NTNU – Trondheim
Norwegian University of
Science and Technology

Relationships Between Environmental and Biological Variables, Plasma Clinical-Chemical Parameters and Persistent Organic Pollutants (POPs) in Polar Bears (*Ursus maritimus*) from Svalbard, Norway

Vidar Torget

Environmental Toxicology and Chemistry
Submission date: May 2015
Supervisor: Bjørn Munro Jenssen, IBI
Co-supervisor: Heli Routti, Norwegian Polar Institute
Anuschka Polder, Norwegian University of Life Sciences

Norwegian University of Science and Technology
Department of Biology

Acknowledgements

This master thesis was conducted at the Department of Biology, the Norwegian University of Science and Technology (NTNU), Trondheim, Norway, included in the degree Master of Science in Environmental Toxicology. The thesis was conducted in collaboration with the Norwegian Polar Institute (NP), Tromsø, Norway. Head supervisor was Professor Bjørn Munro Jenssen at NTNU. Co-supervisors were Heli Routti at NP and Anuschka Polder at the Norwegian University of Life Sciences (NMBU). This master thesis was conducted as a part of the Bear Energy project at NP, and have been funded by NP and NTNU.

I would first like to thank Professor Bjørn Munro Jenssen and Heli Routti for the opportunity to conduct my master thesis on such an interesting animal in such an interesting environment, and for patient guidance and counselling. I would especially like to thank Heli for taking the time to help me with the statistics. I would like to thank Anuschka Polder for guidance and counselling and in addition Katarina Løken and Mahin Karimi at the Laboratory of Environmental Toxicology at NMBU for help, guidance and patience during the POP analysis. I would like to thank Grethe Stavik Eggen for help and guidance during CCP analysis at NTNU. In addition I would like to thank Martin Andersen for help and guidance during the analysis at NMBU, and fellow graduate Astrid Kolind Riemer for the collaboration.

I would like to express my sincere gratitude to my high school biology teacher Håkon Skånland for the inspiring lectures that guided me to become a biologist.

I would like to thank my family and friends support and distractions. I would like thank Niels and Elise for everything, and Christina for something to look forward to.

Trondheim, May 2015

Vidar Torget

Sammendrag

Få tidligere studier har rapportert om nivåer av klinisk-kjemiske parametere i isbjørn (*Ursus maritimus*), og til dags dato har kun ett studie undersøkt nivåer av disse klinisk-kjemiske parameterene og hvilken sammenheng de har med persistente, organsike forurensningsstoffer. I dette studiet ble det analysert for nivåer av klinisk-kjemiske parametere (CCPer), og undersøkt hvordan disse varierte mellom sessong, reproduktiv status, kondisjon og alder. I tillegg ble det undersøkt hvordan CCPer er relatert til polyklorinerte bifenyler (PCBer), hydroksylerte PCB-metabolitter (OH-PCBer), organoklor-pestisider (OCPer) og perfluorerte alkylerstoffer (PFAS). Dette ble gjort ved å analysere plasma fra isbjørnhunner (n = 111) fra Svalbard, innsamlet vår (april) og høst (september) i 2012 og 2013. De analyserte CCPene var: kolesterol (CHOL), HDL-kolesterol (HDLP), triglyserider (TG), aspartataminotransferase (ASAT), alaninaminotransferase (ALAT), γ-glutamyltransferase (GGT), kreatinkinase (CK), kalium (K) og ratioen mellom urea og kreatinine (UCR). Lineære blandet-effekt-modeller og multivariat statistikk ble benyttet for å analysere dataene. Studiet fant sessongforskjeller for UCR, GGT, CK og TG. De lipid-relaterte klinisk-kjemiske parameterene (CHOL, HDLP og TG) var positivt korrelerte, og det samme var leverensymene (ASAT, ALAT og GGT). Alle PFASene var interkorrelerte. Det samme var fem av åtte OH-PCBer. ∑PFAS var positivt korrelert med de lipid-relaterte klinisk-kjemiske parameterene. Det samme var flere andre, individuelle POPer. ∑OH-PCB var negativt korrelert med leverensymene, det var også fler individuelle POPer. Nivåene av CCPene var sammenliknbare med tidligere studier. Sessongforskjellen i UCR støtter opp om tidligere studier som knytter denne til forende/fastende status. Sammenhengen mllom CCPer og POPer støtter opp om tidligere studier som knytter PFASeksponering til lipidmetabolisme. Sammenhengen støtter også opp om tidligere studier som knytter POPer til leverskade.

