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Effects of Persistent Organic Pollutants on Reproductive Hormones in Male Polar Bears (*Ursus Maritimus*) from Svalbard

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Abstract

PCBs and other environmental contaminants have been found to have an effect on steroid hormones in polar bears (*Ursus maritimus*). The purpose of this study was to investigate the effect of persistent organic pollutants (POPs) on the steroidogenesis in male polar bears from Svalbard. Blood samples from male polar bears (n=23) were collected at Svalbard, Norway in April 2008 as a part of the International Polar Year-project, BearHealth. The sampled individuals were between 3-21 years, where individuals under 5 years (n=6) were categorized as subadults. Serum and plasma samples were analysed for steroid hormones (pregnenolone (Pre), progesterone (Pro), androstenedione (AN), dehydroepiandrosterone (DEA), testosterone (TS), dihydrotestosterone (DHT), estrone (E1), estradiol-17α (E2-α) and estradiol-17β (E2-β)) with a recently developed gas chromatography-tandem mass spectrometry (GC-MS/MS) determination method, whereas cholesterol concentrations were measured by Reflotron®. The environmental contaminants (HCB, α-HCH, β-HCH, oxychlordane, trans-nonachlor, mirex, p,p'-DDE, p,p'-DDT, PCB-47, PCB-74, PCB-99, PCB-101, PCB-128, PCB-137, PCB-138, PCB-153, PCB-170, PCB-180, PCB-183, PCB-187, PCB-194, PCB-206, PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167, PCB-189, 4'-OH-CB107, 4'-OH-CB130, 3'-OH - CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187, BDE-47, BDE-154) included in this study were analysed by gas chromatography-electron capture detection (GC-ECD) or GC/MS.

Multivariate regression analysis, principal component analysis (PCA) and orthogonal partial least squares (OPLS) regression, were performed to investigate the influence of the contaminants on the steroid hormones in the adult individuals. Only the OPLS model with DHT as the response variable was significant. Most of the environmental contaminants had a significantly negative contribution on the variation in dihydrotestosterone concentrations. Based on the statistical analyses, the poly-ortho PCBs and HCB might be more central in explaining the variation in DHT concentrations, while PBDEs and OH-PCBs seems to be less important. Androstenedione were found to be the androgen with highest concentration in circulating blood from male polar bears, unlike other studies on mammals where testosterone has been found to be the most abundant androgen. The high level of AN might be connected to the negative effect of contaminants on DEA levels.

The GC-MS/MS method applied in the current study can successfully compete with other frequently used determinations methods, such as RIA. The method is appropriate for the study several steroid hormones and their precursors in male polar bears and can be recommended for studies investigating the effect of persistent organic pollutants on the on the steroidogenesis.

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1. INTRODUCTION

1.1. Pollution in the Arctic

The Arctic region is a seemingly pristine and isolated environment with few local pollution sources. Nevertheless, the region is strongly affected by global pollution (AMAP, 1998). The year 2007-2008 was dedicated to Arctic (and Antarctic) researching, under the name "the International Polar Year", which purpose was to study how long-distance contaminants are affecting the Arctic environment and species (IPY, 2010). The contamination sources are most often located outside the Arctic region and long-range atmospheric transport of pollutants is reported to deliver most of the total quantity of persistent organic pollutants (POPs) measured in the Arctic (AMAP, 1998, 2002). The major route for contaminants is by atmospheric transport from mid-latitude areas to the Arctic (Brunström and Halldin, 2000). However, the atmospheric transport rate differs between seasons, with a higher rate in the winter (Barrie et al., 1992). Other transport routes for contamination are by northbound ocean currents. Furthermore, north-flowing rivers in North-Eurasia and North America transports contaminants and releases them into the Arctic oceans (Brunström and Halldin, 2000).

The Stockholm convention defines POPs as pollutants that remain stable in the environment for several years and can be distributed over long distances by natural processes (Stockholm convention, 2008). Since most POPs are lipophilic they often accumulate in the fatty tissue in living organisms, and tend to have higher concentrations at higher tropic levels compared to lower tropic levels. Arctic top predators live in a cold environment, and rely on energy-rich fatty tissue as their main energy source. Consequently, the POPs present in the fatty tissue will bioaccumulate in the Arctic food chain (AMAP, 2002).

Persistent organics pollutants include many different contamination groups; such as organochlorine pesticides (OCPs), which include dichloro diphenyl trichloroethane (DDT) used for pest control, polybrominated diphenyl ethers (PBDEs), which are used as flame-retardants in different electronics, and organochlorines (OCs), which includes polychlorinated biphenyls (PCBs). The polychlorinated biphenyls are the most common and studied persistent organic pollutants, and were used in insulating fluid and as a coolant in electrical equipment (AMAP, 2002). However, PCBs have been banned since the signing of the Stockholm convention in 2001, due to their resistance against degradation and possible adverse effects to human health and the environment (Stockholm convention, 2008).

Polychlorinated biphenyls include 209 congeners that vary in their degree of chlorination, where number and position of chlorine atoms influence their properties and toxicity (AMAP, 2002). Dependent on the number of ortho-substituted chlorine atoms PCBs can be classified into three groups: non-ortho, mono-ortho and poly-ortho PCBs. Non-ortho PCBs are regarded as the most toxic congeners, mediating toxicity through activation of the aryl-hydrocarbon-receptor (AhR). Mono-ortho PCBs have similar effects, but are weak AhR antagonists, while poly-ortho PCBs are not capable of binding to the receptor. These PCBs elicit toxicity in other ways (Fischer et al., 1998; Hestermann et al., 2000), which has been reported to include interference with Ca^{2+} signalling pathways in the central nervous system, in addition to affecting the availability of steroid and thyroid hormones (Darras, 2008). However, these pathways are not completely understood, and ought to be investigated further.

1.2. POPs in polar bears

Polar bears as top predators generally have a high level of POPs. However, there are geographical differences amongst polar bear populations. Polar bear populations at Svalbard are reported to have some of the highest PCB concentrations in the Arctic compared to populations from North America, which has been reported with much lower concentrations (Bernhoft et al., 1997; AMAP, 2002). The geographical differences are probably caused by the wind systems from Eurasia to Svalbard, which are assumed to be the most contributing sources to Arctic air contamination (Barrie et al., 1992). However, polar bear populations living in the Russian Arctic have been reported to feature higher PCB concentrations compared to polar bear populations from Svalbard (Lie et al., 2003), which is probably due to the local contamination sources.

Polar bears have an efficient cytochrome P450 system, and are able to metabolize POPs to a greater extent compared to its prey, which is mainly ringed seal (Derocher et al., 2002). Therefore, certain compound may be found at lower concentrations in polar bears compared to ringed seals. The more persistent compound will, however, accumulate to high concentrations in the polar bears, due to lack of effective metabolism of these compounds. Furthermore, the high metabolic capacity may lead to accumulation of metabolites, such has hydroxylated PCBs (OH-PCBs), which in some cases may cause greater toxic damage in polar bears than the parent PCB congener. The majority of biotransformed OH-PCBs is easily excreted, while the remaining OH-PCBs are limited to 5-10 single compounds and are mainly transformed from persistent penta-, hexa- and heptachlorinated PCBs (Letcher et al., 2000).

Exposure to OH-PCBs may lead to several possible effects, where those of most concern are effects regarding the thyroid- and reproductive system. Hydroxylated PCBs have been reported to interfere with the thyroid system through binding to plasma transport proteins, which decreases the circulation of thyroid hormones (Darras, 2008). Furthermore, certain hydroxylated PCBs have been reported capable of binding to estrogen hormone receptors (ER). However, they have a low binding affinity compared to the natural estrogens (Letcher et al., 2000). OH-PCBs have also been observed to inhibit estrogen sulfotransferase (hEST), which inactivates estrogens through mimicking estrogenic hEST binding (Kester et al., 2000; Shetsov, 2003). These interferences may lead to an increase of estrogen levels in male polar bears, and affect their reproductivity by disturbing the testosterone-estrogen ratio.

The PCB congeners 99, 118, 153, 156, 180 and 194 have been reported as the most abundant compounds in polar bears. The accumulation of non-ortho PCBs have been found to be low in polar bears, while mono- and poly-ortho PCBs accumulate to greater extent (Bernhoft et al., 1997). Furthermore, there are observed gender differences in polar bear populations in relation to accumulation. Higher ΣPCB levels have been discovered in males compared to females as a consequence of maternal transfer (Bernoft et al., 1997; Lie et al., 2000). However, highly chlorinated compounds and OH-PCBs tend to not be transferred from mother to offspring (Bernoft et al., 1997; Bytingsvik et al., 2012). Consequently, polar bear cubs already possess high levels of POPs by birth, and possibly due to their reduced biotranformation capacity, certain PCB concentrations in cubs and subadults have been reported to exceed concentrations present in adults (Polischuk et al., 2002; Bytingsvik et al., 2012).

Organchlorine pesticides are the most toxic amongst pesticides, which includes dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB). However, most pesticides have been banned for many years and were included in the Stockholm convention signed in 2001. The exception being DDT, which has been reintroduced as malaria control in some places of the world (Klaassen et al., 2008; Stockholm convention, 2008). HCB concentration has been reported to increase in the atmosphere, possible due to continued use of HCB as by products in chlorinated solvents (Hung et al., 2010). Therefore, pesticides are still found in the environment, and reported to possibly interact with reproductive hormones in male polar bears (Oskam et al., 2003; Haugestøl, 2009).

Brominated compounds, such as polybrominated diphenyl ethers (PBDEs), are reported to have similar effects on polar bears as PCBs (Darnerud, 2008). And as for PCBs, the properties of the

PBDE congeners are dependent of the bromination. However, commercial PBDEs products consist generally of penta-, hexa-, hepta- and/or decabromodipenyl ethers (deca-BDE), limiting the variety of properties (Darras, 2008). PBDEs have been reported to interfere with the metabolism of thyroid hormones (Darras, 2008), and act as agonists by binding to ER receptors *in vitro* (Meerts et al., 2001). Most PBDEs have been found to biomagnify in the lower tropical levels in the Arctic food chain. Only BDE-153, however, was reported to biomagnify in polar bears (Sørmo et al., 2006). Commercial PBDE-mixtures pentabromodiphenyl ethers and octabromodiphenyl ethers have been banned from the European Union Marked since 2004 (European Union, 2003), while deca-BDEs are still commercially available. In Norway, however, the use of deca-BDEs were restricted by the Norwegian government in 2008 (Statens forurensningstilsyn, 2008).

1.3. Effects of POPs on reproductive hormones in male polar bears

Male mammals are dependent on an appropriate production and function of reproductive hormones to achieve a normal sexual development. The production and secretion of most sex hormones are controlled by neurons and negative feedback regulation (Hill et al., 2008). However, the regulation of the steroid hormones can be affected by biological and/or environmental factors, which include toxic chemicals in the environment, and cause endocrine disturbance (Klaassen, 2008).

Testosterone (TS) is the primary androgenic steroid in males and is secreted from Leydig cells present in reproductive tissue. When TS has reached its target organ, it may be metabolized into dihydrotestosterone (DHT), which is the active form of testosterone, or bind directly to the androgen receptor (AR). Other androgens, such as androstenedione (AN) and dehydroepiandrosterone (DEA), function mainly as precursors for TS (Nieshlag and Behre, 2004). Testosterone and androstenedione can be further metabolized into estrogens. Estrogens, such as estrone (E1) and estradiol (E2), were earlier classified only as female steroids. However, more recent studies have discovered male reproduction to be dependent on estrogenic influence (Carpino et al., 2001; O′Donnell et al., 2001). Furthermore, estrogens might be responsible for testicular fluid production and intra testicular sperm transport (Carpino et al., 2001). A complete overview of relevant steps in the steroidogenesis is illustrated in Figure 1.

Both TS and DHT elicits their effects through binding to AR. However, DHT amplifies the effect of TS due to a stronger affinity for AR (Hsiao et al., 2000; Hill et al., 2008). Testosterone exerts a wide range of actions, which includes anabolic effects as growth of non-reproductive tissue, such as muscle, kidney, liver and salivary glands. In these tissues TS may bind directly to AR, due to the

limited amount of 5α-reductase in non-reproductive tissue. Anabolic effects are therefore mainly induced by TS, while both DHT and TS induces androgenic effects, which includes growth of the male reproductive tract (Sundaram et al., 1995; Hill et al., 2008). However, contaminants, by acting as agonists, may also bind to the AR and can cause endocrine disrupting effects (Boelsterli, 2007).

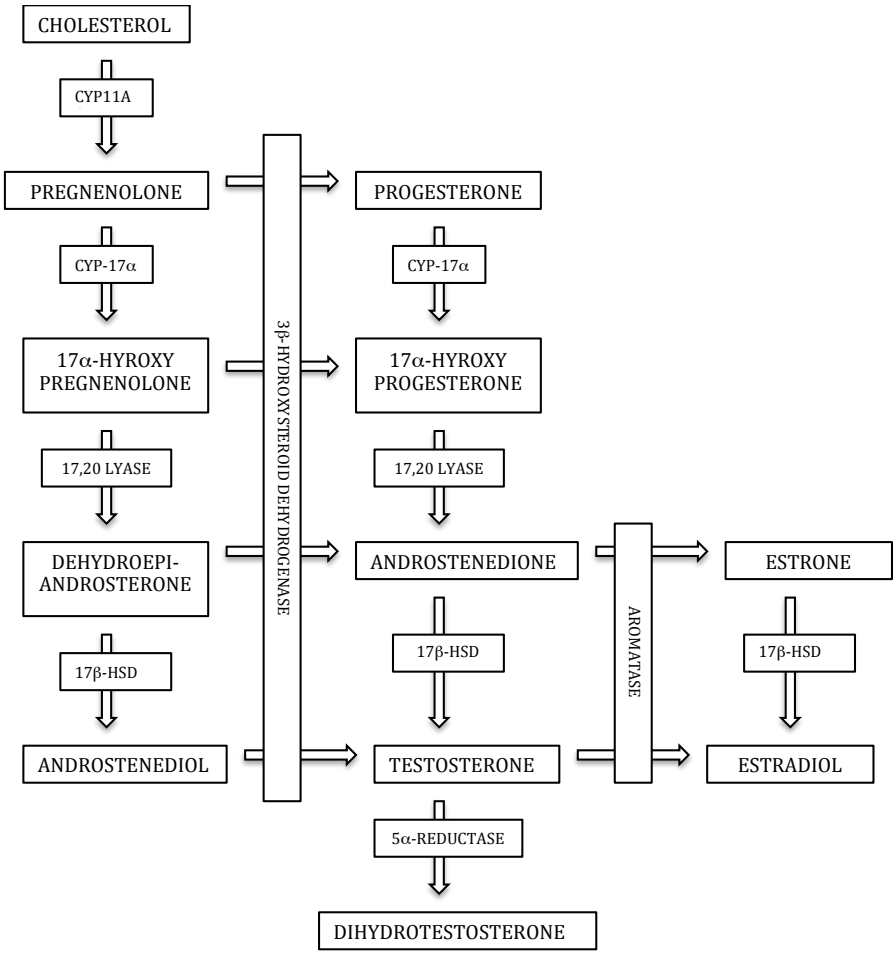

Figure 1. Overview of the relevant steps in the steroidogenesis.

Previous studies on male polar bears from Svalbard have reported that several PCBs have a negative effect on the testosterone concentrations measured in plasma (Oskam et al., 2003; Haugestøl, 2009). Exposure to certain PCBs, such as PCB congener 153 has been reported to damage DNA in goat sperm *in vitro* in addition to decrease testosterone concentration (Ropstad et al., 2006). Changes in the reproductive hormone concentrations in male polar bears may therefore affect their reproductivity and possible the population level (Oskam et al., 2003). The possible effects of PCBs cause concern for the male polar bear cubs due to their high PCB concentrations. Cubs are in a sensitive stage of development where any alterations in steroid hormone levels can disturb the sexual development and affect their reproductive health as adults (Lie et al., 2000).

In addition to effects related to steroid hormones, PCBs have been reported to have a negative effect on the circulating thyroid hormone and retinol levels, by outcompete them for the binding site on transport proteins (TTR) (Skaare et al., 2001; Gutleb et al., 2010).

