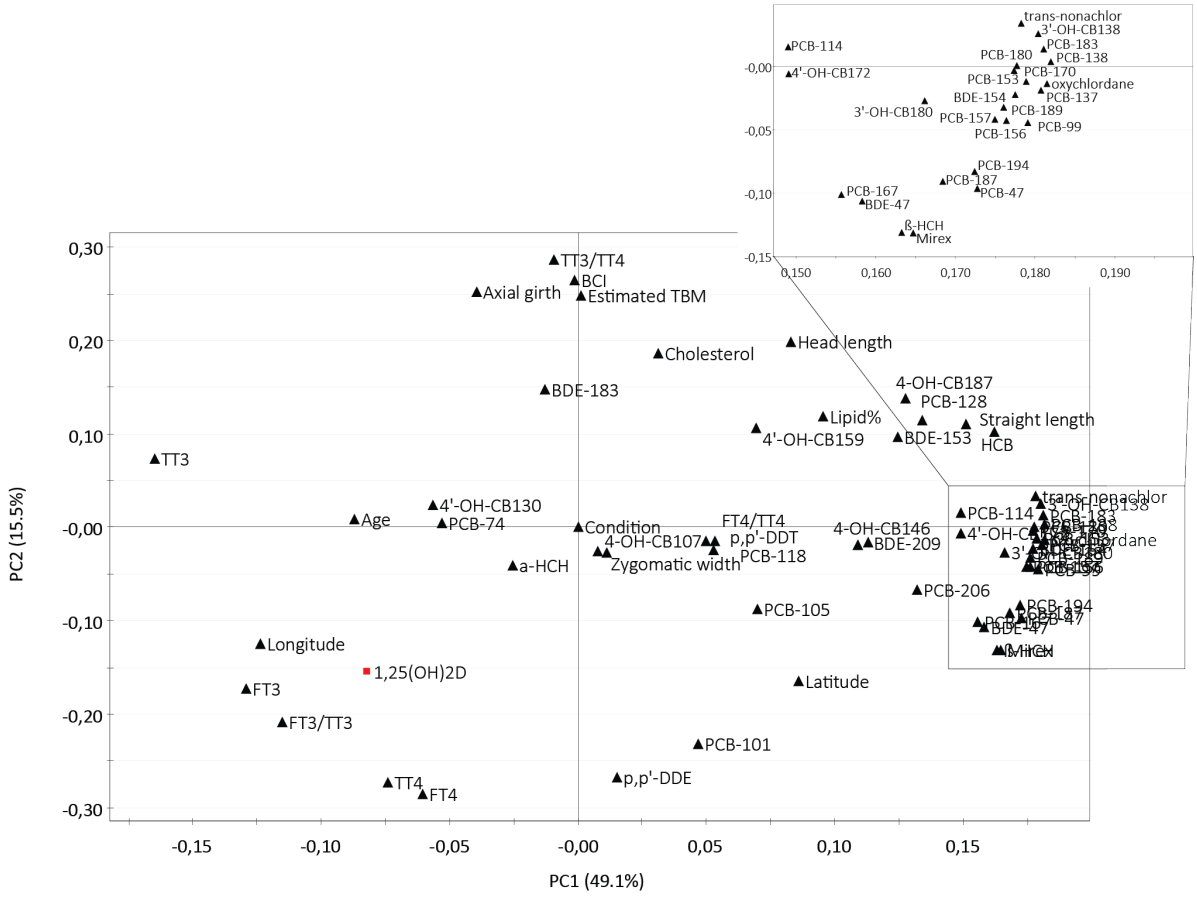

Figure 2: PCA loading plot of the relationships among the observations and the variables in female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY) (n = 8), from Svalbard. A total of 62 variables were included in the model; latitude, longitude, age, condition, straight length, axillary girth, head length, zygomatic width, total body mass, body condition index, lipid content, cholesterol, 8 pesticides, 21 PCBs, 8 OH-PCBs, 5 PBDEs, THs and 1,25$(OH)_2$D.

Orthogonal Projections to Latent Structures (OPLS)

To explore further the relations observed from the PCA-model, a single Y OPLS-model was applied to investigate the relationships between predictor variables (THs, contaminants, biological and environmental factors) and 1,25$(OH)_2$D (Y) (Figure 3). Stepwise removal of the variables of least importance resulted in a significant model (Wold et al., 2001). The OPLS model predicted that the variation in 1,25$(OH)_2$D was significantly explained by 12 variables (R^2X = 0.55, R_2Y = 0.84 and Q^2 = 0.73, CV-ANOVA; p = 0.042). In PLS models, $R^2Y > 0.7$ and a $Q^2 > 0.4$ denote highly significant models when analyzing biological data (Lundstedt et al., 1998). The highest VIP (variable importance in projection) value was

shown by BDE-153, followed by FT3, age, FT3:TT3 ratio, FT4 and HCB (VIP > 1). Hence, these variables were considered to be the most important for explaining the variation in $1,25(OH)_2D$. The information from the variable importance plot (VIP) (Figure 3) is complemented with a coefficient plot (Figure 4). These two plots summarize the overall contribution from each *X* variable, indicating which are correlated with *Y* ($1,25(OH)_2D$), and identifies if the direction of the relationship (between *Y* and the *X* variables) is positive or negative. The coefficient plot showed that FT3, age, FT3:TT3 ratio and FT4 were positively correlated to $1,25(OH)_2D$. In contrast BDE-153 and HCB were inversely associated with $1,25(OH)_2D$ levels in FWCOY (Figure 4). Further testing, using bivariate correlations, confirmed that FT3 ($p = 0.004$, $r_s = 0.879$, n = 8), age ($p = 0.018$, $r_p = 0.797$, n = 8), FT3:TT3 ($p = 0.022$, $r_s = 0.783$, n = 8), FT4 ($p = 0.048$, $r_s = 0.711$, n = 8), and also zygomatic width ($p = 0.000$, $r_s = 0.945$, n = 8) and TT4 ($p = 0.036$, $r_p = 0.739$, n = 8) were positively correlated with $1,25(OH)_2D$ levels. In contrast, BDE-153 ($p = 0.005$, $r_p = -0.941$, n = 6) and HCB ($p = 0.033$, $r_s = -0.747$, n = 8) were inversely correlated with $1,25(OH)_2D$ levels in FWCOY. It should, however, be noted that the BDE-153 concentrations were suspected to have high background levels (see discussion). The PCA plot (Figure 2) indicated negative relationships between PCBs and $1,25(OH)_2D$. However, further correlation tests showed that none of the PCBs correlated with $1,25(OH)_2D$.

The linear relationships between $1,25(OH)_2D$, FT3 and age are presented graphically in Figure 5.

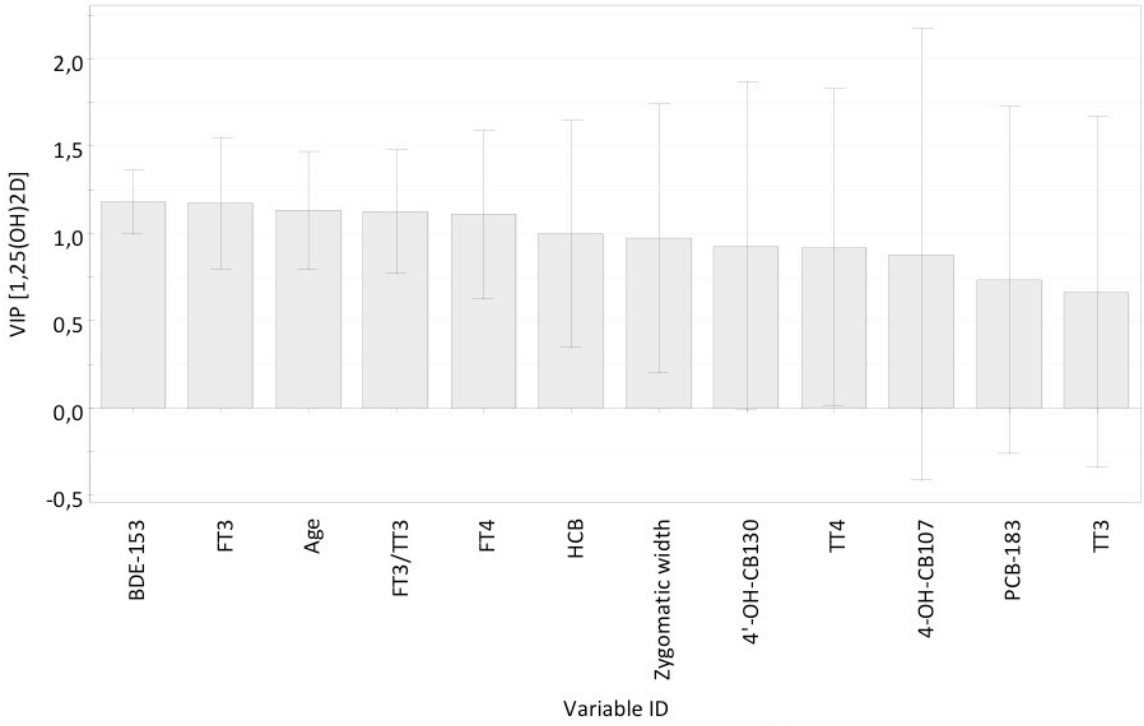

Figure 3: Orthogonal projections to latent structures (OPLS) regression VIP plot with important variables for the explanation of $1,25(OH)_2D$ levels in female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY) (n = 8), from Svalbard. Variables with VIP > 1 are of high importance in the model. All variables are shown with default jack-knife confidence intervals, where larger intervals that cross the 0 line represent the lower reliability. For identification of abbreviations, see Table 1 (POPs) and Table 3 (THs).

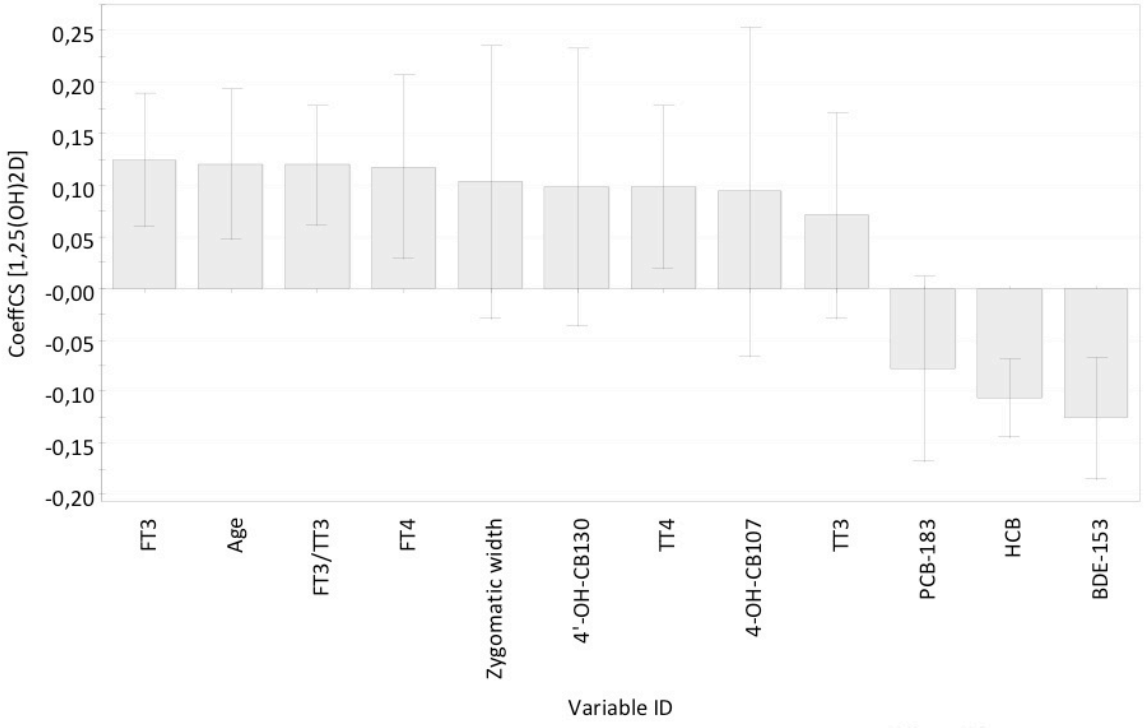

Figure 4: Orthogonal projections to latent structures (OPLS) regression coefficient plot summarizing the relationship between the contaminant levels, TH levels, biological and environmental factors (X-variables) on plasma/serum 1,25$(OH)_2$D levels (Y-variable) in female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY) (n = 8), from Svalbard. Negative coefficients represent inverse relationships and positive coefficients represent positive relationships of the X-variables with 1,25$(OH)_2$D levels. Variables with the highest absolute coefficient values are considered to be most important in explaining the Y-variable 1,25$(OH)_2$D. Jack-knife confidence intervals that cross the 0 line indicate that the predictor is of low importance to the model. For identification of abbreviations, see Table 1 (POPs) and Table 3 (THs).