Abstract

Few previous studies have reported on levels of clinical-chemical parameters (CCPs) in polar bears (*Ursus maritimus*), and to date, only one study has investigated levels of CCPs and how they are related to persistent organic pollutants (POPs) in this species. The present study investigate levels of CCPs and how they vary between seasons, reproductive status, body condition (BCI) and age. In addition, the present study investigate how CCPs are related to polychlorinated biphenyls (PCBs), their hydroxylated metabolites (OH-PCBs), organochlorine pesticides (OCPs) and perfluoroalkyl substances (PFAS). This was done by analyzing plasma samples from female polar bears (n = 111) from Svalbard, Norway, sampled during spring (April) and autumn (September) in 2012 and 2013. The CCPs analyzed were: cholesterol (CHOL), high-density lipoprotein (HDLP), triglycerides (TG), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT), creatine kinase (CK), potassium (K), and the ratio of urea and creatinine (UCR). Linear mixed effect models and multivariate statistics were used to investigate the data. There were found seasonal differences for UCR, CK, TG. The lipid-related CCPs (CHOL, HDLP, TG) were positively correlated, as were the liver enzymes (ASAT, ALAT, GGT). All PFASs were inter-correlated and thus summed, as were five of eight OH-PCBs. ∑PFAS was positively correlated with each lipid-related CCP, as were several other individual POPs. ∑OH-PCB was negatively correlated with the liver enzymes. The levels of CCPs were comparable to previous studies. The seasonal difference seen in UCR supports previous studies linking UCR to fasting/feeding status. The relationships between CCPs and POPs support previous studies linking PFAS exposure to lipid metabolism. It also supports previous studies linking POPs to hepatic dysfunction.

Abbreviations

ALAT	Alanine aminotrasferase
ASAT	Aspartate aminotrasnferase
BCI	Body condition index
BFR	Brominated flame retardant
CCP	Clinical-chemical parameter
CHOL	Cholesterol
CI	Confidence interval
CK	Creatine kinase
COY	Cub-of-the-year
CYP	Cytochrome P450
DDT	Dichloro-diphenyl-trichloro-tehane
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
GGT	γ-glytamyltrasnferase
HCB	Hexachlorobenzene
HDLP	High-density lipoprotein
K	Potassium
KOH	Potassium hydroxide
LOD	Limit of detection
LOQ	Limit of quantification
LRT	Long-range transport
MS	Mass spectrometry
NMBU	Norwegian University of Life Sciences
NTNU	Norwegian University of Science and Technology
NVH	Norwegian Veterinary School
OCP	Organochlorine presticide
OH-PCB	Hydroxylated polychlorinated biphenyl
PBDE	Polybrominated diphenylether
PC	Principal component
PCA	Principal component analysis

PCB	Polychlorinated biphenyl
PFAS	Perfluoroalkyl substances
PFCA	Perfluorinated carboxylated acids
PFOA	Perfluorooctanoic acid
PFOS	Perluorooctane sulfonate
PFOSF	Perluorooctanesulfonyl fluoride
PFSA	Perfluorinated sulfonic acid
POP	Persistent organic pollutant
PPAR	Peroxiosome proliferator-activated receptor
RDA	Multivariate redundancy analysis
TG	Triglycerides
UCR	Urea/Creatinine-ratio
ww	Wet weight
YRL	Yearling