The next step after a possible effect has been discovered is to locate the impact site. *In vitro* studies on male rats has reported decreased steroid enzyme activity due to PCB-mixtures exposure, in addition to reduced testosterone concentrations. The diminished enzyme activity indicated inhibition of the following enzymes: $P450_{scc}$ (CYP11A1), 3β-HSD, 17α-hydroxylase (CYP-17α) and 17β-HSD (Andric et al., 2000; Murugesan et al., 2005, 2008)

1.4. Aim of study

The aim of this study was to investigate the possible influence of POPs (PCBs, hydroxylated PCB metabolites, pesticides and PBDEs) on the levels of reproductive hormones in male polar bears from Svalbard, using a recently developed gas-chromatography tandem mass spectrometry (GC-MS/MS) determination method, capable to simultaneously analyse several steroid hormones. Possible relationships between contaminants and hormones were examined by the multivariate analysis, principal components analysis (PCA) and partial least squares regression (PLS).

2. MATERIALS AND METHODS

2.1. Sampling

The polar bear samples were collected at Svalbard in April 2008 as part of the project BearHealth, financed by the Norwegian Research Council. The aim of the study was to investigate the health status of polar bears in relation to climate change and pollution. The bears were sedated for sampling by remote injection of a dart containing Zoletil® (200 mg/mL; Virbac Laboratories, Carros, France), fired from a helicopter. The date of each individual sampling was recorded as the ordinal date, which is the day of the year ranging between 0-365. The age of the bears was estimated on number of annual growth layer groups (GLGs) in collected teeth. The body length was measured as the dorsal straight line from nose tip to the caudal end of the last tail vertebrae. Axial girth was measured as the circumference around the chest at the axilla. The head length was recorded as the straight line length between the upper middle incisors at the gum line, to the most posterior dorsal scull process of the sagittal crest. Zygomatic width was the maximum width between the zygomatic arches (Haugestøl, 2009).

The collected blood samples were centrifuged with and without anti-coagulant for separation of plasma and serum. Both plasma and serum polar bear samples were used in the steroid hormone determination, when no identified literature could conclude differences between the two sample types. The cholesterol concentrations in plasma samples were measured with clinical chemistry analyser with test-strip device (Reflotron® Cholesterol strips, Roche Diagnostics, Mannheim, Germany) and fat content was determined gravimetrically by drying out the samples. The procedures related to the sampling are described more closely in the study done by Haugestøl (2009).

2.2. Determination of environmental contaminants

Plasma samples were analysed for their contamination load in the Environmental Toxicology Laboratory at the Norwegian School of Veterinary Science, Oslo, Norway, and were performed as part of another project. The methods used were gas chromatography electron capture detection (GC-ECD) and gas chromatography mass spectroscopy (GC-MS/MS). The multicomponent method with extraction and clean up methods, chromatographic separation, equipment and quality control is based on a method originally described by Breivik (1978), and later modified by Bernhoft et al.

(1997). The method were extended to include OH-PCBs, which is described by Løken et al. (2006). A detailed description of the analytical method for the determination of the PBDEs can be found in the study done by Sørmo et al. (2006).

The following groups of contaminants were analysed: pesticides, PCBs, OH-PCBs and PBDEs. With the single contaminants (for complete names, see Appendix 1) included in this study being: HCB, α-HCH, β-HCH, oxychlordane, trans-nonachlor, mirex, p,p'-DDE, p,p'-DDT, PCB-47, PCB-74, PCB-99, PCB-101, PCB-128, PCB-137, PCB-138, PCB-153, PCB-170, PCB-180, PCB-183, PCB-187, PCB-194, PCB-206, PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167, PCB-189, 4'-OH-CB107, 4'-OH-CB130, 3'-OH - CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187, BDE-47, BDE-154.

2.3. Determination of steroid hormones

The serum and plasma samples were analysed by GC-MS/MS for determination of steroid hormone levels at the University of Copenhagen (Department of Pharmaceutics and Analytical Chemistry). Figure 2 illustrates the different steps of the analysis, which includes solid phase extraction (SPE), sample clean up, derivatization and final determination by GC-MS/MS. The following hormones were quantified: pregnenolone (PRE), progesterone (PRO), dehydroepiandrosterone (DEA), androstenedione (AN), testosterone (TS), dihydrotestosterone (DHT), estrone (E1), estradtion-17α (E2-α) and estradiol-17β (E2-β). These hormones cover the majority of the steroidogenesis.

The testosterone concentrations in the same male polar bears has also been analysed with radioimmunoassay (RIA) at the Department of Biology at the Norwegian University of Science and Technology, Trondheim. The RIA analysis was performed using a commercially available kit, a coated-tube radioimmunoassay for quantitative measurement of testosterone concentration in human serum (Spectria Testosterone RIA kit, Orion Diagnostrica, Espoo, Finland). Details about this procedure are found in the study done by Haugestøl (2009). The testosterone concentrations obtained by RIA were compared with the testosterone concentrations achieved by GC-MS/MS analysis, and included in the results.

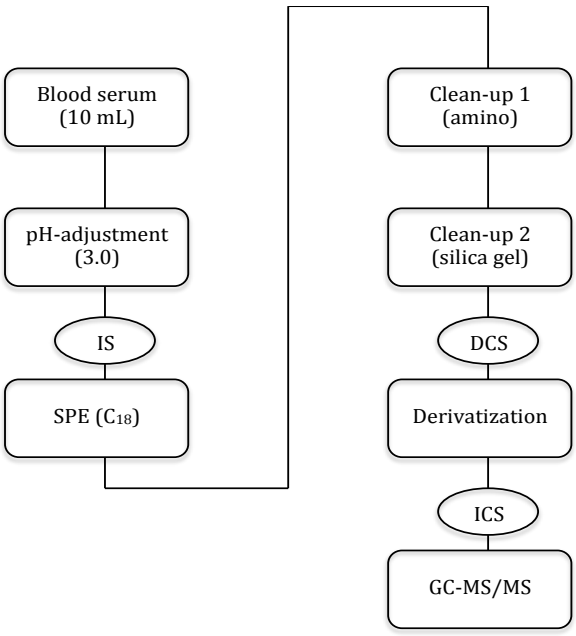

Figure 2. The illustration gives an overview over the different steps included in the steroid hormone determination procedure (Hansen et al., 2011).

2.3.1. Sample preparation

The steroid hormones in plasma/serum samples were stabilized by adjusting the pH to 3.0 with diluted sulphuric acid. Subsequently internal standard (IS) (0.4 ng/μL, 50 μL) were added, where the following deuterated analogues were used; d7-androstenedione (dAN), d4-estrone (dE1) and d5-17β-estradiol (dE2) d9-progesterone (dPRO), d3-testosterone (dTS) and d3-dihydrotestosterone (dDHT). Solid-phase extraction (SPE) was performed with C_{18} cartridges (500 mg, 10 mL reservoir, Varian Inc., Palo Alto, CA, USA) conditioned with heptane (2 × 3 mL), acetone (3 mL), methanol (2 × 3 mL) and finally with pH-fixed (pH 3) tap water (2 × 3 mL). Cartridges were placed in vacuum manifold (IST Vacmaster from Biotage, Uppsala, Sweden), and afterwards the samples were quantitatively transferred to the cartridges. Liquid were removed from the samples at a rate of 1-2 mL/min. After enrichment, the SPE cartridges were dried under complete vacuum for 1 hour. At this point, the cartridges were either stored frozen or analytes were eluted from the SPE cartridges with acetone (5 mL) into a test tube and evaporated to dryness with nitrogen. Finally samples extracts were reconstituted in chloroform (100 μL).

Next in the sample preparation included a two-step clean-up procedure, involving aminoprophyl cartridges (500 mg, Waters Sep-pak, Ireland) and freshly prepared silica gel (60 mesh, Merck, Darmstadt, Germany) filled in glass cartridges (3 mL, LiChrolut, Merck, Darmstadt, Germany) with

a Chromabond filter (Macherey-Nagel, Düren, Germany) in the bottom. The clean-up stages were performed for purification of the steroid hormones from matrix interferences. The first stage was applied for removal of phospholipids and fatty acids, and the second for elimination of steroid esters and triglycerides. The aminoprophyl cartridges were conditioned with heptane (2 × 2 mL) followed by sample application. The test tube was rinsed with additional volume of chloroform:isopropanol (2:1, 5 mL), which was transferred to the same aminoprophyl cartridge. The analytes were eluted with chloroform:isopropanol (2:1, 5 mL) into a new test tube and once more evaporated to dryness. The eluate was reconstituted in chloroform (50 μL) and additionally heptane (450 μL) was added to the solution. Further cleaning of the samples was performed on a silica gel prepared in heptane (silica gel was ensure not to dry out until the final elution). The reconstituted sample was gently added on top of the silica gel. The test tube was rinsed twice with an additional portion of heptane (100 μL) and applied to the same cartridge. Interfering substances were removed from the gel by the addition of heptane (5 mL) followed by heptane:acetone (90:10, 10 mL). Subsequently, the steroid hormones were eluted from the gel with heptane:acetone (65:35, 5 mL) and evaporated to 1 mL.

The extracts were added derivatization control standard (DCS) (17β-estradiol-17-acetate (AE2)) (0.2 ng AE2/μL, 100 μL), followed by evaporation to dryness. The samples were derivatized by adding a derivatization mixture (50 μL) and placed in an oven to initiate the transformation (1 h, 60 °C). The derivatization mixture was made by mixing N-methy-N-trimethylsilyl-trifluoroacetamide (MSTFA) (1000 μL, Sigma-Aldrich, Glostrup, Denmark), N-trimethylsilylimidazole (TMSI) (2 μL, Sigma-Aldrich, Glostrup, Denmark) and 1,4-dithioerythritol) (DTE) (50 μL, Sigma-Aldrich, Glostrup, Denmark). The derivatization step was performed in order to enhance volatility and thermal stability of the steroid hormones. Next the samples were evaporated to dryness and dissolved in heptane (200 μL) containing instrumental control standard (ICS) (estrone-3-methyl ether (MeE1)) (0.1 ng MeE1/μL), and finally transferred to autosampler vials (300 μL) and analysed with GC-MS/MS. The steroid hormone analyse procedure is described in details by Hansen et al. (2011).

2.3.2. Limit of detection

The limits of detection (LOD) were taken from a previous study (Hansen et al., 2011). The values are based on the analysis of plasma samples (10 mL) of male rats (Table 1). They were determined from individual calibration curves and calculated as 3.3 times the residual standard deviation of the regression relative by the slope (Hansen et al., 2011).

Table 1. Limit of detection values for the analysed steroid hormones (Hansen et al., 2011).

Steroid hormones	LOD (nmol/L)
Pregnenolone (Pre)	1.36
Progesterone (Pro)	1.14
Dehydroepiandrosterone (DEA)	1.21
Androstenedione (AN)	0.91
Testosterone (TS)	1.25
Dihydrotestosterone (DHT)	0.69
Estrone (E1)	0.33
Estradiol-α (E2-α)	0.29
Estradiol-β (E2-β)	0.59

2.4. Statistical analysis

In cases where the steroid concentrations were under the detection limit, random numbers between 0 and LOD were used for the statistical analysis. However, random values were not used in the calculation of averages, standard deviation etc. The non-parametric statistical analysis comparing two independent groups (Mann-Whitney U test) was conducted for investigation of concentration differences of contaminants between the age classes (subadults and adults). As a part of the assessment of the GC-MS/MS method, a T-test for independent samples were conducted to test for significant difference between TS concentrations achieved by GC-MS/MS and by RIA.

2.4.1. Multivariate analysis

For the multivariate analysis the software Simca-P version 12 (2008, Umetrics, Umeå, Sweden) was used to perform principal component analysis, and partial least squares regression. The analyses were performed using unit variance (UV) scaling, as suggested by the software manual. Unit variance scaling gives each variable a variance of one and an equal chance of being represented in the models (Simca-P+ 12, 2008).

Principal component analysis

A principal component analysis was performed to get an understanding on how the variables are correlated, and how the variation is distributed amongst the sampled individuals. Principal component analysis can also detect outliers. The procedure converts a set of observations of the possible correlated variables into a set of uncorrelated variables, called principal components. The components are sorted after size, where the first principal component is the most important and explains most of the variability in the data. Two parameters are regarded as important in the principal component analysis: R^2X and Q^2X. R^2X indicates how much of the variation that is explained by the PCA model. Q^2X describes the prediction ability of the model by cross-validation of the data. These parameters vary between 0 and 1, where values close to 1 indicates a model with perfect fit and good prediction power, while values close to zero indicates no fit and low prediction (Simca-P+ 12, 2008).

Orthogonal partial least squares regression

An orthogonal partial least square (OPLS) model, which is an extension of the partial least square (PLS) model, was applied to identify the X-variables which best explained the variation in the Y-variable(s). Partial least square analysis is used to predict possible linear relations between two matrices: the x-variables (predictor variables) and the y-variable(s) (response variables). The relationships can be found by projecting the X and Y space on low dimensional hyper planes. The model finds the multidimensional direction in the X space that explains the maximum multidimensional variance in the Y space. Orthogonal partial least square analysis separates the systematic variation in the X space into two parts, where one part predicts the correlation between X and Y, while the other part is uncorrelated (orthogonal) to Y and expresses the systematic X-variation (Trygg, 2004; Simca-P+ 12, 2008). There are three parameters that can be used to validate the model: R^2X, which explains the variance; R^2Y, which represent the correlation coefficient; and Q^2Y, which represents the prediction coefficient (Simca-P+ 12, 2008). According to guidelines set by Lundstedt et al. (1998), a biological PLS model is considered to have good quality if the R^2 value is above 0.7, and the Q^2 value is above 0.4 (Lundstedt et al., 1998).

2.4.2. Spearman′s rank correlations coefficient test

For further investigation of results achieved from the PCA and OPLS models, Spearman two-tailed correlation analysis, were applied (SPSS version 18.0 for Mac, 2010, IBM, Chicago, Illinois, USA). Spearman correlation test is a non-parametric test, which measured the strength of the relationships between two variables.

3. RESULTS

3.1. Biological variables

The sampled polar bears were divided into two age classes, where those between 5-21 years (n=17) were categorized as adults, while those between 3-4 years (n=6) as subadults. The sample location ranged based on longitudinal gradient from Vitovskybreen in the south (76.7° N) to Waldenøya in the north (80.6 ° N), and based on latitude from Lidefdefjorden in the west (12.6° E) to Duvefjorden in the east (23.8 ° E) of Svalbard. Details on biological variables and location of sampling of the 23 male polar bear individuals are presented in Table 2.

Table 2. Ordinal date (day of sampling (1-365)), latitude, longitude and biological variables of 23 male polar bears from Svalbard.

Variables	Mean	Standard deviation	Median	Minimum	Maximum
Ordinal date (1-365)	105	5.8	104	98.0	115
Latitude (°)	79.0	1.2	79.5	76.7	80.6
Longitude (°)	17.9	3.1	17.0	12.6	23.8
Age (years)	10.3	5.5	10.0	3.0	21.0
Condition (1=poor, 5=good)	3.2	0.5	3.0	2.0	4.0
Body length (cm)	223	21.8	225	170	252
Axial girth (cm)	114	20.5	147	110	176
Head length (mm)	388	29.4	392	321	439
Zygomatic width (mm)	241	33.3	251	174	288
Estimated total body weight (kg) (Derocher and Wiig, 2002)	347	117	346	145	539

3.2. Environmental contaminants levels

The results of the concentrations achieved by the environmental contaminant analysis are presented in Table 3, 4, 5 and 6. Only detectable values were included in the calculations. In cases where the concentrations were below the detection limit, LOD values were set as the minimum value. Table 6 gives the calculated sums of each of the contamination groups and the total load, which is the sum of all the contaminants. Hydroxylated PCBs were found to have the highest average concentrations in the plasma of male polar bears, followed by PCBs, pesticides and PBDEs. 4-OH-CB187 was the single contaminant with the highest average concentrations in both age groups. Amongst the PCBs, PCB153 was found to have the highest concentration.

Table 3. PCB and OH-PCB concentrations measured plasma samples of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard.