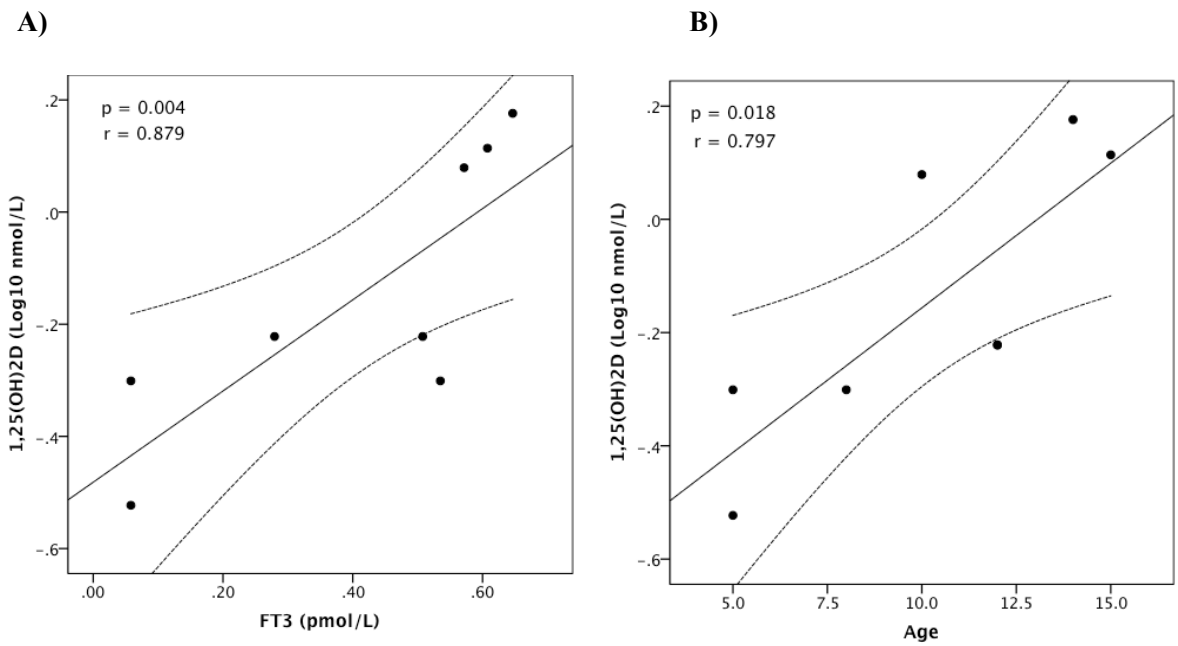

Figure 5: The linear relationship (± 95% confidence interval) between $1,25(OH)_2D$ serum concentrations (nmol/L) and A) FT3 (pmol/L) and B) Age in female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY) (n = 8), from Svalbard, sampled in 2008. The Pearson correlation, p, and r-values are shown in the plot.

3.4.2 Females without cubs of the year, FWOCOY

Principal Component Analysis

The analysis resulted in a PCA model (Figure 6) with 3 significant principal components. PC1 and PC2 explained 35.1 and 14.7 % of the variation, respectively (R^2X = 0.618, Q^2 = 0.052). PC3 explained 1.2 % of the variation. Since all observations were within the Hotellings T2 range, no outliers were present. The loadings of PCA indicated that several of the contaminants were clustered together along PC1 (PC1 = 0.180 – 0.205), and thus, seemed to be associated to the plasma levels of $1,25(OH)_2D$. $1,25(OH)_2D$ grouped closely together with PCB-128, 4-OH-CB187 and 4´OH-CB159, thus indicated a positive relationship between these. In addition, $1,25(OH)_2D$ was negatively associated with BDE-209 and zygomatic width. FT4, TT3 and TT4 were oriented towards the center of the plot, indicating that these were less important to the model. TT3:TT4 was located along PC1 and thus negatively associated with p,p´-DDE. The levels of FT3, FT4:TT4 and FT3:TT3 were clustered together along PC2 and thus negatively associated with PCB-74, BDE-153 and α-HCH.

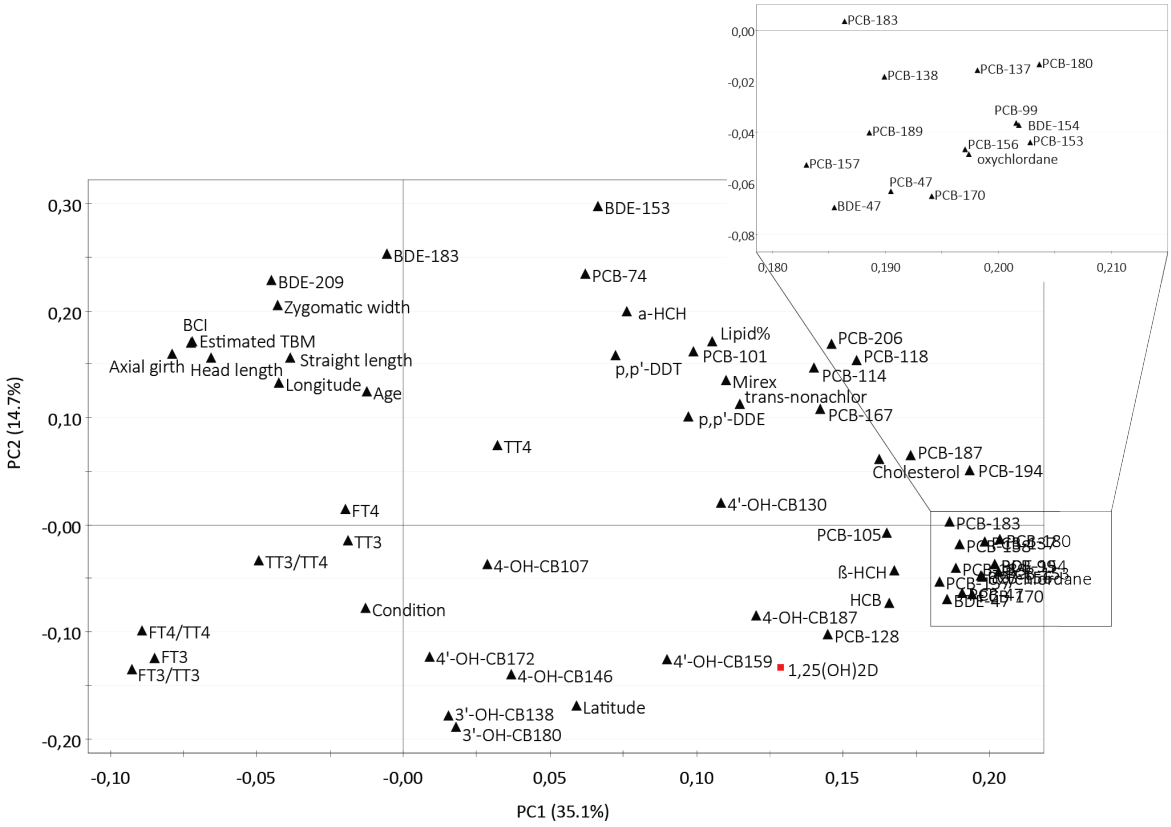

Figure 6: : PCA loading plot of the relationships among the observations and the variables in female polar bears (*Ursus maritimus*) without cubs of the year (FWOCOY) (n = 15), from Svalbard. A total of 62 variables were included in the model; latitude, longitude, age, condition, straight length, axillary girth, head length, zygomatic width, total body mass, body condition index, lipid content (%), cholesterol, 8 pesticides, 21 PCBs, 8 OH-PCBs, 5 PBDEs, THs and 1,25$(OH)_2$D.

Orthogonal Projections to Latent Structures (OPLS)

To explore further the relations observed from the PCA-model, a single Y OPLS-model was applied to investigate the relationships between predictor variables (THs, contaminants, biological and environmental factors) and 1,25$(OH)_2$D (Y) (Figure 7). Stepwise removal of the variables of least importance resulted in a significant model (Wold et al., 2001). The OPLS model predicted that the variation in 1,25$(OH)_2$D was significantly explained by 25 variables ($R^2X = 0.43$, $R^2Y = 0.64$ and $Q^2 = 0.55$, CV-ANOVA; $p = 0.013$). The highest VIP (variable importance in projection) values were shown by 4´OH-CB187, followed by 4´OH-CB172, 4-OH-CB146, 3´OH-CB180, BDE-47, 3´OH-CB138, BDE-209, BDE-154 and PCB-105 (VIP > 1). Hence, these variables were considered to be the most important for explaining the variation in 1,25$(OH)_2$D. The coefficient plot showed that 4´OH-CB187, 4´OH-CB172, 4-OH-CB146, 3´OH-CB180, BDE-47, 3´OH-CB138, BDE-154 and PCB-105 were positively correlated to 1,25$(OH)_2$D. In contrast BDE-209 was inversely associated with 1,25$(OH)_2$D levels in FWOCOY (Figure 8). It should be noted that the validation parameter R^2X was below the value that defines a good model using biological data; $R^2X > 0.7$. However, the criteria of predictability were met; $Q^2 > 0.4$ (Lundstedt et al., 1998).

Further testing, using bivariate correlations, confirmed that 4-OH-CB187 (p = 0,049, r_s = 0.578, n = 12), 4´OH-CB172 ($p = 0.048$, $r_p = 0.580$, n = 12), BDE-47 ($p = 0.012$, $r_p = 0.631$, n = 15), 3´OH-CB138 ($p = 0.047$, $r_s = 0.582$, n = 12), BDE-154 ($p = 0.021$, $r_p = 0.590$, n = 15), and also PCB-47 ($p = 0.025$, $r_p = 0.575$, n = 15), PCB-128 ($p = 0.034$, $r_p = 0.549$, n = 15), oxychlordane ($p = 0.040$, $r_p = 0.534$, n = 15), p,p´DDE ($p = 0.028$, $r_s = 0.567$, n = 15) and PCB-189 ($p = 0.041$, $r_p = 0.532$, n = 15) were positively correlated with 1,25$(OH)_2$D levels. In contrast, BDE-209 ($p = 0.013$, $r_s = -0.623$, n = 15) was inversely correlated with 1,25$(OH)_2$D levels in FWOCOY. It should be noted that 4´OH-CB172 co-eluted with another OH-PCB isomer, and that BDE-209 concentrations were suspected to have high background levels. Hence, these results should be considered as semi-quantitative.