Table of Contents

1. Introduction

Persistent organic pollutants (POPs) are anthropogenic chemical compounds produced for a wide range of applications, such as pesticides, surfactants, impregnation and flame retardants. Others are products and by-products of industrial processes. They persist in the environment for a long time after their release (AMAP, 1998). Due to their persistency, they are very susceptible to long-range transport (LRT). When these POPs reaches the arctic region they will deposit due to "cold condensation". When deposited, the cold temperatures and periods without sun light may "trap" the contaminants and prevent remobilisation, due to the "cold-trapping" effect (Barrie et al., 1992, Mackay and Wania, 1995, Wania and Mackay, 1996, Beyer et al., 2000). After they have deposited in the arctic environment, they have the potential to bioaccumulate in organisms, and biomagnify through the food web (Letcher et al., 2009, Hallanger et al., 2011). After they have been taken up by the organisms, they may cause serious toxic effects such as endocrine disruption, disrupt homeostasis, reproductive effects, neurological effects, teratogenic effects among others (Letcher et al., 2010). There are several groups of POPs. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), chlordanes and hexachlorobenzene (HCB) are referred to as legacy POPs and refers mainly to past releases. These compounds have been banned or are under restriction of use by the Stockholm Convention (Stockholm Convention, 2001). Perfluorinated alkylated substances (PFAS) and flame retardants such as polybrominated diphenylethers (PBDEs) are compounds of more recent concern, and are referred to as emerging POPs (Butt et al., 2010). Limited studies have shown specific mechanisms of toxicity for PFAS, but they are potential endocrine disruptors (Weiss et al., 2009, Boas et al., 2012). Because of their abundance in the environment, and their potential toxicity, PFOS and PFOSF are regulated by the Stockholm Convention (Stockholm Convention, 2009). More recent studies show that many of the legacy contaminants such as PCBs and OCPs are declining in the arctic environment and biota (Hung et al., 2010, Rigét et al., 2010, Bytingsvik et al., 2012a, Dietz et al., 2013). Although decreasing trends have been shown for legacy contaminants it is observed the opposite for emerging contaminants, such as PFOS (Bytingsvik et al., 2012b, Rigét et al., 2013).

The polar bear (*Ursus maritimus*) is the apex predator of the arctic food web. Their diet mainly consists of ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) and

they are dependent on the sea ice to hunt (Thiemann et al., 2008). With annual fluctuations in sea ice cover, there are also an annual fluctuation in prey availability. Prey is abundant when seals give birth on the sea ice during spring. During the summer months when the sea ice have withdrawn, and partly during mid-winter, polar bears may fast for short or longer periods. Pregnant polar bear females will fast from the time they enter dens in late autumn, until they re-emerge with their new-born in the spring (Pilfold et al., 2012, Pilfold et al., 2014).

Polar bears of Svalbard are among the most contaminated bears in the Arctic, and the contaminant levels are among the highest reported in any arctic species (Letcher et al., 2010, McKinney et al., 2011). Lipophilic POPs such as PCBs accumulate in polar bear adipose tissue. Even though the polar bear have a great metabolic capacity to biotransform POPs, products of these processes such as the hydroxylated PCB metabolites (OH-PCBs) may be retained in the body and lead to toxic effects. These metabolites can been found at even higher levels in plasma than their parent compounds (Bytingsvik et al., 2012a). As an emerging contaminant, the information on PFAS transportation, distribution and effect are limited compared to legacy POPs. PFAS are less lipophilic than PCBs, and they bind to proteins, and can thus be found in higher concentrations in protein-rich tissue such as blood, liver and kidney (Conder et al., 2008).

POP levels found in polar bears may vary due to seasonal variability, availability of prey and their condition. The polar bears' capacity for bioaccumulating POPs is due to their high trophic position and that they mainly consume the fatty tissue of their prey. Due to seasonal variability in prey abundance, and thus fasting, polar bears mobilise their fat storage for energy. When fat is mobilised, stored lipophilic contaminants are remobilised into the blood stream. Females will also remobilise stored POPs when they are lactating to nurse their young. Their fat are converted to milk, and contaminants can pass over from mother to offspring through the mother's milk (Bytingsvik et al., 2012a, Bytingsvik et al., 2012b).

Clinical-chemical parameters (CCPs) derived for plasma can provide valuable information in evaluating polar bear health and physiology. Such parameters can also be used as biomarkers for assessing toxicity induced by environmental contaminants (Sonne et al., 2012a). The present study has focused on the lipid-related CCPs cholesterol (CHOL), high-density lipoprotein (HDLP), and triglycerides (TG), the liver enzymes aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT) and creatine kinase (CK) in addition to potassium (K) and the ratio of urea/creatinine (UCR). ASAT and ALAT are both transaminases and together with GGT they are involved in amino acid metabolism

(Cattet, 2000). CK is an enzyme found in the liver but also related to other tissues as skeletal muscles, heart and brain. Increased levels can be related to muscle injuries (Krefetz and McMillin, 2005). K is an important intercellular cation and is essential for a wide variety of functions, such as heart contraction and regulation neuromuscular excitation (Polancic, 2005). Urea are a nitrogenous product of proteins and muscle catabolism. Creatinine is a product from the breakdown of creatine phosphate, and thus a product of muscle catabolism. The ratio of these compounds can be used to indicate the feeding/fasting status of the polar bear (Derocher et al., 1990, Cattet, 2000). Few previous studies have reported information on these CCPs in free-ranging polar bears (Lee et al., 1977, Derocher et al., 1990, Ramsay et al., 1991, Cattet, 2000, Tryland et al., 2002, Ormbostad, 2012). To date, the only known study on assessing links between contaminant exposure and response in CCPs in free-ranging polar bears have been published in a master thesis by Ormbostad (2012).