	Mean (nmol/L)		Standard deviation (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)		N	
Variables	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.
PCB-47	0.56	0.89	0.49	0.63	0.38	0.79	0.12	0.29	1.69	2.01	17	6
PCB-74	0.37	0.36	0.12	0.02	0.35	0.36	<0.22	0.33	0.66	0.40	15	6
PCB-99	8.11	12.11	5.02	7.09	6.99	10.71	2.15	5.25	20.81	23.23	17	6
PCB-101	0.27	0.29	0.08	0.07	0.25	0.27	<0.19	<0.19	0.39	0.38	10	5
PCB-105	1.20	0.31	3.57	0.04	0.29	0.31	0.10	0.25	15.02	0.36	17	6
PCB-114	0.07	0.08	0.02	0.01	0.07	0.08	0.05	0.06	0.13	0.09	17	6
PCB-118	1.38	1.13	0.79	0.07	1.12	1.12	0.76	1.04	3.85	1.25	17	6
PCB-128	0.18	0.09	0.08	0.04	0.17	0.09	0.07	<0.03	0.29	0.13	11	4
PCB-137	0.73	1.20	0.44	0.74	0.60	1.06	0.24	0.40	1.81	2.26	17	6
PCB-138	8.41	11.65	5.17	7.70	7.77	9.54	2.84	4.30	19.74	23.76	17	6
PCB-153	47.79	60.52	31.06	34.19	38.47	48.01	14.58	26.65	141.66	108.05	17	6
PCB-156	2.16	2.49	1.34	1.87	1.88	1.89	0.42	0.92	5.95	6.03	17	6
PCB-157	2.11	2.16	1.14	0.80	1.83	2.00	0.87	1.23	5.04	3.52	17	6
PCB-167	0.09	0.08	0.04	0.02	0.08	0.07	<0.06	<0.06	0.18	0.11	7	4
PCB-170	15.09	15.20	9.96	5.41	11.75	12.44	5.64	10.55	44.95	22.82	17	6
PCB-180	28.17	33.24	18.73	16.71	22.41	24.74	9.46	19.36	88.40	58.10	17	6
PCB-183	0.60	1.00	0.39	0.71	0.46	0.81	0.15	0.29	1.35	2.06	17	6
PCB-187	0.16	0.29	0.11	0.17	0.12	0.31	0.04	0.10	0.43	0.56	17	6
PCB-189	0.57	0.48	0.39	0.18	0.45	0.45	0.19	0.25	1.43	0.79	17	6
PCB-194	9.25	7.29	4.74	2.51	7.17	6.82	4.11	4.55	20.43	11.36	17	6
PCB-206	1.39	1.01	0.63	0.33	1.15	0.96	0.86	0.64	2.85	1.52	17	6
4-OH-CB107	14.61	12.15	8.99	6.49	10.78	10.86	6.03	5.00	37.95	20.44	16	5
4′-OH-CB130	0.50	0.62	0.26	0.27	0.49	0.59	<0.19	<0.23	1.06	0.98	11	4
3′-OH-CB138	3.23	2.11	1.39	0.76	2.97	2.29	1.84	1.27	6.27	3.08	16	5
4-OH-CB146	33.51	66.66	23.66	35.17	24.44	60.94	13.40	18.18	87.06	108.84	16	5
4′-OH-CB159	0.74	0.77	0.37	0.43	0.69	0.67	0.28	0.36	1.64	1.50	16	5
4′-OH-CB172	62.49	68.31	20.87	14.25	71.50	69.89	16.02	51.49	87.34	85.77	16	5
3′-OH-CB180	5.94	2.82	3.45	0.56	4.74	2.63	2.51	2.18	31.80	3.64	16	5
4-OH-CB187	87.24	140.65	70.48	93.04	55.63	111.51	21.09	44.28	248.66	257.91	16	5

Table 4. Pesticide concentrations measured in plasma samples of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard.

	Mean (nmol/L)		Standard deviation (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)		N	
Variables	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.
HCB	5.81	7.37	5.17	6.09	4.16	4.81	0.97	1.57	19.98	18.07	17	6
α-HCH	0.09	0.12	0.02	0.06	0.09	0.08	<0.05	0.07	0.14	0.21	14	6
β-HCH	1.04	1.04	0.50	0.48	0.90	0.92	0.35	0.60	2.29	1.63	17	6
Oxychlordane	4.25	11.29	3.04	5.52	3.13	11.58	1.29	3.04	12.02	18.67	17	6
Trans-nonachlor	1.91	1.20	0.88	0.32	0.97	1.17	0.34	0.88	3.29	1.78	17	6
Mirex	0.14	0.17	0.05	0.05	0.15	0.18	0.04	0.10	0.26	0.23	17	6
p,p′-DDE	0.91	1.65	0.76	0.95	0.50	1.50	0.25	0.54	2.78	2.87	17	6
p,p′-DDT	0.80	0.75	0.72	0.31	0.48	0.73	<0.12	<0.12	1.94	1.22	6	5

Table 5. PBDE concentrations in plasma samples of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard.

	Mean (nmol/L)		Standard deviation (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)		N	
Variables	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.
BDE-47	0.20	0.39	0.13	0.20	0.15	0.36	0.06	0.10	0.45	0.66	17	6
BDE-154	0.17	0.18	0.10	0.06	0.15	0.14	0.07	0.11	0.44	0.27	17	6

Table 6. The sums of pesticides, poly-ortho PCBs, mono-ortho PCBs, PCBs, OH-PCBs, PBDEs and the total load (total sum of all the contaminant concentrations) in plasma samples of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard. The number of individual contaminants is given in the brackets.

	Mean (nmol/L)		Standard deviation (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)	
Variables	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.
Σpesticides (8)	13.75	23.47	8.96	10.01	9.98	25.13	5.06	10.40	36.82	37.77
Σpoly-ortho PCBs (13)	120.57	144.69	72.37	72.30	96.07	112.88	42.19	83.07	340.90	244.94
Σmono-ortho PCBs (8)	7.89	7.07	4.12	2.77	6.71	6.16	3.85	4.84	18.26	12.30
ΣPCB (21)	128.46	151.76	74.36	74.67	100.38	118.64	53.35	88.30	355.50	257.24
ΣOH-PCBs (8)	208.16	293.97	107.25	140.90	186.42	241.60	85.01	142.07	432.00	475.89
ΣPBDEs (2)	0.37	0.57	0.19	0.26	0.29	0.50	0.16	0.21	0.82	0.93
Total load (39)	338.49	420.77	170.72	223.05	305.19	404.16	97.66	142.81	660.20	701.59

The distribution of contaminants between the age classes (adults and subadults) was tested by nonparametric Mann-Whitney U test. A significant difference ($p<0.05$) were found for: oxychordane, p,p′-DDT, 4-OH-CB146, 3′-OH-CB180, BDE-47 and Σpesticides. The average concentrations for these compounds were significantly higher in the subadults compared to the adults, except for 3′-OH-CB180 where the adults had a significantly higher average concentration than the subadults.

3.3. Steroid hormone levels

The mean, standard deviation, median and range of the measured levels of the steroid hormones, in addition to the lipid content and cholesterol levels are presented in Table 7. Not all the individuals had detectable levels of steroid hormones, especially regarding estrogens, where the levels were found to be low. Only detectable values were included in the calculations. In cases where the concentrations were below the detection limit, LOD values were set as the minimum value. However, since these LOD values were taken from a previous study (Hansen et al., 2011), concentrations lower than the given LOD values were detected in this current study. In these cases the lowest detectable value were preferred over the LOD value.

Large variations in the steroid levels were found between the individuals. The largest variations were observed in AN levels (12.60-214.66 nmol/L) and TS levels (<1.25-119.59 nmol/L) in the adult polar bears.

Table 7. Lipid content and hormone levels in plasma and serum samples of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard.

	Mean (nmol/L)		Standard deviation (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)		N	
Variables	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.	Adult	Sub.
Lipid content (%)	0.84	1.11	0.18	0.31	0.83	1.11	0.56	0.74	1.22	1.50	17	6
Cholesterol (mmol/L)	6.47	7.85	1.21	2.36	6.50	7.35	3.90	5.10	8.30	11.50	17	6
Androstenedione (nmol/L)	78.94	12.15	61.88	5.36	50.45	11.56	12.60	6.97	214.66	21.92	17	6
Dehydroepiandrosterone (nmol/L)	2.15	0.86	2.39	0.32	1.16	0.79	0.34	0.48	9.05	1.36	16	6
Dihydrotestosterone (nmol/L)	0.96	0.54	0.64	0.39	0.83	0.60	0.17	0.07	2.32	1.12	17	6
Testosterone (nmol/L)	45.51	2.77	37.06	2.55	45.08	1.85	<1.25	<0.80	119.59	5.64	15	3
Estrone (nmol/L)	0.37	2.99	0.29	3.24	0.31	1.05	<0.08	<0.33	1.05	6.86	11	5
α-Estradiol (nmol/L)	0.15	0.34	0.08	0.24	0.12	0.32	<0.07	0.10	0.30	0.63	8	4
β-Estradiol (nmol/L)	0.09	0.93	0.07	1.92	0.06	0.06	0.02	0.03	0.29	4.82	14	6
Pregnenolone (nmol/L)	1.12	2.31	0.22	0.80	1.08	2.54	0.66	1.08	1.49	3.10	17	6
Progesterone (nmol/L)	1.38	-	1.73	-	1.23	-	<0.13	<1.14	5.08	<1.14	7	0

3.4. Multivariate data analysis

In the PCA and OPLS analysis, the following variables were included: sampling date, sampling location, age, weight (estimated), condition, body length, axial girth, zygomatic width, cholesterol, lipid content, androstenedione, dehydroepiandrosterone, dihydrotestosterone, testosterone, estrone, estradiol-α, estradiol-β, progesterone, pregnenolone, mirex, trans-nonachlor, oxychlordane, HCB, α-HCH, β-HCH, p,p′-DDE, p,p′-DDT, PCB-47, PCB-74, PCB-99, PCB-101, PCB-105, PCB-114, PCB-118, PCB-128, PCB-137, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-170, PCB-180, PCB-183, PCB-187, PCB-189, PCB-194, PCB-206, 4′-OH-CB107, 4′-OH-CB130, 3'OH - CB138, 4-OH-CB146, 4'OH-CB159, 4′-OH-CB172, 4′-OH-CB180, 4'OH-CB187, BDE-47 and BDE-154. According to the Shapiro-Wilk test the following variables was not normally distributed: androstenedione, dehydroepiandrosterone, dihydrotestosterone, estrone, estradiol-β, progesterone, HCB, oxychlordane, trans-nonachlor, DDE, DDT, PCB (47, 128, 153, 170, 180, 183, 194, 206, 105, 114, 118, 157, 167, 189), BDE-47, BDE-154 and all OH-PCB congeners. These variables were log transformed before further statistical analysis.

3.4.1. Principal component analysis (PCA)

Initially, the PCA analysis was first performed including all 23 male polar bears, which resulted in three significant principal components. First component (PC1) explained 33.4%, component 2 (PC2) explained 17.4%, and component 3 (PC3) explained 11.4% of the variation in the data set. As shown in the score plot (Figure 3), there is a clear difference between the two groups, subadult

and adult polar bears. Because the major goal of the analysis was to see the potential effects of contaminants on sex hormones, subadults were excluded form further multivariate data analysis. This approach was applied to eliminate potential confounding factors related to natural differences in male steroid hormone levels between immature and adult animals. Separate multivariate analyses were not performed for subadults due to the low number of immature individuals. However, it has to be mentioned that the different loading plots (Figure 4 and Figure 6) shows similar pattern for both analysis- with and without subadult polar bears.

PCA analysis including only the adult polar bears (R^2X= 0.636, Q^2=0.374) resulted in three significant principal components, where PC1 explained 34.1%, PC2 explained 17.0%, and PC3 explained 12.5% of the variation among the individuals. The principal components with highest explanatory power (PC1 and PC2), where plotted against each other. The score plot, which illustrates the variation between the individuals, is presented in Figure 5. There were observed large variations amongst the individuals, but no outliers were detected. The outliers are defined as individuals that are located outside of the Hotelling T2 range (indicated with the circle). Individual 23949 was the only individual placed in a relative large distance from the other individuals. High value sum of all contaminants (total load) (660.20 nmol/L) that was almost twice the average load (338.49 nmol/L) was found in the plasma of this polar bear. However, this individual is not considered as an outlier.

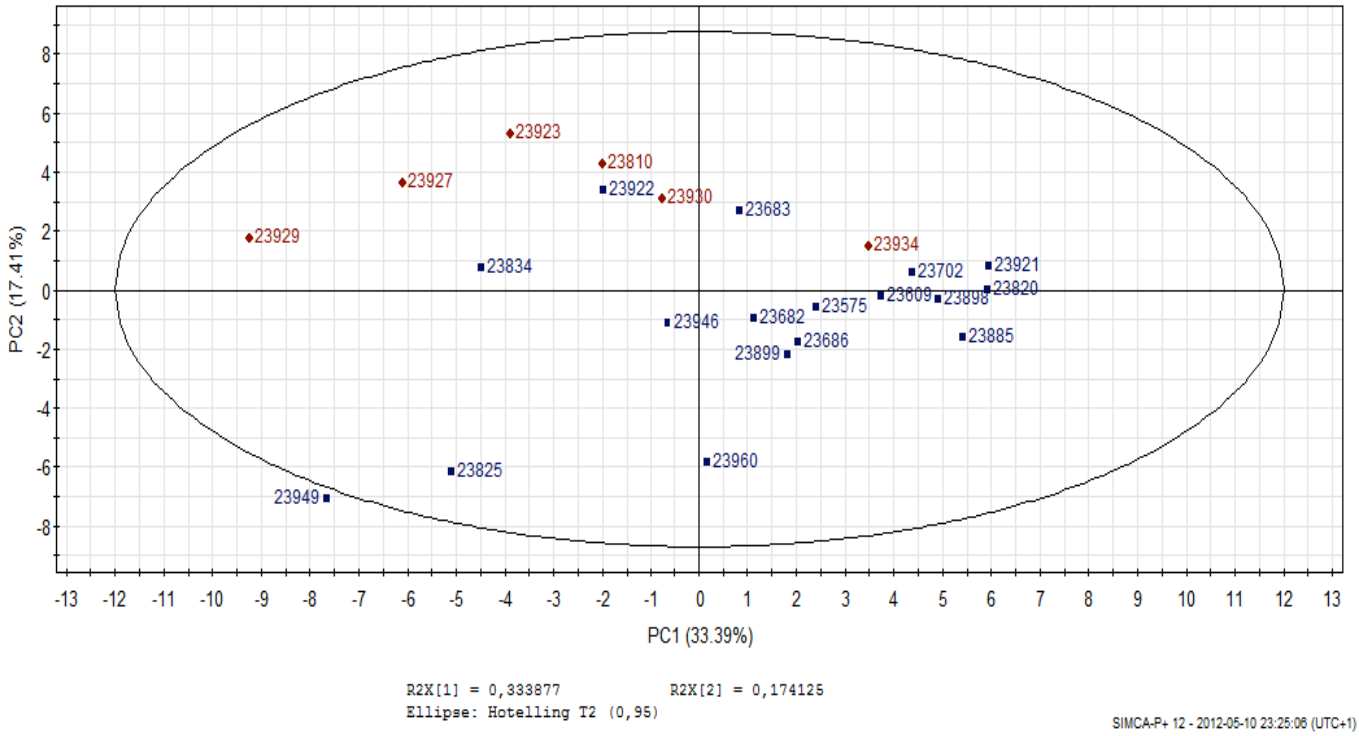

Figure 3. Biplot of scores in PC1/PC2 dimension based on contaminant and steroid hormone concentrations in plasma or serum samples and biological measurements for 23 male polar bears from Svalbard. Subadults (n=6) and adults (n=17) are marked with different colour.

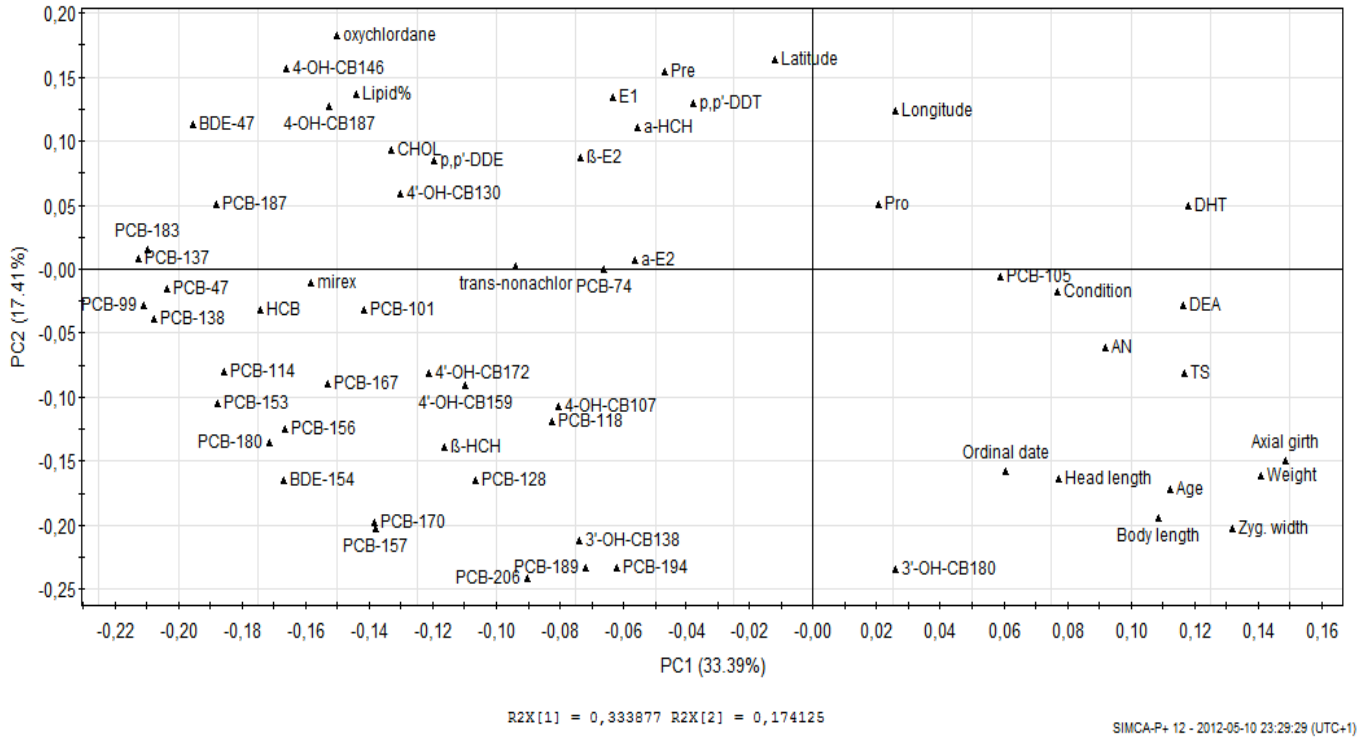

Figure 4. Biplot of loadings in PC1/PC2 dimension based on contaminant and steroid hormone concentrations in plasma or serum samples and biological measurements for 23 male polar bears from Svalbard.