The linear relationships between 1,25$(OH)_2$D, 4-OH-CB187, and BDE-47 are presented graphically in Figure 9.

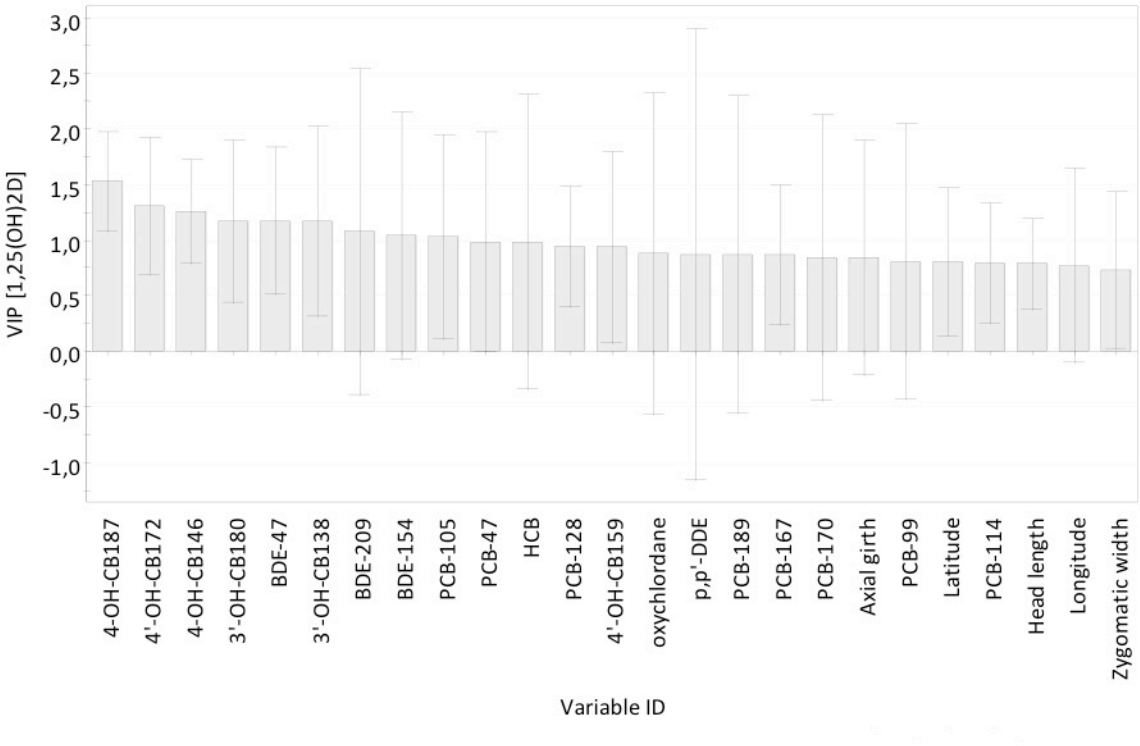

Figure 7: Orthogonal projections to latent structures (OPLS) regression VIP plot with important variables for the explanation of $1,25(OH)_2D$ levels in female polar bears (*Ursus maritimus*) without cubs of the year (FWOCOY) (n = 15), from Svalbard. Variables with VIP > 1 are of high importance in the model. All variables are shown with default jack-knife confidence intervals, where larger intervals that cross the 0 line represent the lower reliability. For identification of abbreviations, see Table 1 (POPs) and Table 4 (THs).

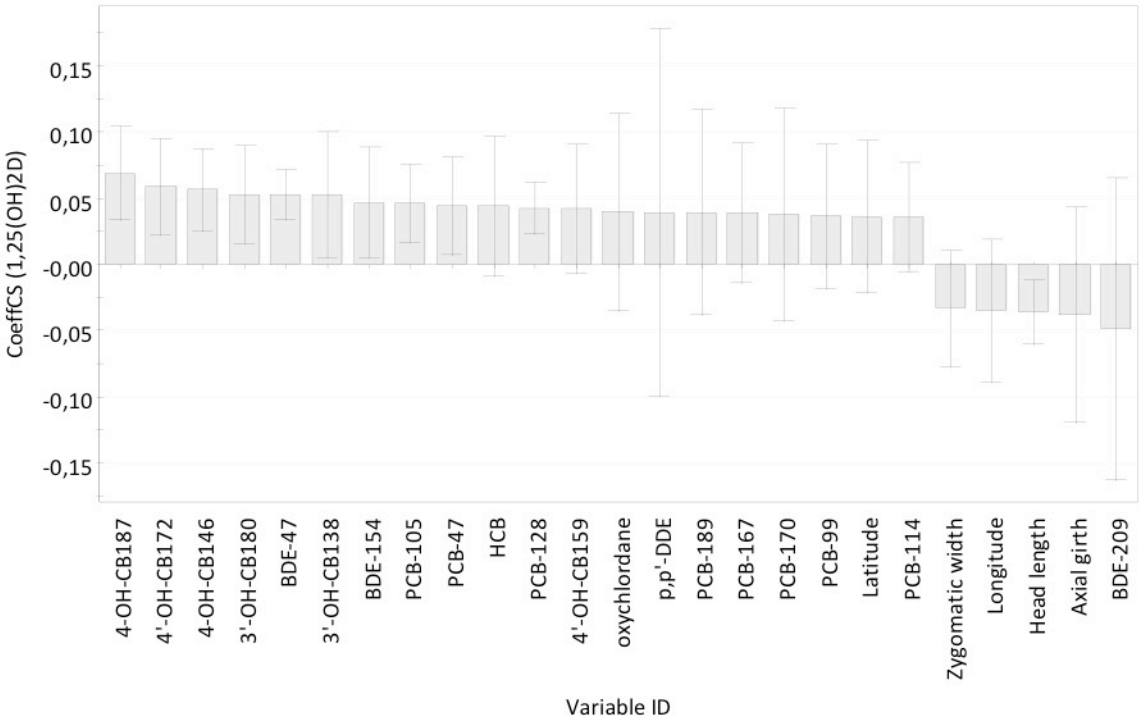

Figure 8: Orthogonal projections to latent structures (OPLS) regression coefficient plot summarizing the relationship between the contaminant levels, TH levels, biological and environmental factors (X-variables) on plasma/serum $1,25(OH)_2D$ levels (Y-variable) in female polar bears (*Ursus maritimus*) without cubs of the year (FWOCOY) (n = 15), from Svalbard. Negative coefficients represent inverse relationships and positive coefficients represent positive relationships of the X-variables with $1,25(OH)_2D$ levels. Variables with the highest absolute coefficient values are considered to be most important in explaining the Y-variable $1,25(OH)_2D$. Jack-knife confidence intervals that cross the 0 line indicate that the predictor is of low importance to the model. For identification of abbreviations, see Table 1 (POPs) and Table 4 (THs).

A) **B)**

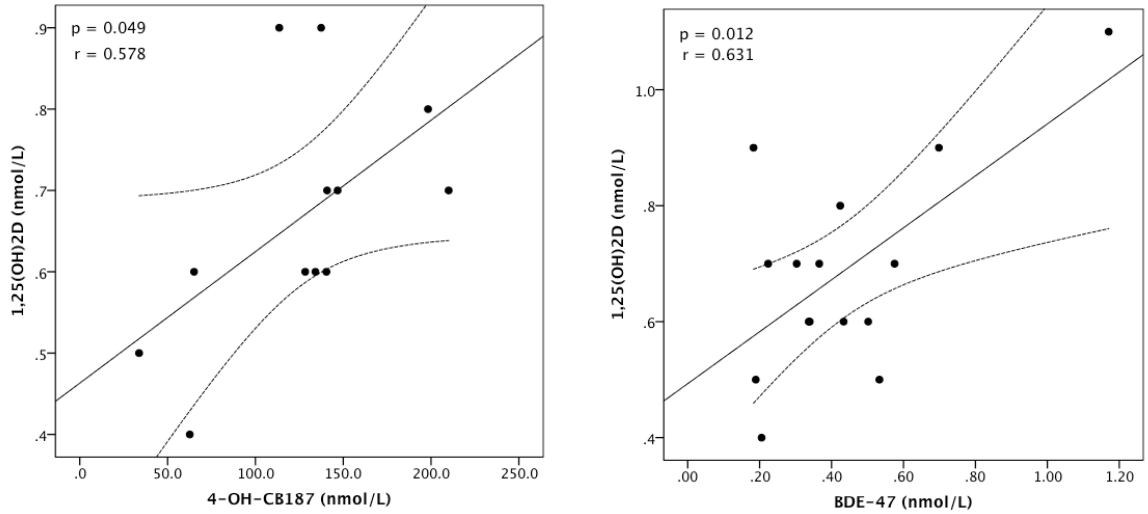

Figure 9: The linear relationship (± 95% confidence interval) between $1,25(OH)_2D$ serum concentrations (nmol/L) and A) 4-OH-CB187 (nmol/L) and B) BDE-47 (nmol/L) in female polar bears (*Ursus maritimus*) without cubs of the year (FWOCOY) (n = 15), from Svalbard, sampled in 2008. The Pearson correlation, p, and r-values are shown in the plot.

3.4.3 Males

Principal Component analysis

The analysis resulted in a PCA model (Figure 10) with 3 significant principal components. PC1 and PC2 explained 34.2 and 17.3 % of the variation, respectively (R^2X = 0.650, Q^2 = 0.379). PC3 explained 13.5 % of the variation. Since all observations were within the Hotellings T2 range, no outliers were present. The loadings of PCA indicated that most of the contaminants were oriented along PC1 (PC1 = -0.18 – -0.14). The plasma/serum levels of $1,25(OH)_2D$ grouped closely together with cholesterol, p,p´-DDE, TT4 and trans-nonachlor, thus indicated a positive relationship between these. In addition, $1,25(OH)_2D$ was negatively associated with TT3:TT4, FT4:TT4, age and several biometric variables (zygomatic width, BDI, estimated TBM and axillary girth). Head length was oriented towards the center of the plot, indicating that it was less important to the model. TT3, FT3, FT3:TT3 and FT4 were negatively associated with several PCBs (PCB-157, -170, -194, -189 and -206).