With the continuing climate change that is especially pronounced in the arctic region, the combined effects of continuing contaminant exposure and the struggle to adapt to climate changes can have detrimental effects for the polar bear population (Jenssen, 2006, Jenssen et al., 2015).

The aim of this thesis was to investigate CCP levels in female polar bears and how they are related to environmental and biological variables and contaminants. The first aim was to examine relationships between CCPs in female polar bear plasma and how they were related to season, reproductive status, age and body condition (BCI). The second aim of this thesis was to examine the relationship between CCPs and POPs.

2. Materials and Methods

2.1 Field sampling

Female polar bears from the Barents Sea population were sampled in 4 periods, April and September 2012 and 2013. 111 samples were collected in total (N=33 in April 2012, N=24 in September 2012, N=28 in April 2013 and N=26 in September 2013), representing 80 individual females of which 26 were captured more than once. The sampling was done

opportunistically throughout the Svalbard archipelago. The bears were immobilized by a dart gun with Zoletil ® 100 (Virbac, Carros, France), from a helicopter (Eurocopter AS350 Ecureuil). After immobilization, a vestigial premolar tooth was used for age estimation. Blood was collected from the femoral vein using heparinised collecting tubes, stored on ice and centrifuged within 10 hours (3500 rpm, 10 minutes). The plasma was frozen and stored at -20^{o}C. Body mass was obtained to the nearest kg by suspending the bear on a stretcher from two spring weights. BCI was calculated using the formula described by (Cattet et al., 2002). Mature females, aging from 4 to 28 years were classified in three groups according to their breeding status: solitary (alone, or together with male in spring), with one or two cubs of the year (COY; cubs younger than 1 year old) or with one or two yearlings (YRL: cub aged between 1 and 2 years old).

2.2 POP Analysis

Extraction

POP analysis were performed at the Laboratory of Environmental Toxicology at The Norwegian University of Life Sciences in Oslo (NMBU), previously the Norwegian School of Veterinary Science (NVH). The laboratory is accredited by the Norwegian Accreditation for analysis of PCBs, OCPs and brominated flame retardants (BFRs) in biological matrices according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137). PFAS and OH-PCBs are not included in the accreditation, but validated according to the same procedures.

The analytical method is based on the principle of liquid/liquid extraction described by Brevik (1978), prior to detection by gas chromatography-mass spectrometry (GC-MS), described by Polder et al. (2008). An additional modification was introduced to the method by Løken et al. (2006) to cover OH-PCB, described in (Gabrielsen et al., 2011).

Before use, all glass equipment used in the procedure was washed with a 1:1 mixture of acetone and cyclohexane. Plasma samples kept at -20^{o}C was thawed in room temperature prior to analysis.) Plasma (2 ± 0.100 g) was weight in centrifuge tubes (80 mL). The samples were spiked with internal standards; PCB-29, -112 and -207 (Ultra Scientific, RI, USA), BDE-77, -119, -181 and -[$^{13}C_{12}$]-209 (Cambridge isotope laboratories, Andover, MA, USA) and 4'-OH-[$^{13}C_{12}$]-CB-159 and 4-OH-[$^{13}C_{12}$]-CB-187. NaCl (6 %, 2 mL), H_2SO_4 (1 M, 10

mL), acetone (15 mL) and cyclohexane (20 mL) were added during the first step of extraction. The samples were sonicated (1 min.) with an ultrasonic homogeniser (Cole Parmer CPX 750, Vernon Hills IL, USA). After sonication, the samples were centrifuged (3000 rpm, 10 min.), (Allegra X-12R Beckman Coulter, Fullerton CA, USA). The supernatants were collected and transferred to Zymark® tubes (50 mL), and then the extraction was repeated with acetone (5 mL) and cyclohexane (10 mL), sonication (30 sec.) and centrifugation (3000 rpm, 10 min.). Supernatants were again transferred to their respective Zymark® tubes. The extracts were then evaporated down to approximately 1 mL aliquots (40^{o}C with continues flow of pure nitrogen gas ,purity: 99.6 %; AGA AS, Oslo, Norway, pressure 0.6 bar), using a Zymark® evaporator (TurboWap II, Zymark Corporation, Hopkinton, MA, USA). The aliquots were quantitatively transferred to pre-weighed glass tubes (10 mL).