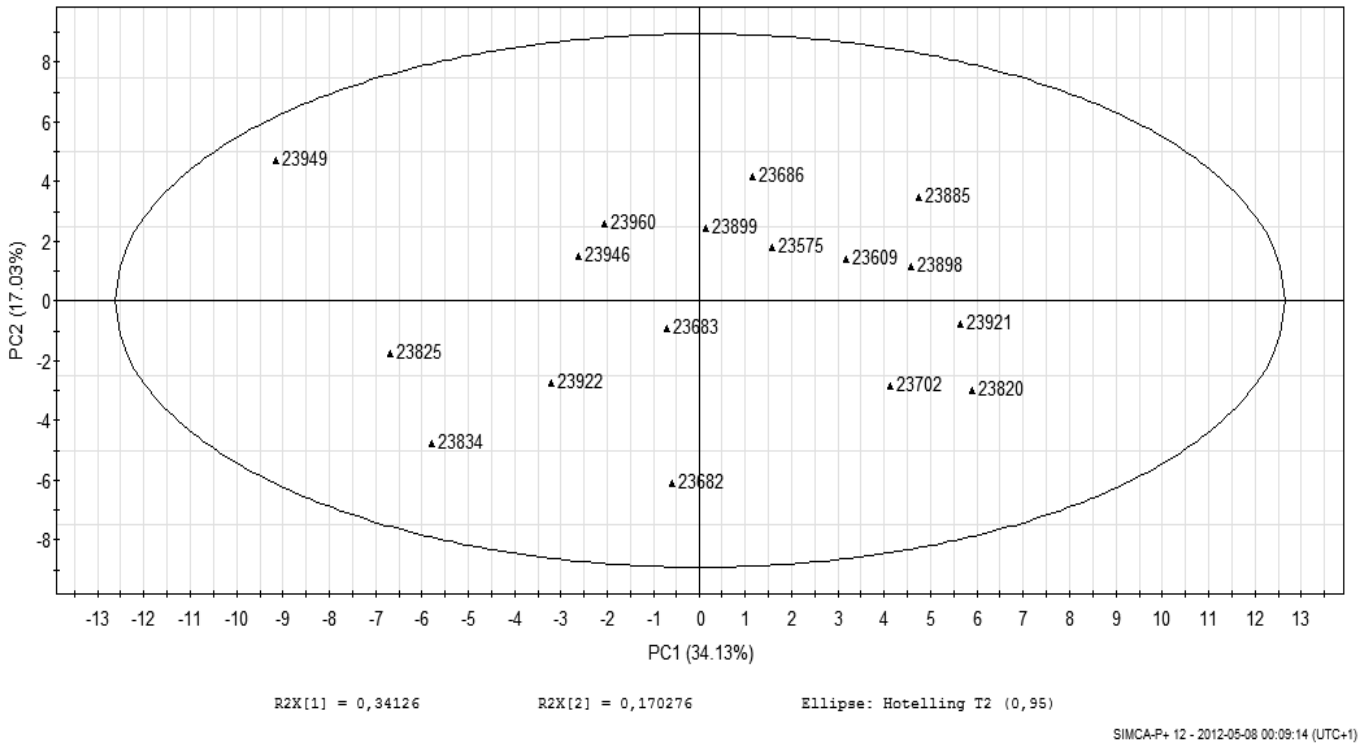

Figure 5. Biplot of scores in PC1/PC2 dimension based on contaminant and steroid hormone concentrations in plasma or serum samples and biological measurements for 17 adult male polar bears from Svalbard.

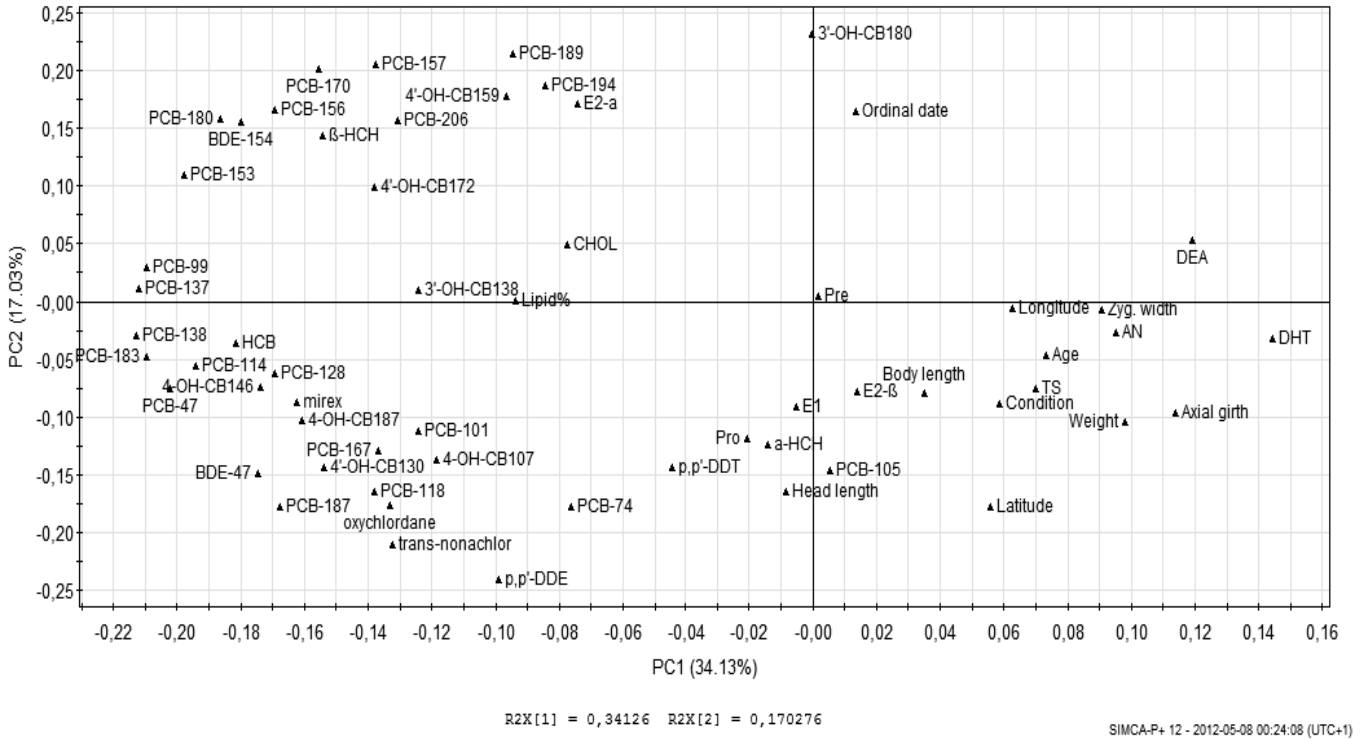

Figure 6. Biplot of loadings in PC1/PC2 dimension based on contaminant and steroid hormone concentrations in plasma or serum samples and biological measurements for 17 adult male polar bears from Svalbard.

The loading plot illustrates the distribution of the variables (Figure 6). The androgens (AN, DEA, TS and DHT) are located relative close to each other, placed horizontally along the PC1. Many biological variables as age, weight, body length and axial girth are also located in this area, indicating a positive correlation between androgens and biological variables. In contrast, the environmental contaminants are placed on the left side of the PC1, indicating a negative correlation between the environmental contaminants and the androgens. Among these, PCB congeners 138, 99, 153 and 114, and HCB are grouped in the largest distance from the androgen group, indicating strong negative correlation to hormones, while the OH-PCBs, the pesticides and the other PCBs are located closer to the androgens, implying weaker correlations. The female steroids (PRE, PRO, E1, E2-α and E2-β) are located either on the left side of PC1 along with the contaminants, or more in the middle of the plot, suggesting no correlations or week positive correlations with the contaminants.

3.4.2. Orthogonal partial least square regression

Orthogonal partial least squares regression was performed with different reproductive steroids as the Y-variable, and all the other variables as X-variables. According to the ANOVA of the cross-validated residuals (CV-ANOVA), which was applied for assessing the reliability of PLS and OPLS models with adult polar bears, only the model with DHT as response variable was found to be significant ($p<0.05$) ($R^2X=0.326$, $R^2Y=0.562$, $Q^2=0.342$). Therefore only this OPLS model was included in the results.

Variable importance in projection (VIP) coefficients was used for classifying the X-variables according to their explanatory power of Y (Figure 7). Variables with a VIP value >0.8 were considered as important variables for describing the variation in DHT concentrations according the SIMCA-P+ 12 tutorial. Based on this, the following variables with values lower than 0.8 were excluded from the OPLS model: Ordinal date, p,p′-DDT, E2-β, weight, zygomatic width, PCB-74, E2-α, testosterone, α-HCH, lipid content, dehydroepiandrosterone, axial girth, head length, progesterone, age, estrone, 3´-OH-CB180, cholesterol, p,p′-DDE, oxychlordane, 4´-OH-CB172, PCB-194, PCB-105, body length, androstenedione, 4-OH-CB146, 4´-OH-CB130 and pregnenolone. A second OPLS model was performed including the remaining variables ($R^2X=0.526$, $R^2Y=0.549$, $Q^2=0.417$).

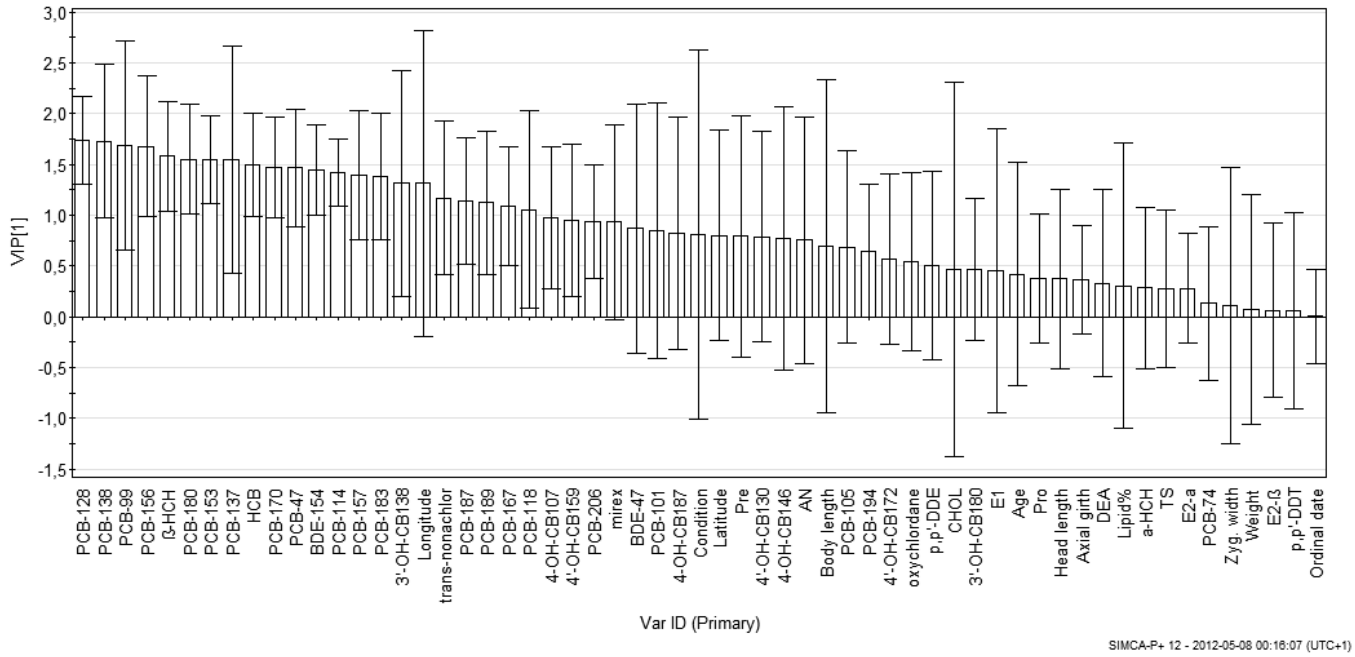

Figure 7. OPLS regression VIP plot, which classifying the X-variables according to their explanatory power of DHT. Variables with a VIP value over 0.8 were regarded as important.

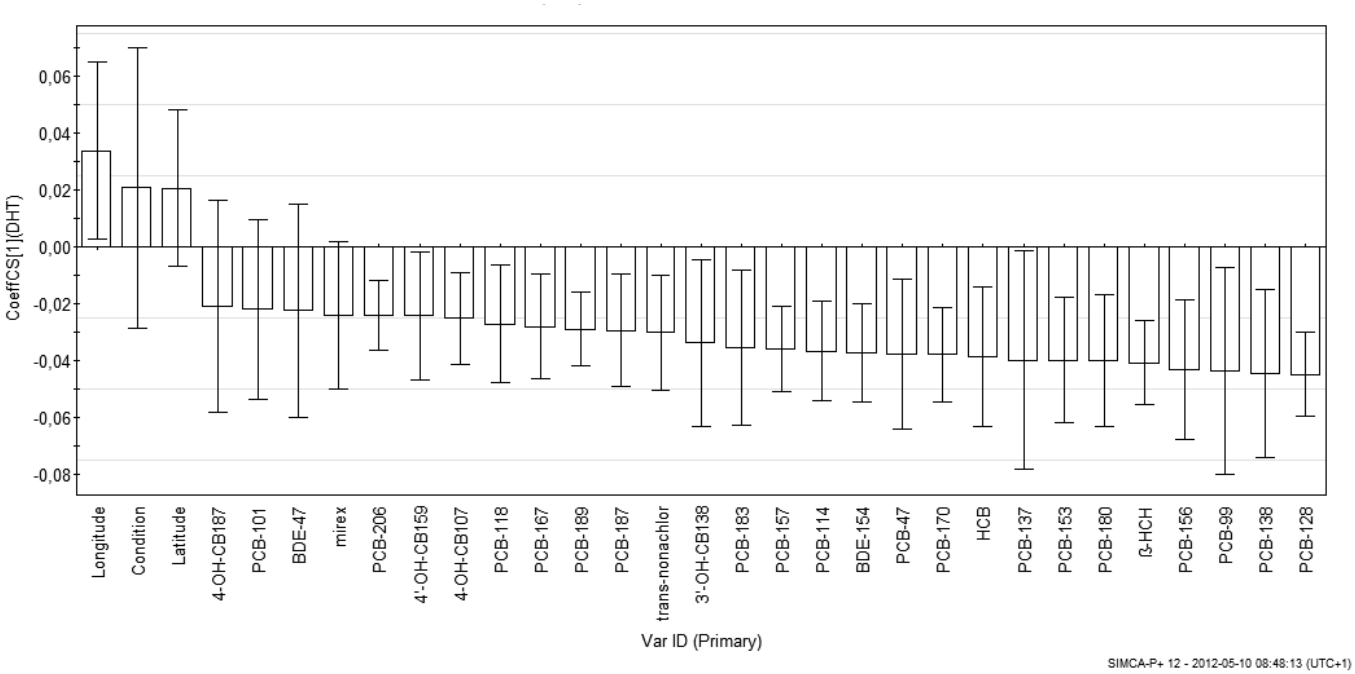

Figure 8. OPLS regression coefficient plot summarizing the relationship between X-variables and DHT. The whiskers represent the 95% confidence interval, and crossing of the zero-line indicates lack of significance.