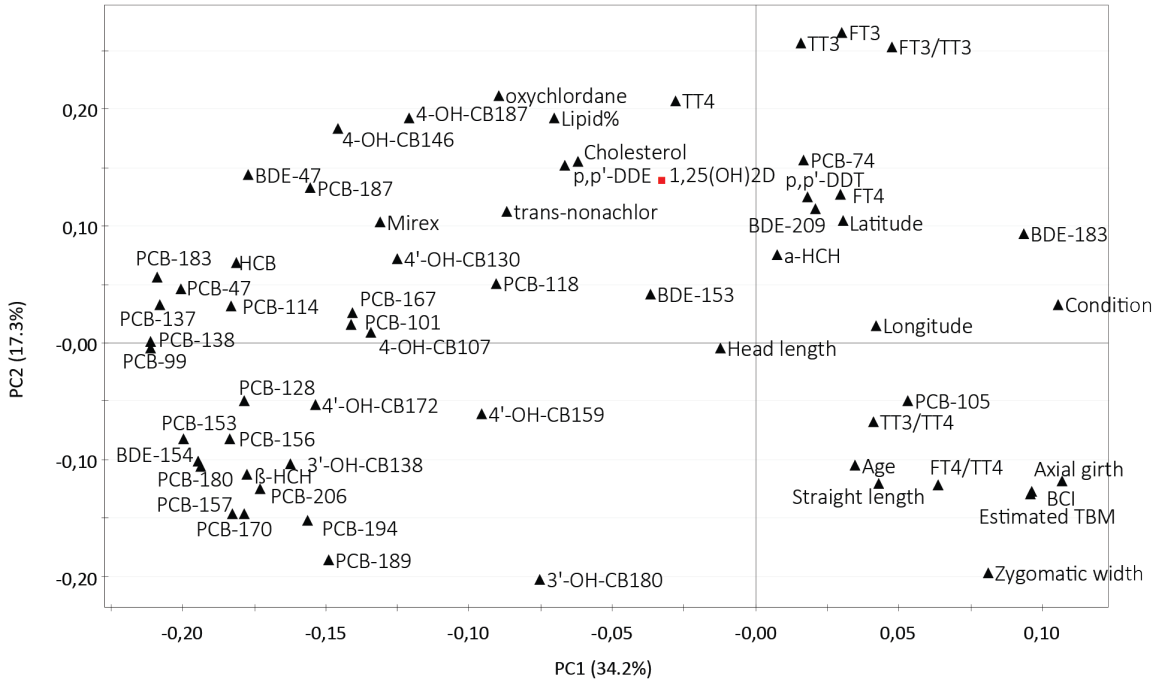

Figure 10: PCA loading plot of the relationships among the observations and the variables in male polar bears (*Ursus maritimus*) (n = 20), from Svalbard. A total of 62 variables were included in the model; latitude, longitude, age, condition, straight length, axillary girth, head length, zygomatic width, total body mass, body condition index, lipid content (%), cholesterol, 8 pesticides, 21 PCBs, 8 OH-PCBs, 5 PBDEs, THs and $1,25(OH)_2D$.

Orthogonal Projections to Latent Structures (OPLS)

To explore further the relations observed from the PCA-model, a single Y OPLS-model was applied to investigate the relationships between predictor variables (THs, contaminants, biological and environmental factors) and 1,25$(OH)_2$D (Y) (Figure 11). Stepwise removal of the variables of least importance resulted in a significant model (Wold et al., 2001). The OPLS model predicted that the variation in 1,25$(OH)_2$D was significantly explained by 20 variables ($R^2X = 0.52$, $R^2Y = 0.30$ and $Q^2 = 0.19$, CV-ANOVA; $p = 0.016$). The highest VIP (variable importance in projection) values were shown by 4-OH-CB146, followed by PCB-183, PCB-137, axillary girth, BDE-47, cholesterol, zygomatic width, lipid %, PCB-99, estimated total body mass (TBM) and body condition index (BCI) (VIP > 1). Hence, these variables were considered to be the most important for explaining the variation in 1,25$(OH)_2$D. The coefficient plot showed that 4OH-CB146, PCB-183, PCB-137, BDE-47, cholesterol, lipid % and PCB-99 were positively correlated to 1,25$(OH)_2$D. In contrast axillary girth, zygomatic width, estimated total body mass (TBM) and body condition index (BCI) were inversely associated with 1,25$(OH)_2$D levels in males (Figure 12). It should be noted that the validation parameters R^2X and Q^2 were below the value that defines a good model when using biological data; $R^2X > 0.7$, $Q^2 > 0.4$ (Lundstedt et al., 1998).

Further testing, using bivariate correlations, showed that the only statistically significant correlations were between 1,25$(OH)_2$D and TT3 ($p = 0.013$, $r_p = 0.545$, n = 20) and between 1,25$(OH)_2$D and TT4 ($p = 0.028$, $r_p = 0.492$, n = 20).

The linear relationships between 1,25$(OH)_2$D, TT3 and TT4 are presented graphically in figure 13.

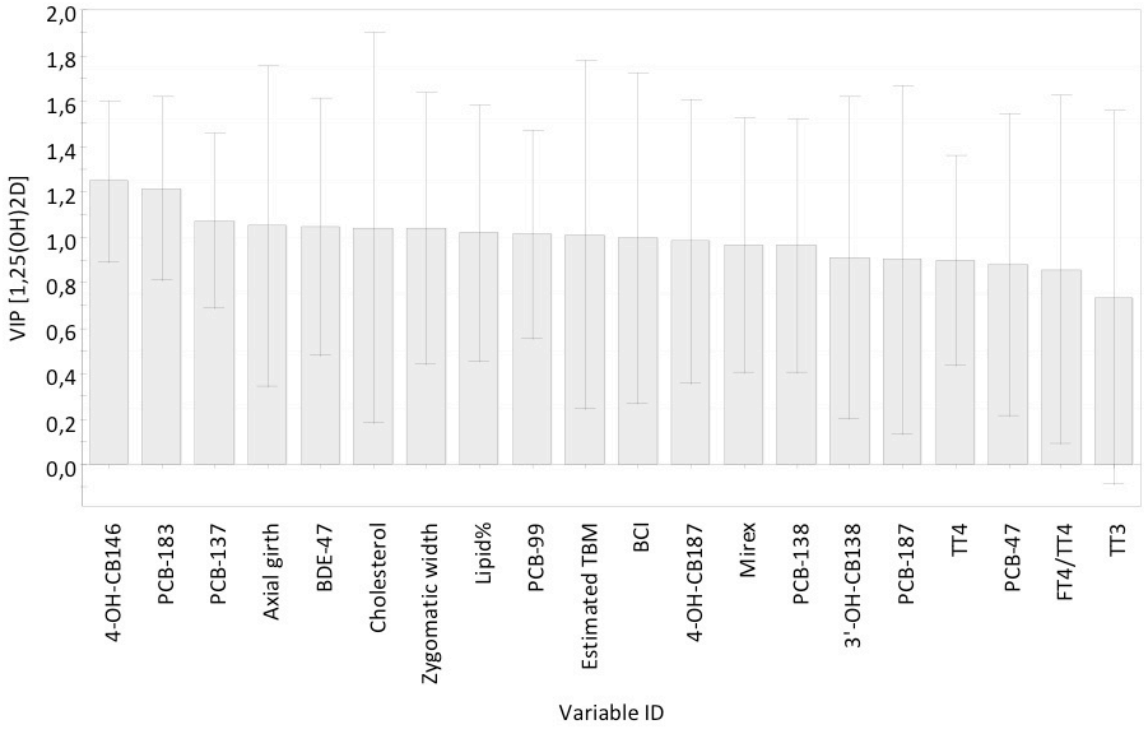

Figure 11: Orthogonal projections to latent structures (OPLS) regression VIP plot with important variables for the explanation of $1,25(OH)_2D$ levels in male polar bears (*Ursus maritimus*) (n = 20), from Svalbard. Variables with VIP > 1 are of high importance in the model. All variables are shown with default jack-knife confidence intervals, where larger intervals that cross the 0 line represent the lower reliability. For identification of abbreviations, see Table 1 (POPs) and Table 5 (THs).

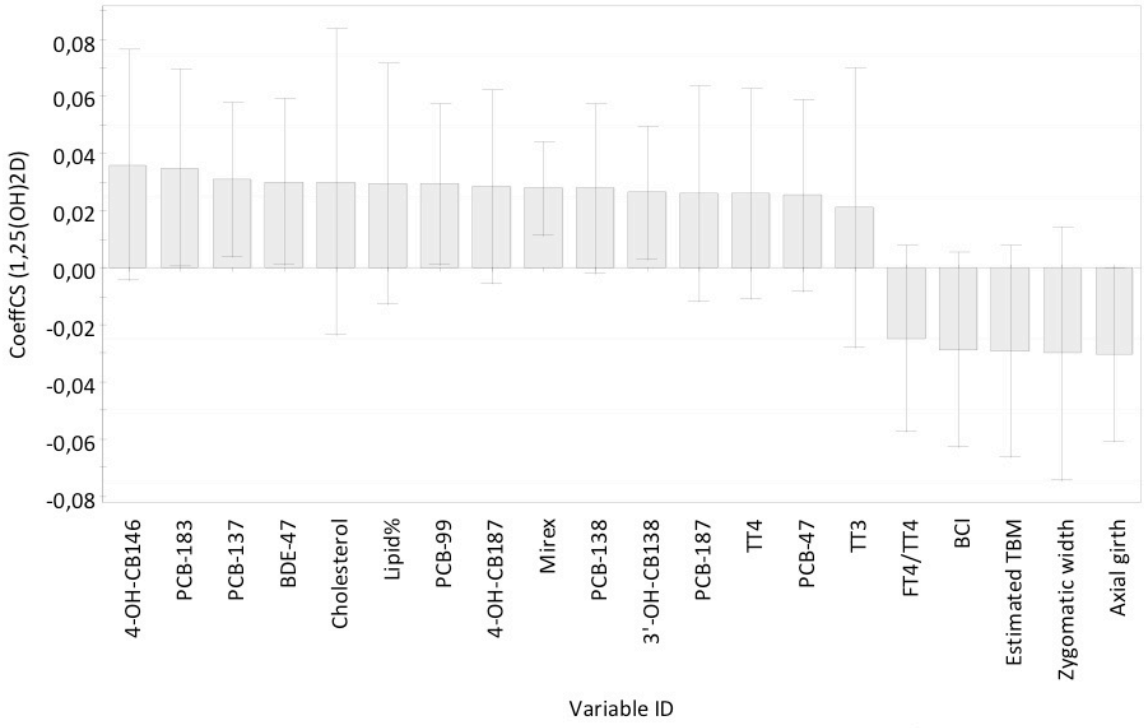

Figure 12: Orthogonal projections to latent structures (OPLS) regression coefficient plot summarizing the relationship between the contaminant levels, TH levels, biological and environmental factors (X-variables) on plasma/serum 1,25(OH)$_2$D levels (Y-variable) in male polar bears (*Ursus maritimus*) (n = 20), from Svalbard. Negative coefficients represent inverse relationships and positive coefficients represent positive relationships of the X-variables with 1,25(OH)$_2$D levels. Variables with the highest absolute coefficient values are considered to be most important in explaining the Y-variable 1,25(OH)$_2$D. Jack-knife confidence intervals that cross the 0 line indicate that the predictor is of low importance to the model. For identification of abbreviations, see Table 1 (POPs) and Table 5 (THs).

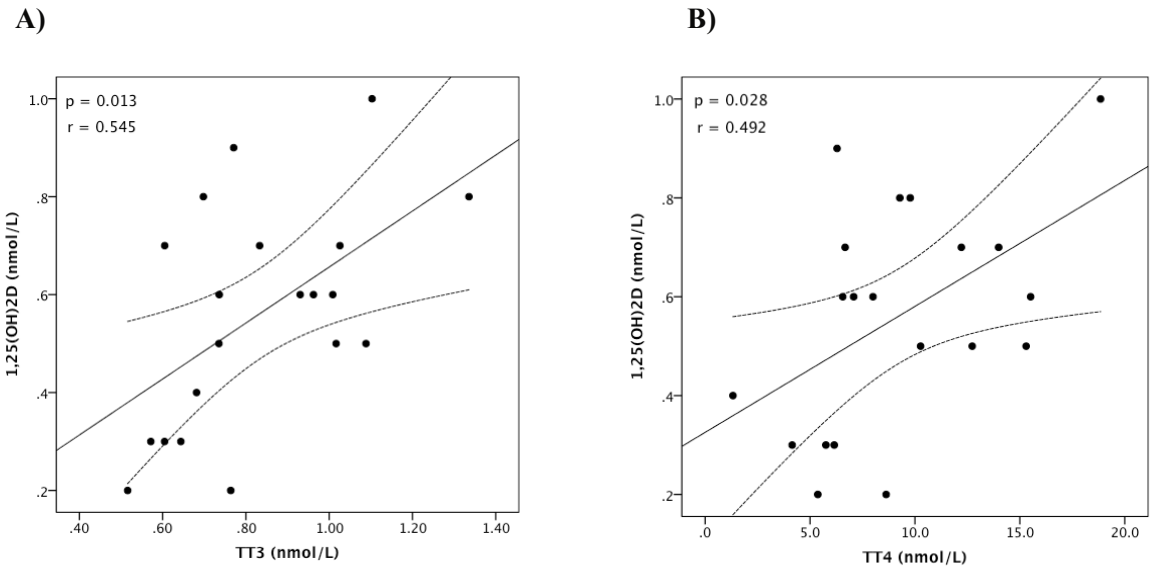

Figure 13: The linear relationship (± 95% confidence interval) between $1,25(OH)_2D$ serum concentrations (nmol/L) and A) TT3 (nmol/L) and B) TT4 (nmol/L) in male polar bears (*Ursus maritimus*) (n = 20) from Svalbard, sampled in 2008. The Pearson correlation, p, and r-values are shown in the plot.