Gravimetric lipid determination

The aliquots were evaporated dry on sand bath (40^{o}C with continuous flow of nitrogen), and then weighed. The lipid concentration was calculated according to the formula:

$$\frac{\text{(weight of 10 mL glass tube with dry sample-weight of empty 10 mL glass tube)*100}}{\text{initially weighted quantity}}$$

Lipid clean-up

The dry samples were added cyclohexane (1 mL), then concentrated sulphuric acid (H_2SO_4, 97.5 %, 6 mL), (Fluka Analytical, Sigma-Aldrich, St. Louis MO, USA), thoroughly mixed and stored in darkness for approximately one hour. The samples were centrifuged (3000 rpm, 10 min.), and the supernatant transferred to new glass tubes (10 mL). The glass tubes containing acid were again added cyclohexane (approximately 1 mL) and centrifuged (3000 rpm, 10 min.) for quantitative transfer of the supernatant.

OH-PCB extraction

The glass tubes were added KOH (1M in 50% ethanol, 5 mL), mixed thoroughly and then centrifuged (3000 rpm, 5 min.). The subnatant was collected and transferred to large test tubes. The extraction was repeated.

The subnatant containing the OH-PCB was added concentrated H_2SO_4 to adjust the pH to a value between 1 and 2. The OH-PCB were extracted with cyclohexane (5 mL) added to the tubes and the tubes stirred gently. After the two phases had separated, the supernatant transferred to Zymark® tubes. This extraction was repeated. The extracts were then evaporated down to approximately 1 mL aliquots (40ºC with continues flow of pure nitrogen gas), using a Zymark® evaporator. The aliquots were quantitatively transferred to test tubes. The aliquots were then evaporated down to approximately 1 mL on sand bath (40ºC with a continuous flow of nitrogen).

Derivatisation

The test tubes containing the OH-PCB were derivatised. A 1:1 solution of pyridine and acetic anhydride was prepared and 60 µL was added to the each test tube. The test tubes were whirl mixed and placed in a 60 ºC hot cabinet for 30 min. After acclimatisation (5 min. in fume hood), the tubes were added grade 1 water (2 mL) and centrifuged (3000 rpm, 5 min.). The supernatants were then transferred to glass tubes (10 mL). The test tubes were washed with one pasteur pipette cyclohexane, centrifuged (3000 rpm, 5 min.), and the supernatant again transferred to its respective glass tube (10 mL). The aliquots were then evaporated down to approximately 400 µL on sand bath (40ºC with a continuous flow of nitrogen). The aliquots were then transferred to GC-glass containers.

PCBs, OCPs and PFAS extraction

The remaining phase from OH-PCB extraction, the supernatant, was added one pasteur pipette grade 1 water and centrifuged (3000 rpm, 10 min.). The supernatant was collected and transferred to glass tubes (10 mL). The remaining phase was then added one pasteur pipette with cyclohexane and centrifuged (3000 rpm, 5 min.), and the supernatant again transferred to

its respective glass tube (10 mL). The aliquots were then evaporated down to approximately 400 µL on sand bath (40ºC with a continuous flow of nitrogen). The aliquots were then transferred to amber coloured GC-glass containers.

Quantification

The quantification of POPs and OH-PCBs was performed using high resolution gas chromatography (high resolution GC) (Agilent 6890 Series GC system, Agilent Technologies, Santa Clara, CA, USA) equipped with an auto sampler (Agilent 7683 Series, Agilent Technologies, Santa Clara, CA, USA). For quantification of PCBs and OCPs, the system was coupled to two ^{63}Ni micro electron capture detectors (Agilent 6890 µ-ECD). For quantification of PBDEs and OH-PCB, the system was configured with a mass spectrometer (MS) detector (Agilent 5973 Network Mass Selective Detector, Agilent Technologies). Details of the methods are described in Polder et al. (2008) and Gabrielsen et al. (2011) with the following modifications in specifications of GC conditions for PCBs and OCPs: constant flow of the hydrogen carrier gas was increased to 1.2 mL/min. and final holding time at 275ºC was increased to 21 min., making the total run time 76.6 min. Level of detection was set to three times the noise level, except for *p,p'*-DDT, *cis*-chlordane, PCB-28, -52 and -101. Due to coeluting compounds, the limit of quantification (LOQ) for these compounds was set to ten times the noise level. None of these compounds was detected in concentrations higher than LOQ in any of the samples (n=111).