The coefficient plot, which indicates how the variables affect DHT levels, is presented in Figure 8. All the environmental contaminants included in the model had negative effects on the DHT level, while the other variables; location, condition and latitude showed positive effects. Thus, the positive coefficients, except for longitude, were found not significant in the OPLS model. The lack of significance is indicated when the error whiskers, which represent the 95% confidence interval, cross the x-axis. Amongst the variables with negative coefficients the following five were not significant: 4'-OH-CB130, 4-OH-CB187, PCB-101, BDE-47 and mirex. The variables with negative coefficients and strongest effects on the DHT levels were the poly-ortho PCBs congeners 128, 138, 99, 180, 153 and 137, mono-ortho PCB 156 and the pesticides β-HCH and HCB.

3.5. Spearman′s rank correlation coefficient test

Possible correlations between significant coefficients and DHT levels were further investigated with Spearman′s rank correlation coefficient test. All variables were included, including the sums of concentrations for the different contaminant groups. Variables found to be significantly correlated ($p<0.05$) with DHT levels are presented in Table 8.

Longitude was the only variable found to have significantly positive effect in both analyses. Amongst the environmental contaminants, most of the poly-ortho PCBs had a significantly negative effect on the DHT levels in the OPLS model and in Spearman. Only two of the poly-ortho PCBs, PCB-187 and PCB-206, were not significantly correlated with DHT in Spearman correlation test. In contrast, few mono-ortho PCBs and none OH-PCBs significant in the OPLS model were significantly correlated with DHT in Spearman. Amongst the pesticides, HCB and β-HCH were significantly correlated in addition to have a significant effect on DHT according to OPLS.

Table 8. The results from Spearman′s rank correlation coefficient test. Only variables significantly correlated ($p < 0.05$) with DHT are included. The significant variables are listed in decreasing significance with significance level (p).

	Dihydrotestosterone	
Variables	r	p
PCB-128	-0.779	0.000
Σmono-ortho PCBs	-0.735	0.001
ΣPCBs	-0.657	0.004
PCB-138	-0.632	0.006
PCB-99	-0.625	0.007
HCB	-0.613	0.009
PCB-153	-0.615	0.009
Σpoly-ortho PCBs	-0.605	0.01
PCB-183	-0.586	0.013
Σpesticides	-0.583	0.014
PCB-167	-0.576	0.016
PCB-47	-0.569	0.017
PCB-180	-0.571	0.017
ΣHCHs	-0.564	0.018
Total load	-0.564	0.018
PCB-137	-0.564	0.018
HCH-β	-0.554	0.021
Trans-nonachlor	-0.547	0.023
Longitude	0.537	0.026
PCB-170	-0.529	0.029
ΣBDEs	-0.529	0.029
PCB-156	-0.515	0.035
BDE-154	-0.512	0.036
PCB-105	-0.5	0.041
PCB-114	-0.494	0.044
PCB-157	-0.488	0.047

3.5.1. Correlation plots; steroids and contaminants

The variables that both contributed most to the variation in the DHT levels (based on OPLS model) and in addition were strongly correlated (based on Spearman′s rank correlation coefficient test), were plotted against DHT (Figure 9). PCB-128 had a significant negative correlation with DHT in the Spearman correlation coefficient test, as well as the strongest negative coefficient in the OPLS model (Figure 8). Followed by PCB-138 and PCB-153 as negative coefficient in the OPLS model, which also were significantly negative correlated in Spearman. The pesticide HCB had negative coefficient in the OPLS model, as well a significant negative correlation in Spearman (Table 8).

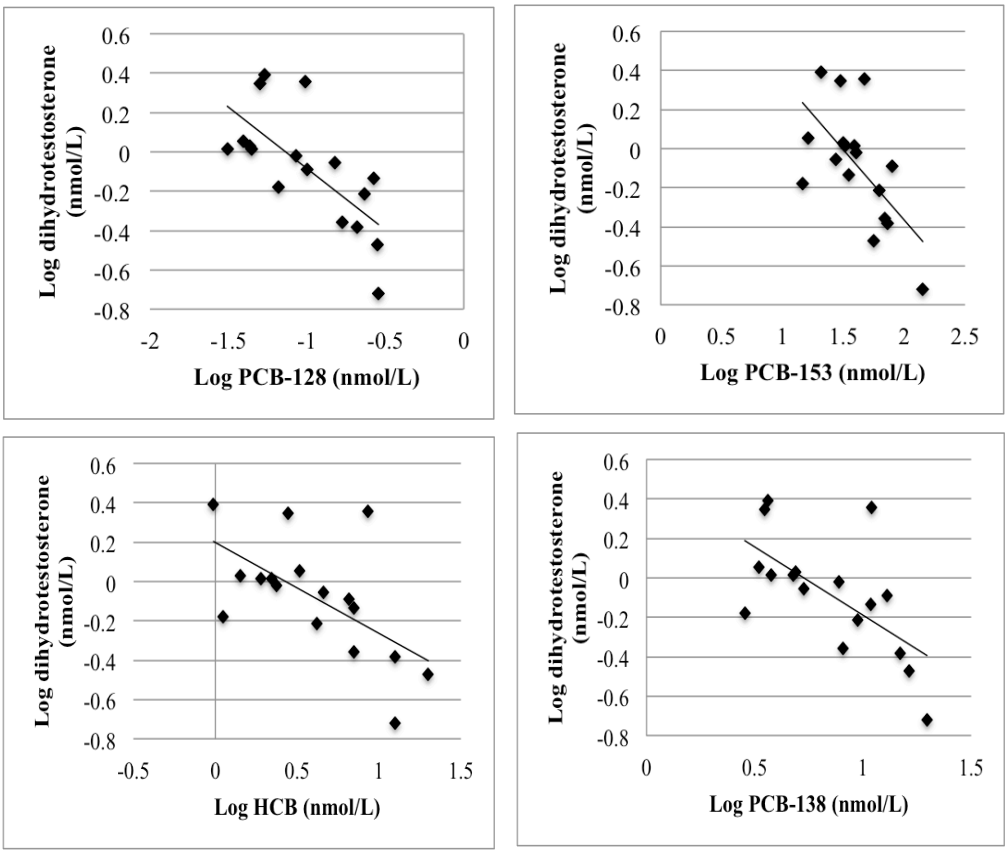

Figure 9. Correlations plot of log PCB-128 (r= -0.779, p<0.001), log PCB-138 (r= -0.632, p=0.006), log PCB-153 (r= -0.615, p=0.009) and log HCB (r= -0.613, p=0.009) against log dihydrotestosterone concentrations from 17 male polar bears from Svalbard. The line represents linear regressions.

3.5.2. Correlation plots; steroids and biological variables

Significantly correlation between age and both TS and AN were found in this study by Spearman correlation test. The correlations plot between some androgens (TS and AN) and biological variables (age, weight and axial girth) are shown in Figure 10. The results of the Spearman′s rank correlation coefficient test, which the plots are illustrating, are given in Appendix 2.

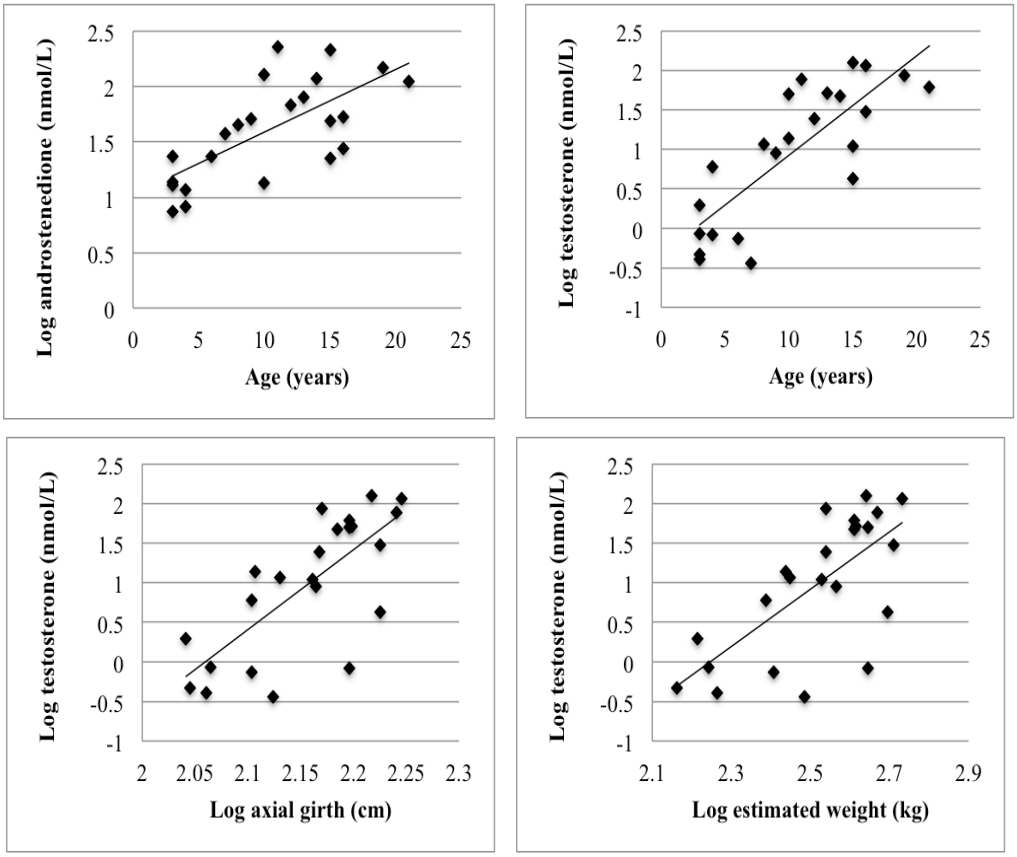

Figure 10. Correlations plots based on biological variables and hormone levels from 23 male polar bears from Svalbard. Age and log AN (r= 0.671, p<0.001) were plotted against each other, and age (r=0.791, p<0.001), log axial girth (r=0.733, p<0.001), log weight (estimated) (r=0.633, p=0.001) were plotted against log TS. The line represents linear regressions.

3.6. Comparison of methods: RIA versus GC-MS/MS

The testosterone levels have been analysed earlier in the same individuals by another method, radioimmunoassay (RIA). This gave an opportunity to test the credibility of this new method involving GC-MS/MS, and consider whether it is an appropriate alternative to RIA. The different testosterone concentrations obtained by both methods are given in Table 9. Figure 11 illustrates the comparison of the average concentrations achieved by the different methods. In addition, a T-test for independent samples was applied, resulting in lack of significant difference (p=0.857).

Table 9. Testosterone levels in plasma and serum samples from 23 male polar bears from Svalbard, measured by RIA and GC-MS/MS.

ID	Method	
	RIA (nmol/L)	GC-MS /MS (nmol/L)
23575	19.15	23.10
23609	69.61	81.10
23682	80.84	110.60
23683	14.77	not detected
23686	12.32	not detected
23702	38.58	57.49
23820	23.33	28.71
23825	35.17	45.08
23834	5.13	8.39
23885	111.84	119.59
23898	54.28	48.23
23899	49.19	49.23
23921	67.31	72.43
23922	7.32	10.86
23946	6.87	10.54
23949	8.58	13.21
23960	3.34	4.07
23923	not detected	not detected
23929	not detected	not detected
23930	not detected	0.80
23810	not detected	1.85
23927	3.55	5.64
23934	76.87	not detected

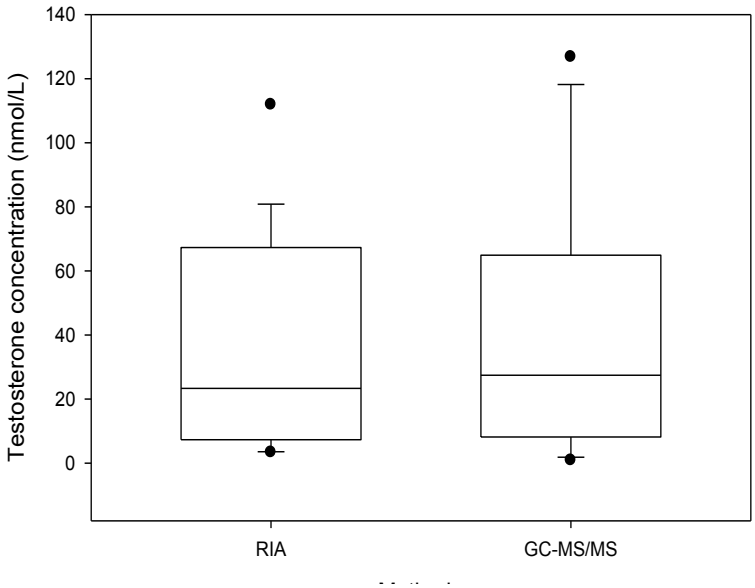

Figure 11. Comparing of testosterone levels obtained by RIA and the GC-MS/MS method. The middle line represent the median value to each of the methods, the upper line of the boxes is the median to the higher values, while the lower line of the boxes the median to the lower values. The whiskers represent the highest and lowest values measured, and the dots possible outliers.

4. DISCUSSION

4.1. Steroid hormones

4.1.1. Adults

Most studies executed on polar bears focus on the testosterone levels, which make it difficult to compare the measured concentrations of the other steroid hormones with other studies. However, basal steroid hormone concentrations have been measured in humans (Burtis et al., 2001). Although, there will be no direct comparison due to the physiological differences. Only the order of the steroid hormones in relation to average concentration will be compared.

The order of the steroid hormones found in adult male polar bears was: AN>TS>DEA>Pro>Pre>DHT>E1>E2-α>E2-β. And the order of the steroid hormone based on concentrations in humans was: TS>AN>DHT>Pro>E1>E2 (Burtis et al., 2008). In human males, testosterone was found to be the major circulation androgen. Androstendione has lower concentration in humans possible due to rapid testosterone and estrone transformation (Nieschlag and Behre, 2004). Higher levels of TS in peripheral plasma compared to AN have also been found in spotted hyenas (*Crocuta crocuta*) and mice (van Jaarsveld and Skinner, 1991; O′Shaughnessy et al., 2000). One would assume testosterone to be the major circulation androgen in polar bears as well. However, from the steroid hormone order found in polar bears, AN was the most abundant androgen in circulating blood. Androstenedione is a weak androgen with no known function in male mammals. Its major biological activity is downstream convention to more potent steroid hormones (Nieshlag and Behre, 2004). As the first study to measure AN concentrations in male polar bears, there is lack of basis for comparison and difficult to determine if the obtained AN concentrations are normal according to polar bears physiology.

The circulating blood concentration of DEA in human blood was not included in Butis et al. (2008). However, a review by Brown et al. (2006) mentioned that similar circulation concentrations of DEA and AN has been found in human blood. There is almost no convention from Pro to AN, which makes AN and TS to mainly be synthesized from DEA. Making DEA one of the most crucial steroid hormones in the steroidogenesis (Nieschlag and Behre, 2004). Pregnenolone is a precursor on the same line as DEA, and similar concentrations would be expected. Only a small percentage of the synthesised TS are found to be converted into DHT, and is reported to have concentration that is approximately 1/10 of the TS concentration in human blood (Hsiao et al., 2000; Nieschlag and

Behre, 2004). The DHT concentration measured in male polar bears was much lower than 1/10 of the TS concentration at approx. 1/45. Testosterone dissociates 3 times faster than DHT, and may be the reason for general low DHT concentrations (Stoker et al., 2000). However, why the DHT concentration is even lower in male polar bears is difficult to explain, and will be further discussed in 4.3.2.

Progesterone, E1 and E2 are all classified as female steroids, and found to have low concentrations in human males (Butis et al., 2008). However, the importance of estrogens in males according to literature is changing, from not relevant to essential for male sexual development. For instance, male fertility has been reported as impaired in mice lacking ER and aromatase (O′Donnell et al., 2001). The levels found in male polar bears were low compared to the androgens.