4. Discussion

The results of the present study show that circulating levels of $1,25(OH)_2D$ did not differ significantly between the three groups of polar bears (Table 6). In addition, the study also shows that circulating concentrations of $1,25(OH)_2D$ in polar bears may be disturbed by contaminants, and relationships between $1,25(OH)_2D$ and THs were observed: POPs affected $1,25(OH)_2D$ levels in both FWCOY and FWOCOY, but not in males, while levels of THs affected $1,25(OH)_2D$ levels in both FWCOY and males, but not in FWOCOY.

4.1 Levels of vitamin D

To my knowledge, this is the first study to examine circulating levels of $1,25(OH)_2D$ in free-ranging polar bears. Only a few studies have examined the plasma levels of vitamin D in polar bears (Crissey et al., 2001, Kenny et al., 1998, Kenny et al., 2004, Lin et al., 2005), and to my knowledge, none have investigated differences in vitamin D levels among females with and without cubs, and males. Only one previous study appear to have reported the serum levels of $1,25(OH)_2D$ in (captive) polar bears (Crissey et al., 2001). The other studies have analyzed milk, serum or blubber levels of 25-OH vitamin D3 ($25\text{-}OHD_3$) in polar bears. (Kenny et al., 1998, Kenny et al., 2004, Lin et al., 2005). $25\text{-}OHD_3$ is metabolized in the liver from 7-dehydrocholesterol, before further metabolized in the kidneys to the active compound $1,25(OH)_2D$ (Vieth, 2005). Lin et al (2005) investigated associations between serum levels of $25\text{-}OHD_3$ in captive polar bears and antebrachial fractures, and reported subnormal vitamin D concentrations in 2 of 3 polar bears with fractures. Kenny et al (1998) reported $25\text{-}OHD_3$ levels in milk from captive and free-ranging polar bears, and later they investigated the $25\text{-}OHD_3$ content in polar bear blubber (Kenny et al., 2004). Due to the lack of a reliable kidney/milk/blubber-to-blood conversion factor for $25\text{-}OHD_3$ to $1,25(OH)_2D$ in polar bears, a direct comparison of levels between the present study (plasma $1,25(OH)_2D$) and previous studies (blubber/milk/plasma $25\text{-}OHD_3$) is not possible. In the present study, the plasma

concentrations of $1,25(OH)_2D$ (Table 3, 4 and 5) were higher (mean = 0.68 nmol/L) than previously reported $1,25(OH)_2D$ levels in captive polar bears (mean = 0.045 nmol/L) (Crissey et al., 2001). The higher plasma concentrations in the present free-ranging polar bears, could be due to different analytical methods, or that the low concentrations reported by Crissey et al. (2001) is due to artificial captivity factors. Levels of $1,25(OH)_2D$ in the present polar bears were similar to those reported in gray seals (*Halichoerus grypus*) (mean = 1.14 nmol/L) and ringed seal (*Phoca hispida botnica*) (mean = 0.26 nmol/L) (Routti et al., 2008). It should however be noted that species differences are likely to exist in marine mammals. These comparative physiological differences are not adressed in the current thesis.

Normally, circulating $1,25(OH)_2D$ levels are strictly controlled (Vieth, 2005) and have been reported to remain stable in hooded seals (*Cystophora cristata*) during vitamin D supplementation (Keiver et al., 1988). Furthermore, plasma levels have been shown not to vary during the molting season in southern elephant seals (*Mirounga leonina*) (Wilske & Arnbom, 1996). This indicates that circulating $1,25(OH)_2D$ levels are strictly controlled independent of some natural physiological constrains. However, the circulating levels of $1,25(OH)_2D$ levels in Baltic ringed seals (*Phoca hispida botnica*), with high body burdens of organochlorinated pollutants, were significantly lower than in ringed seals from two lesser contaminated areas (Sable Island, Canada and Svalbard, Norway) (Routti et al., 2008). It should, however, also be noted that the Baltic ringed seals had higher levels of hepatic vitamin D_3 than the reference seals (Canada and Norway), and did not suffer from vitamin D_3 deficiency.

4.2 Thyroid hormone levels

In FWCOY, the levels of all THs were significantly higher than in males (Table E.1, Appendix). In FWOCOY, levels of TT4 and FT4 were also significantly higher than in males. As discussed later, this could be due to the differences in contaminant levels between the three groups of polar bears, or due to physiological differences between the groups. There were no significant differences between the two female groups of polar bears with regards to the THs (Table E.1).

In the present study, the reported levels of THs measured in plasma of FWCOY (TT4: 18.57 ± 6.16 nmol/L, FT4: 7.04 ± 2.21 pmol/L, TT3: 1.10 ± 0.19 nmol/L, FT3: 0.40 ± 0.24) are comparable with previous reported concentrations of THs in FWCOY at Svalbard sampled in 2008 ((Nilsen, 2011); TT4: 18.8 ± 5.9 (nmol/L), FT4: 7.4 ± 2.1 (pmol/L), TT3: 1.2 ± 0.1 (nmol/L), FT3: 0.5 ± 0.2 (pmol/L)). It should be noted that the present study and that particular study contained some of the same individuals. Thus, the similarities in TH levels between these studies were expected. There have not been any reports of plasma TH concentrations in female polar bears without cubs or in male polar bears from Svalbard during the latest years. However, Braathen et al. (2004) reported levels of plasma THs in the same three groups of polar bears as in the present study, sampled in 1997/1998 (FWCOY: TT4: 15.3 nmol/L; FT4: 4.38 pmol/L; TT3: 1.26 nmol/L; FT3: 1.26 pmol/L; FWOCOY: TT4: 18.5 nmol/L; FT4: 5.20 pmol/L; TT3: 1.58 nmol/L; FT3: 1.42 pmol/L; males: TT4: 13.5 nmol/L; FT4: 3.46 pmol/L; TT3: 1.23 nmol/L; FT3: 0.95 pmol/L). Levels of TT4 and FT4 were higher in FWCOY in the present study compared with the levels reported in FWCOY in 1997/1998 (Braathen et al., 2004). Among FWOCOY and males, levels of FT4 in the present study were also higher than reported in 1997/1998. Furthermore, levels of TT4 in the present study were lower in FWOCOY and males than in 1997/1998. For FWCOY and FWOCOY, levels of TT3 were about the same in 2008 and in 1997/1998. Levels of FT3 were about the same for FWCOY. In FWOCOY the levels of FT3 were lower in the present study than in 1997/1998. For males, TT3 and FT3 were both lower in the present study than in 1997/1998.

POPs have been shown to disrupt the TH homeostasis in polar bears (Braathen et al., 2004, Legler & Brouwer, 2003, Meerts et al., 2001, Verreault et al., 2005a). However, the levels of PCBs in polar bears from Svalbard have decreased the past years (Bytingsvik et al., 2012, Verreault et al., 2005b). Taking into consideration that several factors influence thyroid hormone levels, from food availability, iodine access, season, age, even time of day (McNabb, 2000), it may not be appropriate to draw conclusions on TH levels being higher or lower in this study compared to other studies. On the other hand, when concidering previous reported TH levels in polar bears from Svalbard (Braathen et al., 2004, Skaare et al., 2001), where plasma was sampled during March and April 1991-1994 (Skaare et al., 2001) and during the same time in 1997-1998 (Braathen et al., 2004) the same trend as in the present study appears. When taking into account that levels of organohalogenated compounds in polar bears have decreased significantly during this period (Bytingsvik et al., 2012), this may

indicate that there is a link between contaminant levels and THs in polar bears, as previously suggested by Skaare et al. (2001) and Braathen et al. (2004).

In the present study, FWCOY had significant higher levels of all the THs than males, and the levels of TT4 and FT4 were significantly higher in FWOCOY compared with males (Table E.1). In the study by Skaare et al. (2001) the concentrations of thyroid hormones (TT4, FT4 and FT3) were significantly higher in females than in males. Skaare et al. (2001) did not distinguish between females with and without cubs, as in the present study. In the study by Braathen et al. (2004) they did distinguish between the groups of polar bears, and they reported higher levels of TT3 in FWOCOY, compared to both males and FWCOY, while there were no differences in FT3 levels between the groups. In conclusion, the results on differences in THs between the groups are generally in accordance with previously reported studies (Braathen et al., 2004, Skaare et al., 2001). It is therefore concluded that the higher levels of THs in females compared to males, is due to sex-differences in regulatory mechanisms of THs in polar bears.

Elevated TH levels have been related to an increase in bone resorption and reduction in bone mineral density (Lind et al., 2003). In humans, hyperthyroidism has been associated with osteoporosis (Lakatos, 2003), and thyroid deseases are more common in women than in men, thus women are more likely to develop hypothyroidism, both overt and subclinically (Chiovato et al., 1993, Krueger et al., 2001). Gray seals from British waters have also been suggested to suffer from contaminant-mediated hyperthyroidism (Hall et al., 2003). Thus it is possible that the higher incidence of TH imbalance found in female polar bears (Braathen et al., 2004) compared with males could reflect the greater susceptibility of female polar bears to TH-related effects of POPs.

4.3 Prevalence and patterns of POPs

Polar bears at Svalbard are reported as one of the polar bear population with highest contamination load (de Wit et al., 2004). In the present study the most abundant contaminant group was OH-PCBs, followed by PCBs, pesticides and PBDEs (Table 7, 8 and 9). This reflect the general pattern of POPs reported in Arctic mammals and in polar bear plasma (Letcher et al., 2010). It should be noted that although the PBDEs constituted only 0.2 - 0.28 % of the total POP load in the three groups of polar bears, they were among the most important explanatory variables regarding the variation in $1,25(OH)_2D$. However, since three out of five PBDEs were suspected to have high background levels, these results should be considered as semi-quantitative.

The major compound group detected in the present study in polar bear plasma was OH-PCBs (Table 7), where FWCOY had the highest levels. 4-OH-CB187 was the main contributor to ΣOH-PCBs, and levels in females were almost 2 fold higher as compared to in males. In females, the componds found in second and third highest concentrations among the OH-PCBs were 4-OH-CB146 and 4´OH-CB172. For males 4´OH-CB172 and 4-OH-CB146 had the second and third highest concentrations, respectively. The concentrations of 4´OH-CB172 were higher in males than in females, while levels of 4-OH-CB146 were less than half in males as compared to in females. In all bears, there were detected significant concentrations of co-eluting peak consisting of 4´OH-CB172 and another hepta-chlorinated OH-PCB isomer, Cl_7-OH-PCB (4´OH-CB172/Cl_7-OH-PCB). Of these, Cl_7-OH-PCB was the dominating isomer. 4´OH-CB172 has been detected in earlier polar bear studies (Sandala et al., 2004, Sandau et al., 2000). However, for significant results regarding 4´OH-CB172/Cl_7-OH-PCB, it should be taken into concideration that Cl_7-OH-PCB seemed to be the causative compound. The levels of OH-PCBs in females were similar to those previously reported by Bytingsvik et al. (2012). Since many of the individuals in that particular study and the present study were the same, this was expected. The metabolic capacity has been reported to be age-dependent, where female polar bears were better capable to metabolize PCBs into OH-PCBs compared to polar bear cubs (Bytingsvik et al., 2012).