2.3 Clinical-chemical analysis

Analysis of CCPs in plasma was performed using a "dry" clinical-chemical analyser, Reflotron® (Model IV, Boehringer-Mannheim GmhB, Mannheim, Germany). The system is composed of a reagent carrier (test strip) and a microprocessor controlled reflectance photometer. The system uses individual strips for each parameter, and each strip uses a specific reaction to produce a dye that is measured and evaluated by the reflectance photometer (for reaction principles: Table B1, appendix B). Parameters analysed were cholesterol (CHOL), high-density lipoprotein (HDLP), creatinine (CREA), urea (UREA),

potassium (K), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT), creatine kinase (CK) and triglycerides (TG).

Plasma was thawed in the dark prior to analysis. A 32 µL pipette (Reflotron®) was used to transfer plasma from the cryo-tubes to the test strips (Roche Diagnostics, Mannheim, Germany). The strips were then inserted in the reflectance photometer, and after a specific time (which differed between the parameters) a readout of the quantitative value was obtained. Duplicates was analysed, and in some cases where large variance was observed between duplicates, triplicates were analysed. The mean of the duplicates and triplicates was used for the statistical analysis.

All of the HDLP samples scored above the upper limit of detection (LOD), and had to be diluted. The samples were diluted with 0.9% NaCl physiological saline solution. Trials were performed with several dilution curves to check for linearity and find optimal dilution range. A 1:1 dilution was applied, as it was found optimal.

2.3 Quality Control

POP analysis

For each series of 16 samples, 3 blank samples, one blind, 2 recovery samples and 2 in-house references were included in the analysis. The blank samples consisted of a mixture internal standards and solvents. The blind sample and the two recovery samples consisted of a plasma mixture of mainly cat and dog, spiked with internal standards. The recovery samples were also spiked with known amount of all the analytes. The in-house references consisted of one sample of seal whole blood and one sample of seal fat. Relative recoveries based on internal standards were: PCB: 92 – 121%, OCP: 93 – 14%, PBDE: 92 – 105%, 92 – 124%, OH-PCB: 57 – 100%. The samples were not corrected for recoveries.

Clinical-chemical analysis

For every three to five samples, a Reflotron® Clean + Check strip (Roche Diagnostics, Mannheim, Germany) was used to control that the optical system measured values within the

reference range. Values within the reference range confirmed that the apparatus did not have considerable variance throughout the analysis.

2.4 Data analysis

Only POPs and CCPs detected in 60% or more of the individuals were included in the statistical analysis. Values below the LOD were substituted with half the LOD. Statistical analysis were carried out using R statistical software version 3.1.2 (R Core Team, 2014) and R studio version 0.98.1091 (RStudio, Inc.) with packages nlme version 3.1-120, ade4 version 1.6-2, lsmeans version 2.17. and multcomp version 1.4-0. Linear mixed effect models were constructed and an analysis of variance performed to investigate variance between CCPs and season (April and September) and reproductive status (solitary, COY, YRL). A Tukey's Honest Significant Difference test was run on reproductive status to check for differences between the three different levels. Female ID number was set as the random factor, to account for repeated measures.

Diagnostic plots of residuals against fitted values were used to verify the model assumptions of constant variance and approximately normal distribution (Zuur et al., 2010). UCR, ASAT, ALAT, GGT and CK were ln-transformed to approach the assumptions. Four individuals had extreme concentrations of ASAT, two individuals had extreme concentrations of GGT and four individuals had extreme concentrations of CK. Removing individuals with extreme concentrations of GGT proved GGT to be non-significant between seasons. Removing the individuals with extreme concentrations of ASAT and CK did not affect their relationship with season. The individuals were kept for further analysis.