Several studies on polar bears have measured testosterone level in circulating plasma. When presented on a ng/ml basis, the testosterone concentration in the adult polar bears sampled in April in the current study were 13.61 ng/ml (SD= 10.50 ng/ml, n=17). Seasonal differences in the testosterone levels have been reported in adult polar bears, with serum testosterone concentrations being highest in April (Howell-Skalla et al., 2002; Oskam et al. 2003), corresponding with breeding season. This makes the steroid hormone concentrations dependent on the sampling date, and could explain the variations between the different studies. In addition, during the breeding season male individuals may display aggressive behaviour, which could result in peaks in the testosterone concentrations (Howell-Skalla et al., 2002). This may have contributed to the high TS variations between the individuals found in the current study. Haugestøl (2009) reported a testosterone concentration average of 14.33 ng/ml (SD=10.50 ng/ml, n=15) in male polar bears from Svalbard sampled in March- April. A previous study found an average testosterone value in polar bears samples sampled in April-May on Svalbard of 6.5 ng/ml (SD=9.8 ng/ml, n=121) (Ropstad et al. 2006). Seasonal differences may be the reason for the lower TS concentration reported by Ropstad et al. (2006). The testosterone concentrations in the current adult male polar bears are therefore in general accordance with concentrations reported in previous studies.

4.1.2. Subadults

The average concentration order of the steroid hormones found in subadult male polar bears was: AN>Pre>E1≈TS>DEA>DHT>E2>E2>Pro. The average concentration order of the steroid hormones reported in prepubertal (approx. 6-9 years) humans was: Pro>AN>TS>DHT>E2 (Bortis et al., 2008). The pattern of steroid hormones was not complete in prepubertal humans due to lack of measured concentrations of E1, DEA and pregnenolone. There is a clear difference between the patterns in prepubertal polar bears compared to humans, especially regarding progesterone. However, progesterone levels are reported as relative low in children, and due to the low concentration of most of the steroid hormones measured in immature mammals, a change of order may happen. For instance, progesterone levels in immature humans were much lower compared to human males (Bortis et al., 2008). This is also the case for the polar bears, where progesterone was under the detection limit in subadults compared with detectable levels in most adults.

There are several differences between the steroid hormone pattern in prepubertal and adult male polar bears. Although the high AN concentration in adult polar bears were difficult to explain, high AN concentrations in subadults are more common in mammals. Several studies have reported AN as the major steroid hormone produced by interstitial tissue in prepubertals, for instance *in vitro* in mice, and *in vivo* in spotted hyenas (*Crocuta crocuta*) (van Jaarsveld and Skinner, 1991; O′Shaughnessy et al., 2000). However, when reaching adulthood, TS became the major androgen produced in both species. Most steroid hormone concentration differs from prepubertal to adult stage. However, DHT concentrations found in prepubertal male rats did not change markedly after sexual maturation (Saksena and Lau, 1979), which corresponds with the current study. Estrogens have been found to decrease in male rats when developing from subadult to adult individuals (Saksena and Lau, 1979), same observation was true for the polar bears. The function of estrogen seems also to be age-dependent, where aromatase activity is found to differ between reproductive tissues in relation to age. Strong immunoreactivity of aromatase has been found in Sertoli cells in the testis to prepurbertal male rats, while strong immunoreactivity of aromatase was found in Leydig cells in the testis to mature male rats (Carpino et al., 2001). In immature rats, estrogens produced by Sertoli cells may be important in controlling prepubertal sexual maturation (Dorrington et al., 1978). This, however, is difficult to say anything about in polar bears, since the aromatase activity has not been measured before or in the current study.

The average testosterone hormone level in subadult male polar bears was measured at 0.80 ng/ml (SD=0.74 ng/ml, n=7). The levels were as expected much lower than the adults in the breeding season, and are in accordance with another study, which found average testosterone levels in

subadult polar bears from Svalbard to be 1.0 ng/ml (SD=1.3 ng/ml, n=7) in April-May (Oskam et al., 2003). The TS concentration observed in subadults is similar to the average level observed in adult polar bears off breeding season, which had a range between 0.6-1.7 ng/ml from May to October. Seasonal changes observed in adults do not occur in immature individuals, since the seasonal changes in TS concentrations are connected to breeding season (Howell-Skalla et al., 2002).

4.1.3. Comparison of steroid hormone determination methods: RIA and GC-MS/MS

The testosterone levels obtained by the different methods were very similar, and the T-test confirmed the lack of significant difference (p=0.857) between the average testosterone concentrations. However, there was one individual that clearly stood out and showed a large deviation between the methods. Individual 23934 was reported to have a testosterone concentration at 76.9 nmol/L by RIA, while the testosterone concentration was measured to be below the detection limit by GC-MS/MS. The sample was analysed twice with duplicates with RIA, while the sample (no duplicates) were analysed twice by GC-MS/MS. It is therefore possible, regarding the sample analysed by GC-MS/MS, that an error has occurred under the sample preparation or degradation of testosterone during storage or that it is just a poorly representative sample.

There are several advantages by choosing the GC-MS/MS method over the RIA method. The use of steroid hormone determination by GC-MS/MS allows quantification of several compounds, instead of only a distinct compound (Hoffmann, 1983). In addition, the GC-MS/MS method is applicable for blood from several species, while the RIA method is dependent of the availability of antibodies for the target species (Hansen et al., 2011). Furthermore, there is no risk for cross-reactivity, which is a problem with the RIA method, due to the antibody affinity between steroids with similar structures (Andric et al., 2000; Hansen et al., 2011).

4.2. Environmental contaminants

4.2.1. Adults

Polar bears on Svalbard are reported as one of the polar bear population with highest contamination load (AMAP, 2002). In this current experiment the contamination group observed to be most abundant was OH-PCBs, followed by poly-ortho PCBs, pesticides, mono-ortho PCBs and PBDEs.

The highest concentrations measured in the male polar bears were hydroxylated PCB, and then especially 4′-OH-CB172 with an average concentration at 24.19 ng/g ww, followed by 4-OH-CB187, which had with an average concentration of 33.77 ng/g ww. Similar concentrations have been achieved for 4-OH-CB187 with an average concentration at 58.45 ng/g, while there were measured a much lower concentrations for 4′-OH-CB172, which had an average concentration of 4.54 ng/g (Haugestøl, 2009). However, the peak achieved by the GC-ECD for 4′-OH-CB172 in the current study, was suspected to overlap with another OH-PCBs, which could be the reason for the high concentration compared to the 4′-OH-CB172 concentration obtained by Haugestøl (2009). The relative abundance of OH-PCBs varies significantly dependent on the specie, due to different metabolic capacity (Bergman et al., 1994; Letcher et al., 2000). For instance, OH-PCB-187 is also reported as the major OH-PCB in human plasma, while it is not detected in rat plasma (Bergman et al., 1994). The metabolic capacity has also reported to be age-dependent, where female polar bears were better capable to metabolize PCBs into OH-PCBs compared to polar bear cubs (Bytningsvik et al., 2012). In addition to obtain high ΣOH-PCB concentrations relative to ΣPCBs in mammals, ΣOH-PCB concentrations are reported to range proportionally to ΣPCBs (Letcher et al., 2000).

Samples taken in 1997-1998 report a ΣPCB value (sum of the PCB congeners 99, 118, 153, 156, 189 and 194) of 72.3±43.6 ng/g ww (Braaten et al. 2004). This value was compared to samples taken in 2007 (Haugestøl, 2009) and 2008 (current study), which had a ΣPCB concentration of 30.89 ng/g ww (SD=35.02 ng/g ww) and 20.14 ng/g ww (SD=12.61 ng/g ww). In 10 years the contamination load seems to be halved due to the PCBs restrictions, and the treat from the PCBs toward polar bears has been reduced (Bytningsvik et al., 2012). Since ΣOH-PCB concentration is reported to range proportionally to ΣPCBs (Letcher et al., 2000), ΣOH-PCB was assumed and reported to decrease in polar bears. However, harmful levels are still present in the environment (Bytningsvik et al., 2012). Furthermore, there are indications that PCB-structural differences may have an affect on accumulation in polar bears. There were observed higher levels of poly-ortho PCBs compared to levels of mono-ortho PCBs in adults, which was supported by Haugestøl (2009).

Moreover, non-ortho PCBs have been reported to not bioaccumulate in polar bears (Bernhoft et al., 1997), which enhances the possibility for an ortho-substituted dependent accumulation of PCBs.

The poly-ortho PCBs congener PCB-153 was found to have the highest average concentration with 16.27 ng/g ww (47.79 nmol/L), which is in accordance with other polar bears studies from Svalbard (Braaten et al., 2004; Haugestøl, 2009) and from Alaska (Verreault et al., 2005). PCB-153 is referred to in the literature as the most persistent PCB congener in biota due its unmetabolizable substitution pattern (Bernhoft et al., 1997). PCB-153 is a poly-ortho substituted PCB congener, and has higher possibility for accumulation compared to non-ortho and mono-ortho substituted congeners. However, the concentration of PCB-153 is reported to decrease. In 1997-1998 the average PCB-153 levels were found to be 34.6 ng/g ww in polar bears from Svalbard (Braathen et al., 2004), while in samples from 2007 there was found an average level of 15.37 ng/g ww (Haugestøl, 2009), which was similar to the average level achieved in the present study. Furthermore, the decreasing in concentrations of PCB-153 has also been observed in females and cubs from Svalbard. The PCB-153 concentration has from 1998 to 2008 been reduced by over half in both females and cubs (Bytningsvik et al., 2012). The average concentrations of PCB-153 measured in the male polar bears gives therefore the same indications as the ΣPCBs. As PCBs inter-correlates, PCB-153 may function as a representative for the contamination status (Bernhoft et al., 1997).

Among the pesticides, HCB with an average concentration of 5.81 nmol/L (1.56 ng/g ww), and oxychlordane with an average concentration of 4.25 nmol/L (1.7 ng/g ww), were found to be most abundant in the sampled polar bears, which are in agreement with other studies (Bernhoft et al., 1997; Haugestøl, 2009). When recalculated into a ng/g ww basis, oxychlordane had higher concentration compared to HCB, indicating the relevance of units. In male polar bears samples collected within the same project in 2007, reported higher concentrations of oxychlordane with a mean value of 2.42 ng/g ww (6.05 nmol/L) compared to HCB, which had an average concentration of 1.38 ng/g ww (5.13 nmol/L) (Haugestøl, 2009). Comparing the concentrations from 2007 with those from 2008, the concentrations were slightly higher in the samples from 2008. Air stations at Svalbard (Zeppelin air station) and in Canada (Alert air station) have reported a weak increase of pesticides concentration present in air from 2001 to 2006, including HCB (Hung et al., 2010). A possible explanation for the increased HCB concentration is increased worldwide use of HCB. Although EU has banned the use of HCB, it is still present as by-products in chlorinated solvents and pesticides. Another explanation for the increased concentrations of pesticides is in relation to climate change, including reduced ice cover and increased sea water temperature, which may lead

to increased volatization of previously deposed chemicals from the ocean (Hung et al., 2010).

The ΣPBDE concentration has been found to be 3-4 times less than ΣPCB concentrations in organisms, for instance in Swedish peregrine flacons (AMAP, 2002). The average concentration of the BDE congener 47 and 154 in the present study were found to be 0.19 ng/g ww (SD=0.13 ng/g ww) and 0.17 ng/g ww (SD=0.10 ng/g ww), which was more than 3-4 times lower concentrations compared to PCB concentrations. The levels achieved was supported by levels measured in male polar bear samples the previous year, with a BDE-47 concentration of 0.10 ng/g ww (SD=0.08 ng/g ww) and a BDE-154 concentration of 0.10 ng/g ww (SD=0.15 ng/g ww) (Haugestøl, 2009). In a study by Sørmo et al. (2006) neither BDE-47 nor BDE-154 were found to biomagnify in polar bears. The only PBDE congener found to biomagnifiy in the high tropical levels where BDE-153. Polar bears are therefore able to metabolize most PBDEs and to avoid high concentrations (de Wit et al., 2004). However, air concentrations of PBDEs have been reported higher than for PCBs, and there are reports on an increased presence of PBDEs in Arctic biota (AMAP, 2002).

4.2.2. Subadults

The average contamination concentrations were found to be higher in the subadults compared to the adults. The contaminants that showed significant difference between age classes where oxychlordane, p,p′-DDT, 4-OH-CB146, Σpesticides and ΣDDTs, which all were significantly higher in subadults, while 3′-OH-CB180 was significantly higher in adults. These findings correspond with a study by Norstrom et al. (1998), where the organochlorine concentrations were observed to be 2 times higher in subadults compared to adults. Other studies have found cubs and subadults to have higher levels of PCBs compared to adults (Polischuk et al., 2002; Bytningsvik et al., 2012). In contrast to these results, previous studies have observed the adult PCB-levels to be significantly higher compared to levels measured in subadult (Bernhoft et al., 1997; Dietz et al., 2004). In the study by Bernhoft et al. (1997) PCBs concentrations were also reported to positively correlate with age. The concentration increased until the male polar bears reached approximately 14 years, after which the concentration starts to decrease (Bernhoft et al. 1997).

Furthermore, hydroxylated-PCBs have been found to have higher concentrations in subadults compared to adults (Polischuk et al., 2002). 4-OH-CB187 was observed to have an average concentration of 140.65 nmol/L in the subadults compared to 87.24 nmol/L in the adults in the current study. However, only 4-OH-CB146 were significantly higher in subadults, with an average concentration at 66.66 nmol/L (SD=35.17 nmol/L) compared to 33.51 nmol/L (SD=23.66 nmol/L) in adults. Cubs have been reported to have lower OH-PCB concentrations compared to adults due to

lower metabolic capacity (Bytningsvik et al., 2012). However, this was not the case for subadults, which gives an indication of improved metabolic capacity of subadults compared to cubs.

4.3. Determinants for variation in steroid hormone levels

4.3.1. Correlations between reproductive hormones and biological variables

The longitude of the sample collection location was the only biological variable observed to have a significant effect on the DHT concentrations. However, in the OPLS model it was one of the weaker coefficients. A possible explanation for the positive correlation between DHT and longitude is that the correlation may be affected by age. Most of the old males were located in the west of Svalbard, and DHT increased slightly by age. Another explanation is in relation to diet. On the east side of Svalbard, a high number of polar bears feed on harper seals. The harper seals often migrate from the Russian White Sea and may serve as a biological pathway from Russia to Svalbard. The blubber in harper seal is found to contain much higher concentrations of PCBs, compared to the ringed seal (AMAP, 2002) However, longitude was not significantly correlated to the contaminants, weakening the explanation.

The orthogonal partial least square (OPLS) model, with testosterone as the Y-variable, was not significant according to CV-ANOVA. However, there were found several significant correlations between biological variables and testosterone in Spearman. According to the correlation test, axial girth was significant positive correlated with testosterone concentrations, which is supported by another study where the axial girth were found to be the most important biological variable in male polar bears for explaining the variation in TS concentrations (Oskam et al., 2003). Given that TS exerts growth of non-reproductive tissue as muscles, this makes sense since the chest is full of muscles and will effect measurements of the axial girth. Significant positive correlations were found between age and AN, as well as age and TS, which are supported with similar finding for testosterone in Haugestøl (2009). In addition, the highest TS concentrations were measured in animals older than 8-10 years. Most male polar bears don't start mating until they are of that age, which may explains the lower TS concentrations found in the younger male polar bears (Howell-Skalla et al., 2002).

4.3.2. Correlations between reproductive hormones and contaminants

Through assessing the reliability of the OPLS models with CV-ANONA, only the model with DHT as response variable was confirmed as significant and therefore the only one presented in the results. Of the androgens (AN, DEA, TS and DHT) in the PCA loading plot (Figure 6), DHT was the androgen furthest away from the contaminants. This indicates a stronger negative correlation compared to the other androgens, which were located closer to the middle. This may be the reason why CV-ANOVA only registered the OPLS with DHT as the response variable as significant, and not models with the others steroid hormones.

It should be noted that the steroids and the majority of biological variables were not included in the final significant OPLS-model. However, all contamination groups were included, where most of the single contaminants where PCBs. According to the OPLS analysis (Figure 8), the majority of the PCB congers were poly-ortho PCBs, which had a negative effect on the DHT concentration. In addition, the poly-ortho PCBs were the strongest coefficients in OPLS model. They were also significantly correlated with DHT according to the Spearman correlation test. The sum of mono-ortho PCBs also had a strong correlation with DHT according to Spearman correlation test. Previous study has reported poly-ortho PCBs to contribute most to the variation in TS concentration (Haugestøl et al., 2009). However, since the contaminants seem to affect both TS and DHT, there should be a positive correlation between these androgens, especially as TS is the precursor to DHT. An explanation for the lack of significant relationship between DHT and TS may be the large variations observed in the TS concentrations.

There are not many studies that have observed the effects of environmental contaminants on DHT concentration, which hampers comparison with other studies. However, in studies where testosterone levels were analysed, many of the same relations have been reported. This includes *in vivo* studies done on polar bears (Oskam et al., 20003; Haugestøl, 2009), *in vivo* studies on male rats (Han et al., 2010) and *in vitro* studies on cultured male rat Leydig cells (Murugesan et al., 2005, 2008) which all observed significant negative correlations between the testosterone level and concentrations of individual PCBs and PCB-mixtures.