The second most abundant compound group was PCBs, with PCB-153 as the main contributor for all three groups of polar bears. PCB-180 and PCB-170 had the second and third highest concentrations in all groups. The following compound group was the pesticides

(Table 8). Oxychlordane was main contributor in the females, while HCB had highest levels in males. Oxychlordane-levels was 7-8 fold higher in females compared to males. The levels of the other pesticides were comparable between the three groups of polar bears. PBDEs was the compound group with the lowest concentrations in the polar bear plasma from all three groups (Table 9). BDE-47 was the main contributor to ΣPBDE in the female groups, and BDE-183 was the main contributor in males. BDE-47 levels were 2-3 fold lower in males compared to FWCOY and FWOCOY. The pattern of the other PBDE congeners varied between the three groups. The reason for these differences may be due to the reported high background levels, or due to maternal transfer of POPs and/or group differences in capacity to metabolize the PBDEs (i.e P450 enzyme activities), or exposure related to diet differences between the groups (McKinney et al., 2009). Because elucidating differences in contaminant burdens between groups was not the main aim of the present study, these aspects are not discussed further. Thus, the reader is referred to Letcher et al. (2010) for a more detailed discussion of these aspects.

In the present study, negative relationships between biometric variables and mainly OH-PCBs were detected for FWOCOY (Figure 6). Age was inversely correlated with 4-OH-CB146, 3´OH-CB138 and 3´OH-CB180. Since the contaminants are persistent, it was expected that age would be positively correlated with some of the POPs. The inverse relationship between age and the above mentioned OH-PCBs may be due to concentration dependent toxicokinetics or age related changes in the metabolism of PCBs to OH-PCBs. However, the focus of the present study was to elucidate the combined effects of POPs, THs and biometric and environmental variables on 1,25(OH)2D levels. Thus, associations between POPs and biometric variables are not discussed in more detail herein.

4.4 Effects of POPs, THs and biometric variables on vitamin D

Both biometric variables, POPs and THs affected $1,25(OH)_2D$ levels. However, there were large differences between the groups. In the OPLS model for FWCOY, there was a strong inverse correlation between $1,25(OH)_2D$, and BDE-153 and HCB, while there were positive correlations between $1,25(OH)_2D$, and age, TT4, zygomatic width, FT4, FT3, and FT3:TT3 (Figure 4). In the OPLS model for FWOCOY, several of the POPs correlated positively with

1,25$(OH)_2$D, while none of the THs were correlated with 1,25$(OH)_2$D. Oxychlordane, p,p´DDE, PCBs (-47, -128, -189), 4´OH-CB172, 3´OH-CB138, 4-OH-CB187, BDE-47, and BDE-154 were positively correlated to 1,25$(OH)_2$D, while BDE-209 were negatively correlated to 1,25$(OH)_2$D in FWOCOY (Figure 8). In the OPLS model for males, none of the contaminants correlated significantly with levels of 1,25$(OH)_2$D. However, TT3 and TT4 were positively correlated with 1,25$(OH)_2$D (Figure 12).

In the OPLS models for all groups, OH-PCBs seemed to be important predictors for the circulating levels of 1,25$(OH)_2$D. It is known that OH-PCBs are thyroid endocrine disrupters, and the results from the present study may indicate that they also are important vitamin D disruptors. To my knowledge this has not been reported previously. As discussed below, it is possible that there are interacting effects of OH-PCBs and THs on levels of 1,25$(OH)_2$D. Routti et al. (2008) has previously reported that levels of circulating 1,25$(OH)_2$D and THs were negatively related with hepatic levels of POPs. It has also been suggested that low levels of 1,25$(OH)_2$D could be compensated for by elevated TH levels (Mohan et al., 2004), or that excess THs results in a decrease in 1,25$(OH)_2$D production (Epstein & Schneider, 2005). However, in the present study, THs was shown not to be a predictor for the levels of 1,25$(OH)_2$D in FWOCOY. This may indicate that the interacting effects between POPs and THs on 1,25$(OH)_2$D, not necessarily are related to THs and their role in the regulation of vitamin D. The OPLS models indicates that POPs played a larger role for the concentrations of 1,25$(OH)_2$D in FWOCOY, than in FWCOY and in males. The reason for this is not known but may, as discussed below, be due to the differences in the physiology between the groups.

Previously, levels of THs have shown to be both positively and negatively related with POP levels in polar bears (Braathen et al., 2004, Skaare et al., 2001), and levels of 1,25$(OH)_2$D have been reported to be negatively associated with POPs levels in ringed seals (Routti et al., 2008). Previous studies have suggested that excess TH levels may cause a reduction in 1,25$(OH)_2$D levels (Epstein & Schneider, 2005), and that low levels of 1,25$(OH)_2$D may lead to elevated TH levels (Mohan et al., 2004). However, it is possible that there is complex relationships in which POPs can lead to reduced levels of TH, as shown for FWCOY in the present study, and that this is a contributing factor to reduced vitamin D levels in this group. However, the OPLS models showed that in males and in FWOCOY, THs played little or no role in explaining the variations in levels of 1,25$(OH)_2$D, respectively. This may indicate that THs plays a larger role for the vitamin D homeostasis (i.e. 1,25$(OH)_2$D plasma

concentrations) in FWCOY than in the other groups. This could be related to the physiological status of this group, possibly in relation to lactation, and/or that they recover from giving birth to the cubs in the den during the winter. In this study it is not possible to give an exact explanaition of possible mechanisms involved in the combined effects of POPs and THs on plasma concentrations of $1,25(OH)_2D$ in polar bears.

In FWCOY, age and size (zygomatic width) seemed to be a relatively strong predictor of levels of $1,25(OH)_2D$. In contrast, biometric variables were less important for FWOCOY. In males, several biometric variables (age, size [BCI and zygomatic width]) and physiological variables such as lipid % and cholesterol were important predictors in explaining $1,25(OH)_2D$ levels. This indicates that the biometric variables, in interaction with POPs, affect levels of $1,25(OH)_2D$, especially in FWCOY and males. In addition, the result indicates that for these two groups (FWCOY and males), a more complex interaction exists, were levels of $1,25(OH)_2D$ is affected by both THs, biometric variables (particularly age and size) and POPs.

It is challenging to elucidate effects from POPs on wildlife. The sample size in each of the groups in the present study represent a small section of polar bears from the population at Svalbard. The present study focus on effect assessment in three groups of polar bears, and it is therefore possible to assess differences in levels and effects between sexes. However, there is limited knowledge about the life history and generel physiological conditions of these randomly captured individuals. The period the study was conducted represents a short time window of the polar bear life cycle; in addition there are logistical constraints associated with the study area and season. Arctic biota is exposed to a complex mixture of anthropogenic contaminants, of which the quantitatively and qualitatively composition is not fully elucidated (de Wit et al., 2004). For instance, the POPs analysed in the present study represents a selection of compounds within a selection of all compound groups that polar bears are exposed to. For this reason, it is not possible to account for the possible interactions from non-analysed contaminants in the present study. Furthermore, the contaminants in the present study showed a high degree of covariation. Such strong intercorrelations in a dataset can complicate the multivariate data analysis when elucidating responses (Trygg & Wold, 2002). Multivariate data analysis methods, such as the OPLS, are developed to better handle the challenges regarding intercorrelations between variables. However, there are still uncertainties associated with the interpretation of data. For instance, the non-analysed

compounds may contribute to the observed indications of effects on $1,25(OH)_2D$, while others merely covary with the compound explaining the variation. Since no one previously have analysed levels of $1,25(OH)_2D$ in polar bears from Svalbard, it is not possible to conclude on a time trend for polar bears with regards to plasma concentrations of vitamin D, even though we know that the levels of POPs are declining. When assessing levels and effects of POPs in polar bears, it is essential to allow for differences in tissue, analysis procedures, data handling, sample size and study area. As strongly indicated in the present study, dissimilarities between sexes, species, lipid content and time of sampling should also be considered.

The present study documents that there are complex interactions between POPs, THs and biometric variables on the $1,25(OH)_2D$ status in polar bears, and that there are differences in the importance of these different predictors in FWCOY, FWOCOY and males. It is proposed that the differences in the importance of the various predictors, is due to the very different physiology of the animals. By nature, males and females have a completely different genetic physiology and hormone regulation. It is also likely that there are large differences in physiology between FWCOY and FWOCOY. FWCOY have been fasting in the den during the winter, and were lactating actively when sampled. It is likely that this affected the complex interactions of POPs, THs and biometric variables on $1,25(OH)_2D$ levels differently in the two groups of females. Furthermore, the sampled FWOCOY had essentially stopped lactating, and had probably not fasted to the same extent throughout the winter (because they do not go to den). They are therefore not prone to the same physiological stress that FWCOY, and it is possible that this could explain the differences in the complex relationships between POPs, THs and biometric variables on $1,25(OH)_2D$ in these two groups of female polar bears.

5. Conclusions

In conclusion, the present study strongly indicates that there are interactive effects of plasma levels of POPs and plasma levels of THs on plasma levels of $1,25(OH)_2D$ in polar bears. There were, however, some differences between the three groups of polar bears. Thus, the present study demonstrates that it is important to take into account differences in sex and life stages when assessing endocrine effects of POPs.

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Appendices

A. Sampling Information

Table A.1: Date, sex, age and location (latitude and longitude) in female (FWCOY and FWOCOY) and male polar bears (*Ursus maritimus*) from Svalbard, sampled in 2008.

ID	Date	Sex	Latitude	Longitude	Age
FWCOY					
23958	24.04.08	F	78.21	21.94	5
23689	13.04.08	F	79.21	15.85	5
23781	20.04.08	F	77.05	16.99	8
23966	25.04.08	F	77.75	18.48	14
23962	25.04.08	F	77.34	17.77	12
23703	21.04.08	F	78.58	21.00	15
23909	14.04.08	F	79.33	13.93	12
23924	09.04.08	F	79.49	17.96	10
FWOCOY					
23802	13.04.08	F	79.01	16.12	9
23831	14.04.08	F	79.66	12.16	6
23881	16.04.08	F	79.65	12.23	6
23931	11.04.08	F	79.69	21.58	8
23882	14.04.08	F	79.61	13.00	6
23719	21.04.08	F	78.52	20.14	7
23500	24.04.08	F	78.12	22.31	10
23942	16.04.08	F	79.75	12.10	11
23637	18.04.08	F	77.07	16.00	7
23714	19.04.08	F	77.74	18.44	8
23731	20.04.08	F	77.04	16.32	8
23824	14.04.08	F	79.73	12.22	14
23945	19.04.08	F	77.55	22.12	9
23688	13.04.08	F	79.08	16.04	12
23948	20.04.08	F	76.72	16.16	16
Males					
23834	13.04.08	M	79.30	15.80	9
23683	14.04.08	M	79.63	12.81	6
23921	08.04.08	M	80.62	19.80	11
23922	08.04.08	M	80.25	23.70	8
23682	13.04.08	M	79.01	16.12	16
23946	19.04.08	M	77.77	18.52	15
23686	14.04.08	M	79.54	12.60	7
23949	20.04.08	M	76.72	16.16	10
23960	24.04.08	M	77.39	18.03	15
23575	25.04.08	M	77.46	18.16	12
23830	09.04.08	M	79.97	18.40	13
23899	20.04.08	M	77.04	16.32	13
23825	14.04.08	M	79.45	13.22	14
23820	14.04.08	M	79.63	14.10	16
23809	13.04.08	M	79.10	16.18	17
23702	21.04.08	M	78.58	20.87	21
23855	25.04.08	M	77.73	18.42	15
23609	11.04.08	M	79.69	21.58	19
23898	21.04.08	M	78.58	20.87	10
23934	11.04.08	M	79.77	20.24	4

B. Individual biometric measurement

Table B.1: Individual biometric measurements of female (FWCOY and FWOCOY) and male polar bears (*Ursus maritimus*) from Svalbard, sampled in 2008.