The PCB congener PCB-153 was found to have the highest concentrations among PCBs during this study, in addition to significantly contribute negatively on the DHT levels. Other studies have reported decrease in testosterone levels by PCB-153 exposure in an *in vitro* study on human cell line (Kragerud et al., 2010). Furthermore, it has been found that PCB-153 exposure cause smaller testis diameter in male goat offspring, combined with decreased testosterone levels (Ropstad et al.,

2006). However, there are conflictions between studies on this topic. Some studies have found POP exposure to decrease testis size and reduce sperm count, without decreased testosterone levels. For instance, rats and hamsters exposed for TCDD were found to have reduced sperm without TS decrease. Dioxins like TCDD elicit their toxicity through AhR instead of AR, and hormone levels will not directly be affected (Gray et al., 2001). Testis size has been observed to correlate with testosterone level in male polar bears (Howell-Skalla et al., 2002), which makes testosterone necessary for the maintenance of the testis, in addition to the development (Hsiao et al., 2000).

HCB was found to have a negative effect on the DHT concentration in the current study. A study by Ralph et al. (2003) found HCB to have a dose-dependent influence on the androgenic action in *in vitro* cell cultures. At low doses, HCB were reported to act as an agonist influencing androgenic action by altering steroid transport, while high HCB doses suppressed the transcriptional activity (Ralph et al., 2003). Sonne et al. (2006) reported inverse relationships for baculum and testis size with HCB concentrations in male polar bears from East-Greenland. A decrease in testis size may further lead to reduce sperm quality due to testicular dysfunction (Sonne et al., 2006). Steroid hormone levels were not measured in the mentioned study, which makes it difficult to say if the reduction of the reproductive organs is an effect of decreased steroid hormone concentrations. However, TS and DHT are found in reproductive tissue, where they stimulate development of reproductive organs, such as penis, scrotum and prostate gland (Sundaram et al., 1995; Shabsigh, 1997, Hill et al., 2008). In addition, DHT has been found to be the active androgen in maintaining erectile function in male rat penis (Shabsigh, 1997). A decrease in androgen levels, especially in TS and DHT concentrations, due to HCB exposure may cause severe harm to the reproductive functions in male polar bear. HCB was found to have a negative effect on the DHT concentration in the current study, which may have affected the reproductive organs in the male polar bears. These finding cause concerns for the increased HCB concentrations reported in air, and the possible increased concentrations in polar bears.

According to the multivariate analysis and the Spearman test, BDE-154 had a significant effect on the DHT level and was significant correlated to DHT, while BDE-47 had not a significant effect on the DHT levels. In contrast, other studies have reported BDE-47 to have a significant contribution on the TS, and significant correlation with TS (Haugestøl et al., 2009). However, both BDE-47 and BDE-154 has been banned from the European Union Marked since 2004 (European Union, 2003), and concentrations are assumed to decrease. Furthermore, in a study by Sørmo et al. (2006) neither BDE-47 nor BDE-154 were found to accumulate in polar bears, indicating that polar bears are able

to metabolize most BDEs. This was supported by a review by de Wit et al. (2010) where most PBDEs were found in lower concentrations in polar bears than in ringed seals, indicating biotransformation differences between the two species. The same review also mentioned a reported stagnation and decreases in PBDE levels from 2000 to 2003 in Canadian Arctic ringed seal blubber in a study by Ikonomou et al. (2005).

4.4. Mechanisms involved in endocrine disruption

It is difficult to conclude which pathway that could be inhibited by the POPs, there are several pathways and most likely the contaminants affect several of them. Studies that have found a decrease in testosterone levels due to exposure to certain PCBs or PCB-mixtures, also find that several of the steroid enzymes gets affects (Andric et al., 2000; Murugesan et al., 2005, 2008; Han et al., 2010). However, since it in the current study only has been measured steroid hormone and contaminant concentrations, one can only assume which pathway that may be inhibited.

According to the OPLS, none of the other steroid hormones could explain the variations in DHT, nor were any of the steroids significantly correlated to DHT. This makes it difficult to assume were the inhibition may have taken place in order to have a negative effect on the DHT levels. The only analyses, which can give some assumptions about possible inhibited pathways, were the PCA loading plot and Spearman correlation test.

A possible explanation of the negative correlation between DHT and contaminants is an inhibition of 5α-reductase, which metabolizes TS into DHT. A study on male rats reported that when 5α-reductase were inhibited by finasteride, which is a synthetic 5α-reductase inhibitor, TS was converted into AN instead of DHT (George, 1997). Resulting in elevated levels of TS and AN, which may explain the high levels of AN compared to TS in the current study. However, inhibition of 5α-reductase has only been observed by non-ortho PCBs exposure, not by ortho-substituted PCBs exposure (Endo et al., 2003). Poly-ortho PCBs were assumed to have largest effect on the DHT concentrations, and since ortho-substituted PCBs don't inhibit 5α-reductase, one can assume this were not the reason for negative correlation between DHT and contaminants. However, it would be interesting to see if HCB is able to inhibit 5α-reductase at high doses. The study by Ralph et al. (2003) did not analyse the enzyme levels, and there are no identified literature on this topic.

It has been reported that certain hydroxylated PCBs and other POPs are capable to bind to estrogene hormone receptors (ER), although with very low affinity relative to the endogenous hormones such

as E1 and E2-α (Letcher et al., 2000; Sanderson, 2006). Another study has observed that PCBs inhibit estrogen sulfotransferase (hEST), which inactivates estrogens (Kester et al., 2000). Certain OH-PCBs have been reported to mimic the binding of estrogenic compounds to hEST (Shevtsov, 2003). The inhibition of hEST will increase the level of estrogens in male reproductive tissue (Kester et al., 2000). The study found 4-OH-CB107 to be one of the hydroxylated PCBs with high inhibitory activities. In addition to 4-OH-CB107, also 4-OH-CB146 and 4-OH-CB187 were included in the study by Kester et al., (2000). 4-OH-CB187 did not have the strongest inhabitation properties, however, the contaminant showed an affect at concentrations between 20-30 nmol (Kester et al., 2000). The present study found an average concentration of 4-OH-CB187 of 87.24 nmol/L and a concentration range between 21.09-248.66 nmol/L in the adult polar bears. 4-OH-CB187 could therefore inhibit EST activity, and cause increased estrogen concentrations in the male polar bears.

Coplanar mono-ortho PCBs can elicit toxicity through binding to the AhR (Fischer et al., 1998; Hestermann et al., 2000). The low contribution of mono-ortho PCBs on DHT levels indicates that the major impact on the DHT levels probably not occurs through the AhR receptor, but through other pathway. Both DEA and AN were positively correlated to all of the androgens, while negative correlated to estrogens, progesterone and pregnenolone according to Spearman correlation test ($p<0.05$) (Appendix 3). Pregnenolone is the precursor to dehydroepiandrosterone, while progesterone is the precursor to androstenedione. The weak negative relationship between precursors and product may indicate an inhibition of the enzyme that performs the conversion, 17α-hydroxylase (CYP-17α). Possible inhibition of CYP-17α was found in studies on male adult rats exposed to PCB mixtures (Andric et al., 2000; Murugesan et al., 2005, 2008), in addition, also possible inhibitions of CYPscc, 3β-HSD and 17β-HSD were reported (Andric et al., 2000; Murugesan et al., 2005, 2008). However, the effect from single contaminants is difficult to predict from these kinds of studies, and the possibility of synergetic effects is present. A study done by Han et al. (2010) found some of the same indications when exposing rats for PCB-126 and PCB-114. Where groups exposed to the PCBs, transcription and translation levels of CYP-17α and $P450_{scc}$ were significantly reduced (Han et al., 2010).

It is difficult to predict if an effect is caused by PCB and enzyme interaction, or if steroid hormone decline only is the last step of a bigger cascade. The studies done by Murugesan et al. (2005, 2008), also discovered elevated levels of reactive oxygen species, and that testosterone decline can be the case of interaction between reactive oxygen species and enzymes (Murugesan et al., 2005, 2008).

5. CONCLUSION

The results from the present study found that several of the persistent organic pollutants analysed had a negative effect on the concentration variation of at least one of the reproductive hormones in male polar bears from Svalbard. Most of the environmental contaminants had a significantly negative contribution on the variation in dihydrotestosterone concentrations, where poly-ortho PCBs and HCB were found to be most central. While PBDEs and OH-PCBs seems to be less important in explaining the variations in DHT concentrations. Androstenedione were found to be the most abundant androgen in circulating blood from male polar bears, unlike other studies on mammals where testosterone was most abundant. The high level of AN could possibly be connected to the negative effect of contaminants on DEA levels.

There was observed a significant difference in the concentrations measured in the different age classes, where the concentrations of p,p′-DDT, 4-OH-CB146, Σpesticides and ΣDDT were significantly higher in subadults compared to adults, suggesting the metabolic capacity to be age-dependent. All of the contaminants included in this study are banned in EU. The PCB and OH-PCB concentration were found to decrease by comparison with previous studies. While HCB concentrations are suggested to increase, despite EU restriction. Therefore, stricter restrictions concerning HCB by-products seem to be necessary.

The GC-MS/MS method applied in the current study was found to be appropriate for the study of several steroid hormones and their precursors in male polar bears. This method can successfully compete with other used determination methods, such as RIA, and can be recommended for other studies investigating the effect of endocrine disruptive chemicals on the steroidogenesis.

REFERENCES

Andric, S.A., Kostic, T.S., Stojilkovic, S.S., Kovacevic, R.Z. 2000. Inhibition of rat testicular androgenesis by a polychlorinated biphenyl mixture arochlor 1248. Biology of reproduction. 62:1882-1888.

AMAP. 1998. Arctic Monitoring and Assessment Programme: AMAP assessment report- Arctic Pollution Issues.

AMAP. 2002. Arctic Monitoring and Assessment Programme: AMAP assessment report - Persistent Organic Pollutants in the Arctic.

Barrie, L.A., Gregor, D., Hargrave, B., Lake, R., Muir, D., Shearer, R., Tracey, B., Bidleman, T. 1992. Arctic contaminants: sources, occurrence and pathways. The Science of the Total Environment. 122:1-74.

Bergman, Å., Klasson-Wehler, E., Kuroki, H. 1994. Selective retention of hydroxylated PCBs metabolites in blood. Environmental Health Perspect. 102:464-469.

Bernhoft, A., Wiig, Ø., Skaare, J.U. 1997. Organochlorines in polar bears (*Ursus maritimus)* at Svalbard. Environmental Pollution. 95(2):159-175.

Boelsterli, U.A. 2007. Mechanistic toxicology: The molecular basis of how chemicals disrupt biological targets. Second edition. CRC.

Braathen, M., Derocher, A.E., Wiig, Ø., Sørmo, E.G., Lie, E., Skaare, J.U., Jenssen, B.M. 2004. Relathionships between PCBs and thyroid hormones and retinol in female and male polar bears. Environmental Health Perspect. 112:826-833.

Breivik, E.M. 1978. Gas chromatographic method for the determination of organochlorine pesticides in human milk. Bulletin of environmental contamination and toxicology. 19:281.

Brown, G.A., Vukovich, M., King, D.S. 2006. Testosterone prohormone supplements. Medicine and Science in Sport and Exercise. 38(8):1451-1461.

Brunström, B., Halldin, K. 2000. Ecotoxicological risk assessment of environmental pollutants in the Arctic. Toxicology Letters. 112-113:111-118.

Burtis, C.A., Ashwood, E.R., Bruns, D.E. 2008. Fundamentals of clinical chemistry. Sixth edition. Saunder Elsevier.

Bytingsvik, J., Lie, E., Aars, J., Derocher, A.E., Wiig, Ø., Jenssen, B.M. 2012. PCBs and OH-PCBs in polar bear mother-cub pairs: A comperative study based on plasma levels in 1998 and 2008. Science of the Total Environment. 417-418:117-128.

Carpino, A., Pezzi, V., Rago, V., Bilinska, B., Ando, S. 2001. Immunolocalization of cytochrome P450 aromatase in rat testis during postnatal development. Tissue & Cell. 33(4):349-353.

Darnerud, P.O. 2008. Brominated flame retardants as possible endocrine disruptors. International Journal of Andrology. 31(2):152-160.

Darras, V.M. 2008. Endocrine disrupting polyhalogenated organic pollutants interfere with thyroid hormone signalling in the developing brain. The Cerebellum. 26-37.

Derocher, A.E., Wiig, Ø., Andersen, M. 2002. Diet composition of polar bears in Svalbard and the western Barents Sea. Polar biology. 25:448-452.

Dietz, R., Riget, F.F., Sonne, C., Letcher, R., Born, E.W., Muir, D.C.G. 2004. Seasonal and temporal trends in polychlorinated biphenyls and organochlorine pesticides in East Greenland polar bears (*Ursus maritimus*), 1990-2001. Science of the Total Environment. 331:107-124.

Dorrington, J.H., Fritz, I.B., Armstrong, D.T. 1978. Control of testicular estrogen synthesis. Biology of reproduction. 18:55-64.

Endo, F., Monsees, T.K., Akaza, H., Schill, W.-B., Pflieger-Bruss, S. 2003. Effect of single non-*ortho*, mono-*ortho*, and di-*ortho* chlorinated biphenyls on cell functions and proliferation of the human prostatic carcinoma cell line, LNCaP. Reproductive Toxicology. 17:229-236.

European Union. 2003. Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003. Official Journal of the Europian Union. L42/45-46.

Fischer, L.J., Seegal, R.F., Ganey, P.E., Pessah, I.N., Kodavanti, P.R.S. 1998. Symposium overview: Toxicity of non-coplanar PCBs. Toxicological Science. 41:49-61.

George, F.W. 1997. Androgen metabolism in the prostate of the finasteride-treated, adult rat: A possible explaination for the

differential action of testosterone and 5α-dihydrotestosterone during development of the male urogenital tracts. Endocrinology. 138(3):871-877

Gray, L.E.Jr., Ostby, J., Furr, J., Wolf, C.J., Lambright, C., Parks, L., Veeramachaneni, D.N., Wilson, V., Price, M., Hotchkiss, A., Orlando, E., Guillette, L. 2001. Effects of environmental antiandrogens on reproductive development in experimental animals. Human Reproductive Update. 7(3):248-264.

Gutleb, A.C., Cenijn, P., Van Velzen, M., Lie, E., Ropstad, E., Skaare, J.U., Malmberg, T., Bergmann, Å., Gabrielsen, G.W., Legler, J. 2010. *In vitro* assay shows that PCB metabolites completely saturate thyroid hormone transport capacity in blood of wild polar bears (*Ursus maritimus*). Environmental Science & Technology. 44:3149-3154.

Han, D.-Y., Kang, S.-R., Park, O.-S., Cho, J.-H., Won, C.-K., Park, H.-S., Park, K.-I., Kim, E.-H., Kim, G.-S. 2010. Polychlorinated biphenyls have inhibitory effects on testicular steroidogenesis by downregulation of $P450_{17\alpha}$ and $P450_{scc}$. Toxicology and Industrial Health. 26(5):287-296.

Hansen, M., Jacobsen, N., Nielsen, F., Björklund, E., Styrishave, B., Halling-Sørensen, B. 2011. Determination of steroid hormones in blood by GC-MS/MS. Analytical and Bioanalytical Chemistry. 400(10):3409-17.

Haugestøl, G.L. 2009. Effects of endocrine disrupting chemicals in male polar bears (*Ursus maritimus*) at Svalbard. Master thesis at the department of biology, NTNU, Trondheim, Norway.

Hill, R.W., Wyse, G.A., Anderson, M. 2008. Animal physiology. Second edition. Sinauer.

Hestermann, E.V., Stegeman, J.J., Hahn, M.E. 2000. Relative contributions of affinity and intrinsic efficacy to aryl hydrocarbon receptor ligand potency. Toxicology and Applied Pharmacology. 168(2):160-172.

Hoffmann, B. 1983. Use of radioimmunoassay procedures for the determination of sex hormones in animal tissues. The Journal of Steroid Biochemistry. 19(1):947-951.

Howell-Skalla, L.A., Chattet, M.R.L., Ramsay, M.A., Bahr, J.M. 2002. Seasonal changes in testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (*Ursus maritimus*). Reproduction. 123:729-733.

Hsiao, P.-W., Thin, T.W., Lin, D.-L., Chang, C. 2000. Differential regulation of testosterone vs. 5α-dihydrotestosterone by selective androgen response elements. Molecular and Cellular Biochemistry. 206:169-175.