ID	Head length (mm)	Zygomatic width (mm)	Estimated total body mass (TBM)	BCI	Zoologic length (cm)	Straight length (cm)	Axillary girth (cm)
FWCOY							
23958	329	186	153.0	144.34	195	183	108
23689	346	196	158.9	147.48	215	205	101
23781	346	196	194.9	162.38	209	195	118
23966	349	203	183.2	157.85	208	197	113
23962	342	199	186.6	159.12	200	191	117
23703	336	201	165.1	150.06	208	189	110
23909	351	199	260.5	183.88	212	201	136
23924	335	200	139.3	137.55	204	191	99
FWOCOY							
23802	353	197	188.9	160.11	210	197	115
23831	336	190	166.5	150.80	205	197	107
23881	331	189	161.5	148.53	205	195	106
23931	334	185	134.0	134.56	193	181	101
23882	341	183	159.6	147.59	207	191	107
23719	333	196	163.7	149.43	205	190	109
23500	354	199	203.6	165.62	205	195	121
23942	339	198	164.8	149.98	205	191	109
23637	349	209	205.6	166.49	220	205	117
23714	359	209	160.4	147.99	204	194	106
23731	343	199	189.2	160.34	215	204	112
23824	346	212	237.3	177.10	215	205	127
23945	366	208	196.5	162.93	204	192	120
23688	357	207	207.5	167.15	214	202	119
23948	345	216	169.9	152.24	204	193	110
Males							
23834	422	226	367.3	209.95	255	236	146
23683	377	215	254.9	182.57	231	216	127
23921	387	258	467.9	227.64	243	225	174
23922	384	227	280.1	189.50	234	214	135
23682	439	280	539.3	238.58	263	246	176
23946	408	245	337.9	203.55	245	224	145
23686	400	242	304.8	196.06	251	232	133
23949	380	230	273.3	187.87	237	225	128
23960	399	288	494.4	232.11	266	245	168
23575	388	257	346.1	205.33	237	224	147
23830	408	250	344.1	204.96	246	227	145
23899	392	258	412.1	218.38	247	232	158
23825	415	269	408.0	217.80	256	240	153
23820	427	277	513.8	235.10	266	252	168
23809	405	259	384.5	213.13	248	226	155
23702	398	253	407.5	217.56	244	232	157
23855	404	265	436.8	222.62	245	229	165
23609	358	251	346.0	205.26	240	222	148
23898	405	275	441.5	223.76	264	246	157
23934	403	267	441.5	223.76	257	246	157

C. Individual $1,25(OH)_2D$ concentrations

Table C.1: Individual serum $1,25(OH)_2D$ measurements of female (FWCOY and FWOCOY) and male polar bears (*Ursus maritimus*) from Svalbard, sampled in 2008.

ID	$1,25(OH)_2D$ (nmol/L)	ID	$1,25(OH)_2D$ (nmol/L)
FWCOY		**Males**	
23958	0.521	23834	1.031
23689	0.326	23683	0.777
23781	0.474	23921	0.859
23966	1.517	23922	0.520
23962	0.619	23682	0.258
23703	1.266	23946	0.647
23909	0.592	23686	0.472
23924	1.171	23949	0.197
FWOCOY		23960	0.337
23802	0.507	23575	0.675
23831	0.669	23830	0.771
23881	1.054	23899	0.705
23931	0.904	23825	0.706
23882	0.857	23820	0.314
23719	0.488	23809	0.493
23500	0.412	23702	0.582
23942	0.738	23855	0.648
23637	0.702	23609	0.373
23714	0.626	23898	0.615
23731	0.599	23934	0.202
23824	0.641		
23945	0.605		
23688	0.779		
23948	0.652		

D. Individual Thyroid hormone concentrations

Table D.1: Individual concentrations of Thyroid hormones of female (FWCOY and FWOCOY) and male polar bears (*Ursus maritimus*) from Svalbard, sampled in 2008.

ID	TT4 (nmol/L)	FT4 (pmol/L)	TT3 (nmol/L)	FT3 (pmol/L)	FT3/TT3* (x100)	FT4/TT4* (x100)	TT3/TT4 (x100)
FWCOY							
23958	18.11	7.73	1.15	0.53	0.046	0.043	0.064
23689	11.31	5.02	0.69	0.06	0.008	0.044	0.061
23781	13.32	5.06	1.27	0.06	0.005	0.038	0.095
23966	18.12	8.63	1.25	0.65	0.052	0.048	0.069
23962	20.10	5.51	1.19	0.51	0.043	0.027	0.059
23703	21.55	7.60	1.24	0.61	0.049	0.035	0.058
23909	14.84	5.49	1.01	0.28	0.028	0.037	0.068
23924	31.24	11.30	1.01	0.57	0.057	0.036	0.032
FWOCOY							
23802	15.48	6.73	1.19	0.74	0.062	0.043	0.077
23831	10.52	6.41	1.30	0.74	0.057	0.061	0.123
23881	20.43	7.40	1.49	0.44	0.029	0.036	0.073
23931	10.15	4.63	0.79	0.27	0.033	0.046	0.078
23882	24.83	12.65	1.62	1.49	0.092	0.051	0.065
23719	19.85	9.34	1.31	0.39	0.030	0.047	0.066
23500	19.64	7.72	1.55	0.88	0.057	0.039	0.079
23942	11.59	6.97	0.92	0.24	0.026	0.060	0.080
23637	8.75	3.51	1.02	0.33	0.033	0.040	0.116
23714	19.73	9.95	1.13	0.48	0.043	0.050	0.057
23731	14.15	7.23	1.21	0.50	0.041	0.051	0.085
23824	18.57	9.21	1.39	0.72	0.052	0.050	0.075
23945	7.78	6.27	1.44	0.64	0.044	0.081	0.185
23688	37.78	14.83	1.61	0.72	0.045	0.039	0.043
23948	11.56	4.78	1.25	0.46	0.037	0.041	0.108
Males							
23834	18.85	5.40	1.10	0.47	0.043	0.029	0.059
23683	9.77	5.40	1.34	0.70	0.053	0.055	0.137
23921	6.29	6.41	0.77	0.15	0.020	0.102	0.123
23922	12.73	4.82	1.09	0.53	0.048	0.038	0.085
23682	4.15	3.13	0.60	0.06	0.010	0.075	0.146
23946	6.56	3.36	1.01	0.06	0.006	0.051	0.154
23686	10.27	7.31	0.74	0.18	0.024	0.071	0.072
23949	5.37	4.26	0.52	0.06	0.011	0.079	0.096
23960	5.76	3.00	0.64	0.06	0.009	0.052	0.112
23575	13.99	4.04	1.03	0.31	0.030	0.029	0.073
23830	9.28	3.15	0.70	0.06	0.008	0.034	0.075
23899	6.67	3.28	0.60	0.06	0.010	0.049	0.091
23825	12.21	6.49	0.83	0.06	0.007	0.053	0.068
23820	6.15	4.08	0.57	0.06	0.010	0.066	0.093
23809	15.30	5.62	1.02	0.29	0.029	0.037	0.066
23702	15.51	8.58	0.96	0.41	0.043	0.055	0.062
23855	8.00	4.50	0.93	0.23	0.024	0.056	0.116
23609	1.33	1.51	0.68	0.06	0.009	0.114	0.514
23898	7.08	4.52	0.74	0.19	0.026	0.064	0.104
23934	8.63	3.82	0.76	0.23	0.029	0.044	0.089

* FT3 and FT4 were converted to nmol/L before calculation of ratio.

E. Thyroid concentrations with significant differences between groups

Table E.1 : Mean (X) concentrations of 1,25(OH)2D (nmol/L), cholesterol (mmol/L), lipid content (%), total (nmol/L) and free (pmol/L) T4 and T3, and FT3:TT3, FT4:TT4, TT3:TT4 mole ratios (x100) ± standard deviaton (SD) and range (min – max), measured in serum from three groups of polar bears (*Ursus maritimus*) from Svalbard in 2008, with F-statistics and significance level *(p)* from analysis of variance. For significant differences, *p*-values are given in bold.

	FWCOY (n=8)			**FWOCOY (n=15)**			**Males (n=20)**			**ANOVA**	
	X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	F	p
1,25(OH)2D (nmol/L)	0.81 ± 0.43	0.61	0.33 – 1.52	0.68 ± 0.16	0.65	0.41 – 1.05	0.56 ± 0.23	0.59	0.19 – 1.03	2.76	0.076
Cholesterol (mmol/L)	9.24 ± 1.98[a]	9.25	5.40 – 11.50	9.02 ± 1.61[a]	9.1	5.7 – 13.10	6.29 ± 1.19[b]	6.25	3.9 – 8.3	18.53	**< 0.001**
Lipid (%)	1.30 ± 0.23[a]	1.21	1.0 – 1.59	1.34 ± 0.21[a]	1.36	0.82 – 1.56	0.82 ± 0.17[b]	0.79	0.56 – 1.22	35.44	**< 0.001**
THs											
TT4[1] (nmol/L)	18.57 ± 6.16[a]	18.12	11.31 – 31.24	16.71 ± 7.74[a]	15.48	7.78 – 37.78	9.19 ± 4.39[b]	8.31	1.33 – 18.85	9.93	**< 0.001**
FT4[2] (pmol/L)	7.04 ± 2.21[a]	6.56	5.02 – 11.30	7.84 ± 2.99[a]	7.23	3.51 – 14.83	4.63 ± 1.67[b]	4.38	1.51 – 8.58	8.90	**0.001**
TT3[3] (nmol/L)	1.10 ± 0.19[a]	1.17	0.69 – 1.27	1.28 ± 0.24[a,b]	1.3	0.79 – 1.62	0.83 ± 0.21[b]	0.77	0.52 – 1.34	17.59	**< 0.001**
FT3[4] (pmol/L)	0.40 ± 0.24[a]	0.52	0.06 – 0.65	0.60 ± 0.31[a,b]	0.5	0.24 – 1.49	0.21 ± 0.18[b]	0.17	0.06 – 0.70	10.72	**< 0.001**
FT3:TT3	0.03 ± 0.02[a,b]	0.04	0.01 – 0.06	0.04 ± 0.01[a]	0.04	0.03 – 0.09	0.02 ± 0.01[b]	0.02	0.01 – 0.05	8.26	**< 0.001**
FT4:TT4	0.03 ± 0.01[a]	0.04	0.03 – 0.05	0.04 ± 0.01[a,b]	0.05	0.04 – 0.08	0.05 ± 0.02[b]	0.05	0.03 – 0.11	3.74	**0.032**
TT3:TT4	0.06 ± 0.01	0.06	0.03 – 0.10	0.08 ± 0.03	0.08	0.04 – 0.18	0.11 ± 0.09	0.09	0.06 – 0.51	1.83	0.173

[1] total thyroxine
[2] free thyroxine
[3] total triiodothyronine
[4] free triiodothyronine
[a,b] different letters denote significant differences between groups

F. Contaminant concentrations with significant differences between groups

Table F.1: Concentrations of contaminant compounds (nmol/L) in plasma of Svalbard female polar bears (*Ursus maritimus*) with cubs of the year (FWCOY) females without cubs of the year (FWOCOY) and males. N denotes the number of observations per variable. For significant differences, *p*-values are given in bold.

		Females WCOY (n=8)			Females WOCOY (n=15)			Males (n=20)			ANOVA	
Analyte (nmol/L)		X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	F	p
Pesticides	oxychlordane	34.4 ± 35.7[a]	23.7	7.26 - 118	30.4 ± 20.8[a]	26.3	10.9 - 97.1	3.89 ± 2.65[b]	2.99	1.21 - 11.3	53.8	**< 0.00**
	trans-nonachlor	1.23 ± 0.96	1.19	0.06 - 3.13	1.49 ± 0.99	1.39	0.34 - 3.69	1.08 ± 0.77	0.93	0.32 - 3.10	0.69	0.509
	Mirex	0.23 ± 0.14[a]	0.17	0.13 - 0.55	0.18 ± 0.07[a,b]	0.19	0.07 - 0.32	0.13 ± 0.04[b]	0.14	0.03 - 0.24	3.74	**0.032**
	HCB	3.23 ± 2.62	1.87	1.05 - 8.62	3.56 ± 3.00	2.72	1.01 - 12.9	5.57 ± 4.77	3.86	0.92 - 18.9	1.31	0.281
	α-HCH	0.08 ± 0.04	0.09	0.02 - 0.16	0.12 ± 0.04	0.11	<0.00 - 0.20	0.07 ± 0.02	0.08	<0.00 - 0.13	1.51	0.233
	β-HCH	0.72 ± 0.42	0.56	0.35 - 1.57	0.81 ± 0.46	0.70	0.37 - 2.24	1.07 ± 0.70	0.84	0.33 - 3.39	1.72	0.193
	p,p'-DDT	0.32 ± 0.31	0.26	0.01 - 0.85	0.32 ± 0.44	0.16	0.01 - 1.35	0.30 ± 0.48	0.09	<0.00 - 1.83	0.09	0.912
	p,p'-DDE	1.05 ± 2.01	0.35	0.10 - 6.02	0.81 ± 0.43	0.62	0.25 - 1.80	0.80 ± 0.67	0.49	0.24 - 2.62	1.03	0.367
	Σpesticides[1]	41.2 ± 41.9[a]	28.2	9.83 - 138	37.7 ± 25.6[a]	31.8	13.3 - 118	12.9 ± 9.68[b]	9.26	3.06 - 39.3	7.05	**0.002**
PCBs	PCB-47	0.53 ± 0.46	0.41	0.13 - 1.51	0.55 ± 0.34	0.52	0.14 - 1.53	0.54 ± 0.48	0.34	0.11 - 1.59	0.32	0.726
	PCB-74	0.38 ± 0.14	0.39	0.22 - 0.58	0.33 ± 0.08	0.36	0.20 - 0.55	0.31 ± 0.12	0.32	0.04 - 0.62	0.89	0.417
	PCB-99	12.0 ± 12.5	7.90	3.41 - 41.3	11.70 ± 7.20	10.1	3.79 - 31.0	7.93 ± 5.31	5.86	2.03 - 20.1	2.01	0.148
	PCB-101	0.22 ± 0.24	0.18	<0.00 - 0.80	0.21 ± 0.11	0.21	0.06 - 0.54	0.17 ± 0.11	0.18	0.03 - 0.37	0.90	0.413
	PCB-105	0.24 ± 0.15	0.23	0.05 - 0.46	0.20 ± 0.10	0.20	0.09 - 0.51	1.00 ± 3.10	0.28	0.10 - 14.2	2.88	0.068
	PCB-114	0.07 ± 0.03	0.08	0.01 - 0.13	0.07 ± 0.04	0.07	0.04 - 0.20	0.06 ± 0.01	0.06	0.05 - 0.12	0.62	0.542
	PCB-118	1.23 ± 0.66	1.13	0.33 - 2.19	1.19 ± 0.42	1.19	0.50 - 2.06	1.30 ± 0.69	1.07	0.72 - 3.64	0.25	0.782
	PCB-128	0.13 ± 0.11	0.08	0.01 - 0.31	0.14 ± 0.10	0.13	<0.00 - 0.37	0.12 ± 0.09	0.09	0.01 - 0.30	0.31	0.738
	PCB-137	1.15 ± 1.16	0.80	0.30 - 3.87	1.13 ± 0.67	1.02	0.28 - 2.68	0.68 ± 0.42	0.52	0.23 - 1.71	2.91	0.066
	PCB-138	9.66 ± 10.1	7.52	1.72 - 32.8	10.7 ± 6.79	8.43	3.95 - 25.8	8.35 ± 5.79	6.19	2.68 - 23.3	0.96	0.392
	PCB-153	60.4 ± 68.7	33.2	13.7 - 223	58.2 ± 36.4	48.7	27.8 - 162	48.3 ± 36.1	34.6	13.8 - 148	0.70	0.503
	PCB-156	2.56 ± 2.80	1.53	0.83 - 9.29	2.21 ± 1.48	1.83	0.56 - 6.34	2.09 ± 1.27	1.71	0.39 - 5.61	0.03	0.973
	PCB-157	1.66 ± 1.94	0.81	0.51 - 6.34	1.57 ± 1.41	1.04	0.44 - 5.86	2.11 ± 1.18	1.77	0.82 - 4.86	2.56	0.090
	PCB-167	0.06 ± 0.03	0.06	0.02 - 0.12	0.05 ± 0.04	0.05	0.01 - 0.17	0.04 ± 0.04	0.05	<0.00 - 0.17	1.26	0.295
	PCB-170	14.7 ± 16.3	7.72	3.65 - 53.1	15.0 ± 12.3	12.6	5.46 - 54.5	17.0 ± 12.9	12.2	5.82 - 53.6	0.66	0.521
	PCB-180	36.8 ± 43.5	19.6	9.39 - 140	35.0 ± 21.1	26.6	14.2 - 87.8	28.7 ± 21.8	20.9	8.93 - 89.0	0.67	0.516
	PCB-183	0.99 ± 1.13[a,b]	0.69	0.16 - 3.65	1.00 ± 0.59[a]	0.87	0.44 - 2.42	0.57 ± 0.40[b]	0.42	0.14 - 1.45	3.48	**0.041**
	PCB-187	0.19 ± 0.14	0.17	0.04 - 0.47	0.22 ± 0.11	0.19	0.08 - 0.46	0.14 ± 0.10	0.1	0.04 - 0.41	2.53	0.093
	PCB-189	0.39 ± 0.37	0.25	0.14 - 1.28	0.38 ± 0.25	0.32	0.13 - 1.23	0.55 ± 0.37	0.44	0.18 - 1.35	1.74	0.189
	PCB-194	9.74 ± 7.99	6.97	3.89 - 27.4	8.45 ± 3.98	8.05	3.62 - 18.2	9.22 ± 5.51	6.73	3.88 - 24.4	0.04	0.958
	PCB-206	1.70 ± 1.12	1.31	0.43 - 3.54	1.51 ± 0.59	1.55	0.61 - 2.64	1.36 ± 0.73	1.07	0.81 - 3.33	0.32	0.731
	ΣPCBs[2]	155 ± 167	86.9	40.5 - 550	150 ± 91.2	116	68.0 - 405	131 ± 88.7	91.9	51.0 - 379	0.21	0.812

Table F.1. Continued

Analyte (nmol/L)		Females WCOY (n=8)			Females WOCOY (n=15)			Males (n=20)			ANOVA	
		X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	X ± SD	Median	Min - Max	F	p
OH-PCBs	4-OH-CB107	15.8 ± 13.6	12.0	1.83 - 42.7	9.41 ± 4.97	8.61	1.83 - 17.42	13.44 ± 8.39	9.40	4.71 - 35.8	0.93	0.403
	4'-OH-CB130	0.51 ± 0.53	0.24	0.21 - 1.47	0.42 ± 0.18	0.39	0.20 - 0.75	0.51 ± 0.27	0.47	0.18 - 1.00	0.27	0.764
	3'-OH-CB138	1.71 ± 1.19[a]	1.66	0.52 - 4.32	2.11 ± 1.08[a,b]	1.95	0.91 - 4.50	3.24 ± 2.13[b]	2.71	1.19 - 10.2	4.72	**0.015**
	4-OH-CB146	75.4 ± 41.9[a]	75.2	16.4 - 137	66.1 ± 20.2[a]	68.7	22.0 - 93.5	31.1 ± 21.7[b]	22.4	12.6 - 81.9	11.4	**< 0.00**
	4'-OH-CB159	2.00 ± 2.63[a]	0.83	0.50 - 8.25	0.59 ± 0.28[b]	0.51	0.19 - 1.13	0.66 ± 0.33[b]	0.64	0.26 - 1.54	5.03	**0.012**
	4'-OH-CB172	43.9 ± 31.5	41.8	7.45 - 99.8	46.9 ± 14.3	47.5	21.9 - 74.3	60.3 ± 19.8	66.8	15.6 - 91.9	2.95	0.065
	3'-OH-CB180	2.43 ± 1.00[a]	2.31	1.26 - 4.60	2.34 ± 1.02[a]	2.09	1.05 - 4.72	5.58 ± 3.22[b]	4.06	2.36 - 12.99	13.2	**< 0.00**
	4-OH-CB187	137 ± 88.2	134	24.6 - 260	126 ± 52.0	136	33.8 - 210	78.5 ± 62.2	50.2	19.9 - 234	3.57	**0.038**
	ΣOH-PCBs[3]	279 ± 151	302	57.2 - 484	254 ± 82.4	264	83.7 - 378	225 ± 104	211	81.9 - 407	2.28	0.116
PBDEs	BDE-47	0.49 ± 0.31[a]	0.44	0.11 - 0.99	0.43 ± 0.25[a]	0.37	0.18 - 1.17	0.18 ± 0.12[b]	0.13	0.06 - 0.42	11.1	**< 0.00**
	BDE-153	0.11 ± 0.05	0.10	0.05 - 0.20	0.13 ± 0.06	0.13	0.05 - 0.30	0.10 ± 0.04	0.11	0.04 - 0.17	1.28	0.291
	BDE-154	0.23 ± 0.23	0.15	0.06 - 0.80	0.21 ± 0.13	0.18	0.08 - 0.63	0.17 ± 0.12	0.14	0.06 - 0.55	0.71	0.497
	BDE-183	0.16 ± 0.19	0.08	0.03 - 0.58	0.40 ± 0.37	0.2	0.05 - 1.04	0.30 ± 0.27	0.28	0.04 - 1.01	1.78	0.181
	BDE-209	0.04 ± 0.01	0.05	0.02 - 0.07	0.04 ± 0.03	0.03	0.01 - 0.10	0.06 ± 0.05	0.05	0.01 - 0.21	1.48	0.240
	ΣPBDEs[4]	0.96 ± 0.63[a]	0.93	0.15 - 2.12	1.22 ± 0.54[b]	1.02	0.33 - 2.07	0.87 ± 0.32[a]	0.89	0.30 -1.39	3.17	0.053

[1] Σpesticides include oxychlordane, *trans*-Nonachlor, Mirex, HCB, α-HCH and β-HCH, p,p'-DDT and p,p'-DDE

[2] ΣPCBs the 21 PCB congeners, PCB – 47, -74, -99, -101, -105, -114, -118, -128, -137, -138, -153, 156, -157, -167, -170, -180, -183, -187, -189, -194 and -206.

[3] ΣOH-PCBs include the 8 metabolites: 4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187

[4] ΣPBDEs include the congeners BDE -47, -153, -154, -183 and -209.

[a,b] different letters denote significant differences between groups.