Hung, H., Kalleborn, R., Breivik, K., Su, Y., Brorström-Lundén, E., Olafsdottir, K., Thorlacius, J.M., Leppänen, S., Bossi, R., Skov, H., Mano, S., Patton, G.W., Stern, G., Sverko, E., Fellin, P. 2010. Atmospheric monitoring of organic pollutants in the Arctic under the Arctic Monitoring and Assessment Programme (AMAP): 1993-2006. Science of the Total Environment. 408:2854-2873.

Ikonomou, M.G., Kelly, B.C., Stern, G.A. 2005. Spatial and temporal trends of PBDEs in biota from the Canadian Arctic marine environment. Organohalogen Compounds. 67:950–953.

IPY (International Polar Year). 2010. About IPY. Available: http://ipy.arcticportal.org/about-ipy (accessed 05 June 2011).

van Jaarsveld, A.S., Skinner, J.D. 1991. Plasma adrogens in spotted hyaenas (*Crocuta crocuta*): Influence of social and reproductive development. Journals of Reproductive and Fertility. 93:195-201.

Kester, M.H.A., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C.N., Coughtrie, M.W.H., Bergman, Å., Safe, S.H., Kuiper, G.G.J.M., Schuur, A.G., Brouwer, A., Visser, T.J. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activity of PCBs. Endocrinology. 141(5):1897-1900.

Klaassen, C.D. 2008. Casarett and Doull′s Toxicology: The basic science of poisons. 7th edition. The McGraw-Hill companies.

Kraugerud, M., Zimmer, K.E., Dahl, E., Berg, V., Olsaker, I., Farstad, W., Ropstad, E., Verhaegen, S. 2010. Three structurally different polychlorinated biphenyl congeners (PCB 118, 153 and 126) affect hormone production and gene expression in the human H295r in vitro model. Journal of Toxicology and Environmental Health. Part A. 73:1122-1132.

Letcher, R.J., Klaason-Wehler, E., Bergman, Å. 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. Handbook of Environmental Chemistry. Part K. 3:315-359.

Lie, E., Bernhoft, A., Riget, F., Belikov, S.E., Boltunov, A.N., Derocher, A.E., Garner, G.W., Wiig, Ø., Skaare, J.U. 2003. Geographical distribution of organochlorine pesticides (OCPs) in polar bears (Ursus maritimus) in the Norwegian and Russian Arctic. The Science of the Total Environment. 306:159-170.

Lundstedt, T., Seifert, E., Abramo, L., Thelin, B., Nyström, Å., Pettersen, J., Bergman, R. 1998. Experimental design and optimization. Chemometrics and Intelligent Laboratory Systems. 42(1-2):3-40.

Løken, K.B., Lie, E., Lundanes, E., Skaare, J.U. 2006. Extension of a multicomponent method to include the determination of OH-PCBs and OH-PBDEs in biological matrices. Organohalogen Compound. 68:2430-2433.

Meerts, I.A.T.M., Letcher, R.L., Hoving, S., March, G., Bergman, Å., Lemmen, J.G., van der Burg, B., Brouwer, A. 2001. *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. Environmental Health Perspect. 109:339-407.

Murugesan, P., Kanagaraj, P., Yuvaraj, S., Balasubramanian, K., Aruldhas, M.M., Arunakaran, J. 2005. The inhibitory effect of polychlorinated biphenyl Aroclor 1254 on Leydig cell LH receptors, steroidogenic enzymes and antioxidant enzymes in adult rats. Reproductive Toxicology. 20:117-126.

Murugesan, P., Muthusamy, T., Balasubramanian, K., Arunakaran, J. 2008. Polychlorinated biphenyl (Aroclor 1254) inhibits testosterone biosynthesis and antioxidant enzymes in cultured rat Leydig cells. Reproductive Toxicology. 25:447-454.

Nieschlag, E., Behre, H.M. 2004. Testosterone; action, deficiency, substitution. Third edition. Cambridge University Press.

Norstrom, R.J., Belikov, S.E., Born, E.W., Garner, G.W., Malone, B., Olpinski, S., Ramsay, M.A., Schliebe, S., Stirling, I., Stishov, M.S., Taylor, M.K., Wiig, Ø. 1998. Chlorinated hydrocarbon contaminants in polar bears from Eastern Russia, North America, Greenland, and Svalbard: Biomonitoring of Arctic pollution. Archives of Environmental Contamination and Toxicology. 35:354-367.

O′Donnell, L., Robertson, K.M., Jones, M.E., Simpson, E.R. 2001. Estrogen and spermatogenesis. Endrocrine Reviews. 22(3):289-318.

O′Shaughnessy, P.J., Baker, P.J., Heikkilä, M., Vainio, S., McMahon, A.P. 2000. Localization of 17β-hydroxysteroid dehydrogenase/17-ketosteroid reductase isoform expression in the developing mouse testis- androstenedione is the major androgen secreted by fetal/neonatal leydig cells. Endocrinology. 141(7):2631-2637.

Oskam, I.C., Ropstad, E., Dahl, E., Lie, E., Derocher, A.E., Wiig, O., Larsen, S., Wiger, R., Skaare, J.U. 2003. Organochlorines affect the major androgenic hormone, testosterone, in male polar bears (Ursus maritimus) at Svalbard. Journal of Toxicology and Environmental Health. Part A. 66(22):2119-2139.

Polischuk, S.C., Norstrom, R.J., Ramsay, M.A. 2002. Body burdens and tissue concentration of organochlorines in polar bears (Ursus maritimus) vary during seasonal fasts. Environmental Pollution. 118(1):29-39.

Ralph, J.L., Orgebin-Crist, M.-C., Lareyre, J.-J., Nelson, C.C. 2003. Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. Environmental Health Perspectives. 111(4):461-466.

Ropstad, E., Oskram, I.C., Lyche, J.L., Larsen, H.J., Lie, E., Haave, M., Dahl, E., Wiger, R., Skaare, J.U. 2006. Endocrine disrubtion induced by organochlorines (OCs): Field studies and experimental models. Journal of Toxicology and Environmental Health. Part A. 69:53-76.

Saksena, S.K., Lau, I.F. 1979. Variations in serum androgens, estrogens, progestin, gonadotropins, and prolactin levels in male rats from prepubertal to advanced age. Experimental Aging Research. 5(3):179-194.

Sanderson, J.T. 2006. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicological sciences. 94(1):3-21

Shabsigh, R. 1997. The effects of testosterone on the cavernous tissue and erectile function. World Journal of Urology. 15(1):21-26.

Shevtsov, S., Petrotchenko, E.V., Pedersen, L.C., Negishi, M. 2003. Crystallographic analysis of a hydroxylated polychlorinated biphenyl (OH-PCB) bound to the catalytic estrogen binding site of human estrogen sulfotransferase. Environmental Health Perspect. 111:884-888.

Simca-P+12. 2008. User guide and tutorial.

Skaare, J.U., Bernhoft, A., Wiig, Ø., Norum, K.R., Haug, E., Eide, D.M., Derocher, A. 2001. Relationships between PCBs, retinol and thyroid hormone levels in plasma of polar bear (Ursus maritimus) at Svalbard. Toxicology and Environmental Health. 24:231-238.

Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Riget, F.F., Kirkegaard, M., Muir, D.C.G. 2006. Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (*Ursus maritimus*). Envirnomental Science and Technology. 40(18):5668-5674.

Statens forurensningstilsyn. 2008. Ban on deca-BDE. Official publication. TA-2401.

Stockholm Convention. 2008. Stockholm Convention on persistent organic pollutants (POPs). Available: http://chm.pops.int/

(accessed 02 June 2011).

Stoker, T.E., Parks, L.G., Gray, L.E., Cooper, R.L. 2000. Endocrine-disruptive chemical chemicals: prepubertal exposures and effects on sexual maturation and thyroid function in the male rat. A focus on the EDSTAC recommendations. Critical Reviews in Toxicology. 30(2):197-252.

Sundaram, K., Kumar, N., Monder, C., Bardin, C.W. 1995. Different patterns of metabolism determine the relative anabolic activity of 19-norandrogens. Journal of Steroid Biochemistry and Molecular Biology. 53(1-6):253-257.

Sørmo, E.G., Salmer, M.P., Jenssen, B.M., Hop, H., Baek, K., Kovacs, K.M., Lydersen, C., Falk-Petersen, S., Gabrielsen, G.W., Lie, E., Skaare, J.U. 2006. Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar beat food chain in Svalbard, Norway. Environmental toxicology and chemistry. 25(9):2502-2511.

Trygg, J. 2004. Prediction and spectral profile estimation in multivariate calibration. Journal of chemometrics. 18:166-172.

Verreault, J., Muir, D.C.G., Norstrom, R.J., Stirling, I., Fisk, A.T., Gabrielsen, G.W., Derocher, A.E., Evans, T.J., Dietz, R., Sonne, C., Sandala, G.M., Gebbink, W., Riget, F.F., Born, E.W., Taylor, M.K., Nagy, J., Letcher, R.J. 2005. Chlorinated hydrocarbon contaminants and metabolites in polar bears (*Ursus maritimus*) from Alaska, Canada, East Greenland, and Svalbard: 1996-2002. Science of the Total Environment. 351-352:369-390.

de Wit, C.A., Alaee, M., Muir, D. 2004. Brominated flame retardants in the Arctic- an overview of spatial and temporal trends. Organohalogen compounds. Volume 66.

de Wit, C.A., Herzke, D., Vorkamp, K. 2010. Brominated flame retardant in the Arctic environment- trends and new candidates. Science of the Total Environment. 408:2885-2918.

APPENDIX 1: Full names of analyzed environmental contaminants

a= Mono-ortho substituted PCBs

α-HCH: alpha-Hexachlorocyclohexane

β-HCH: beta-Hexachlorocyclohexane

HCB: hexachlorobenzene

Mirex

Oxychlordane

Trans-nonachlor

pp-DDE: p,p'-Dichlorodiphenyldichloroethylene

pp-DDT: p,p'-Dichlorodiphenyltrichloroethane

3'-OH-CB138: 3'-OH-2,2',3,4,4',5-hexachlorobiphenyl

3'-OH-CB180: 3'-OH-2,2',3,4,4',5,5'-heptachlorobiphenyl

4-OH-CB107: 4-OH-2,3,3', 4', 5-pentachlorobiphenyl

4'-OH-CB130: 4'-OH-2,2',3,3',4,5'-hexachlorobiphenyl

4-OH-CB146: 4-HO-2,2',3,4',5,5'-hexachlorobiphenyl

4'-OH-CB159: 4'-HO-2',3,3',4,5,5'-hexachlorobiphenyl

4'-OH-CB172: 4'-HO-2,2', 3,3',4,5,5'-heptachlorobiphenyl

4-OH-CB187: 4-HO-2,2',3,4',5,5',6'-heptachlorobiphenyl

PCB-47: 2,2',4,4'-tetrachlorobiphenyl

PCB-74: 2,4,4',5-tetrachlorobiphenyl[a]

PCB-99: 2,2'4,4',6-pentachlorobiphenyl

PCB-101: 2,2',4,5,5'-pentachlorobiphenyl

PCB-105: 2,3,3',4,4'-pentachlorobiphenyl[a]

PCB-114: 2,3,4,4',5-pentachlorobiphenyl[a]

PCB-118: 2,3',4,4',5-pentachlorobiphenyl[a]

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[a]

PCB-157: 2,3,3',4,4',5'-hexachlorobiphenyl[a]

PCB-170: 2,2',3,3',4,4',5-heptachlorobiphenyl

PCB-180: 2,2',3,4,4',5,5'-heptachlorobiphenyl

PCB-183: 2, 2',3,4,6 ,4',5'-heptachlorobiphenyl

PCB-187: 2,2',3,4',5,5',6-heptachlorobiphenyl

PCB-189: 2,3,3',4,4',5,5'-heptachlorobiphenyl[a]

PCB-194: 2,2',3,3',4,4',5,5'-octachlorobiphenyl

PCB-206: 2,2',3,3',4,4',5,5',6-nonachlorobiphenyl

BDE-47: 2, 2'4, 4'-tetrabromodiphenyl ether

BDE-154: 2,2',4,4',5,6'-hexabromodiphenyl ether

APPENDIX 2: Spearman′s rank correlation test results for AN and TS

Table 10 and 11 shows the coefficient (r) and the significant level (p) of correlations between the variables (environmental contaminants, biological variables and other steroid hormones) and androgen (AN and TS) in all the sampled male polar bears (n=23) from Svalbard, analysed by the Spearman′s rank correlations coefficient test.

Table 10. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p<0.05$) to AN in 23 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

	Androstendione	
Variables	r	p
Age	0.671	0
Testosterone	0.652	0.001
Pregnenolone	-0.590	0.003
Estrone	-0.549	0.007
Estradiol-α	-0.545	0.007
4-OH-CB146	-0.571	0.007
Axial girth	0.523	0.010
ΣBDEs	-0.504	0.014
DEA	0.493	0.017
Oxychlordane	-0.477	0.021
Weight	0.472	0.023
BDE-47	-0.460	0.027
Head length	0.456	0.029
Zygomatic width	0.452	0.030
PCB-187	-0.453	0.030
PCB-137	-0.429	0.041
p,p′-DDE	-0.427	0.042
Σpesticides	-0.415	0.049

Table 11. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p<0.05$) to TS in 23 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

	Testosterone	
Variables	r	p
Age	0.791	0.000
Axial girth	0.733	0.000
Weight	0.633	0.001
Androstenedione	0.652	0.001
Zygomatic width	0.610	0.002
Dehydroepiandrosterone	0.575	0.004
Mirex	-0.571	0.004
4-OH-CB146	-0.600	0.004
Estrdiol-α	-0.511	0.013
Estrone	-0.500	0.015
PCB-137	-0.495	0.016
PCB-183	-0.491	0.017
ΣBDEs	-0.489	0.018
pp-DDT	-0.486	0.019
PCB-180	-0.481	0.020
Oxychlordane	-0.459	0.027
PCB-187	-0.460	0.027
4-OH-CB187	-0.469	0.032
Head length	0.434	0.039
Σpoly-ortho PCBs	-0.434	0.039
Body length	0.425	0.043
BDE-154	-0.421	0.045
ΣPCBs	-0.414	0.050

APPENDIX 3: Spearman′s rank correlation test results for steroid hormones in adult polar bears

Table 12-16 shows the coefficient (r) and the significant level (p) of correlations between the variables (environmental contaminants and biological variables) and steroid hormones(AN, DEA, TS, E1 and E2-α) in all the sampled male adult polar bears (n=17) from Svalbard, analysed by the Spearman′s rank correlations coefficient test.

Table 12. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p<0.05$) to AN in 17 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

	Androstenedione	
Variables	r	p
DEA	0.618	0.008

Table 13. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p<0.05$) to DEA in 17 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

	Dehydroepiandrosterone	
Variables	r	p
TS	0.718	0.001
4-OH-CB146	-0.644	0.007
AN	0.618	0.008
ΣOH-PCBs	-0.615	0.011
PCB-183	-0.574	0.016
ΣBDEs	-0.576	0.016
PCB-118	-0.564	0.018
PCB-187	-0.546	0.023
4-OH-CB187	-0.559	0.024
3-OH-CB138	-0.55	0.027
PCB-137	-0.522	0.032
BDE-47	-0.51	0.037
PCB-101	-0.506	0.038
Girth	0.49	0.039
PCB-167	-0.493	0.045

Table 13. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p < 0.05$) to TS in 17 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

Variables	Testosterone	
	r	p
DEA	0.718	0.001
Girth	0.658	0.004
Age	0.624	0.007
Mirex	-0.614	0.009
PCB-180	-0.596	0.012
PCB-137	-0.569	0.017
PCB-183	-0.561	0.019
BDE-154	-0.544	0.024
ΣBDEs	-0.537	0.026
Weight	0.532	0.028
ΣPCBs	-0.517	0.034
Σpo-PCBs	-0.505	0.039
4-OH-CB146	-0.518	0.04
Zygomatic width	0.486	0.048

Table 14. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p < 0.05$) to E1 in 17 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

Variables	Estrone	
	r	p
PCB-189	-0.665	0.004
4-OH-CB187	0.559	0.024
DEA	-0.519	0.033

Table 15. The results from Spearman′s rank correlation coefficient test, showing the different variables, which were significantly correlated ($p < 0.05$) to E2-α in 17 male polar bears. The significant variables are listed up in decreasing significant with significance level (p).

Variables	Estradiol-α	
	r	p
TS	-0.632	0.007
PCB-180	0.543	0.024
4′-OH-CB159	0.547	0.028
3′-OH-CB180	0.519	0.039
4′-OH-CB172	0.513	0.042
PCB-206	0.487	0.048