Multivariate analysis were performed to illustrate the relationships between CCPs, season, reproductive status, BCI, age and POPs (Quinn and Keough, 2002). First a principal component analysis (PCA) was performed on the CCPs, with BCI and age as supplementary variables. Sample scores were plotted by season and status. Second, multivariate redundancy analysis (RDA) was used to look at the relationship between CCPs and POPs, with CCPs as response variables and POPs as predictor variables, and with BCI and age as supplementary variables. The significance of the RDA plot was tested using a Monte-Carlo permutation test (1000 replicates, RV coefficient 0.105, $p = 0.005$). All the data included in the multivariate

analysis was centred and scaled prior to analysis. Only variables with a loading >|0.30| relative to each other along the same PC in the RDA plot were considered correlated and included in further statistical investigation. Linear mixed effect models were used to statistically test relationships between selected CCPs and POPs based on the RDA plot. To reduce the number of explanatory variables, contaminants with similar structure were summed if the individual compounds were highly correlated ($r > 0.4$). Contaminants with similar structure which were not correlated ($r > 0.4$) were not further statistically pursued and were discussed on the basis of the RDA plot. Effect of explanatory variables on response variables are reported as parameter estimates (β) from the mixed models with 95 % confidence intervals (95% CI).

3. Results

3.1 Relationships between CCPs and environmental and biological variables

Concentrations of CCPs in blood plasma of female polar bears from Svalbard ($n = 111$) are presented in Table 1. Biological measurements and mean CCP levels for each individual are in Table A1, appendix A. All parameters were detected in more than 60 % of the individuals.

Table 1: Concentrations of clinical-chemical parameters in female polar bear plasma (n = 111), sampled at Svalbard, Norway during spring (April) and autumn (September), 2012 and 2013. Data is presented as mean ± standard deviation, median and range, by season. All values are presented as their unit on a wet weight (ww) basis. Number of detections (n) and limit of detection (LOD) are also shown. Significant differences between seasons are marked with an asterisk.

Clinical-chemical	Spring			Autumn				
parameter (ww)[1]	Mean + SD	Median	Range	Mean + SD	Median	Range	n	LOD
CHOL (mmol/L)	8.0 ± 1.5	7.9	5.3 – 11.7	8.6 ± 1.9	8.7	4.8 – 12.2	111	2.59
HDLP (mmol/L)	2.8 ± 0.7	2.8	1.5 – 4.6	3.0 ± 0.6	3.0	1.1 – 4.2	111	0.26
UCR* (mmol/L/µmol/L)	0.08 ± 0.07	0.08	0.01 – 0.2	0.04 ± 0.04	0.03	0.01 – 0.15	85	3.33
K (mmol/L)	3.9 ± 0.3	3.9	3.2 – 4.7	3.9 ± 0.3	4.0	3.3 – 4.7	111	2.00
ASAT (U/L)	59 ± 17	57	30 – 106	71 ± 66	52	29 – 403	111	5.00
ALAT (U/L)	13 ± 6	13	3 – 28	14 ± 9	12	3 – 51	108	5.00
GGT (U/L)	83 ± 76	57	18 – 459	161 ± 364	74	29 - 1965	111	5.00
CK* (U/L)	107 ± 29	101	48 - 215	209 ± 209	143	83 - 1160	111	24.40
TG* (mml/L)	1.4 ± 0.5	1.4	0.4 – 3.6	2.0 ± 0.5	2.0	1.0 - 3	109	0.80

[1]Abberivations: CHOL: cholesterol, HDLP: high-density lipoprotein, UCR: urea/creatinine-ratio, K: potassium, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, GGT: γ-glutamyltransferase, CK: creatine kinase, TG: triglycerides

UCR was significantly higher in polar bears sampled in the spring compared to those sampled in the autumn (Table 1, $F = 21.75$, $p = 0.0001$). CK ($F = 34.46$, $p < 0.0001$) was the only enzyme having significantly lower levels in polar bears sampled in the spring compared to those sampled in the autumn (Table 1). When removing the outliers, GGT was not significantly different between seasons ($F = 3.32$, $p = 0.0787$). TG ($F = 27.67$, $p < 0.0001$) was also significantly lower in the spring compared to the autumn. As illustrated by the PCA plot, CCPs were not affected by age or BCI (Figure 1A). Significant differences was not found for CCPs between statuses (solitary, COYs, YRLs), BCI or age.

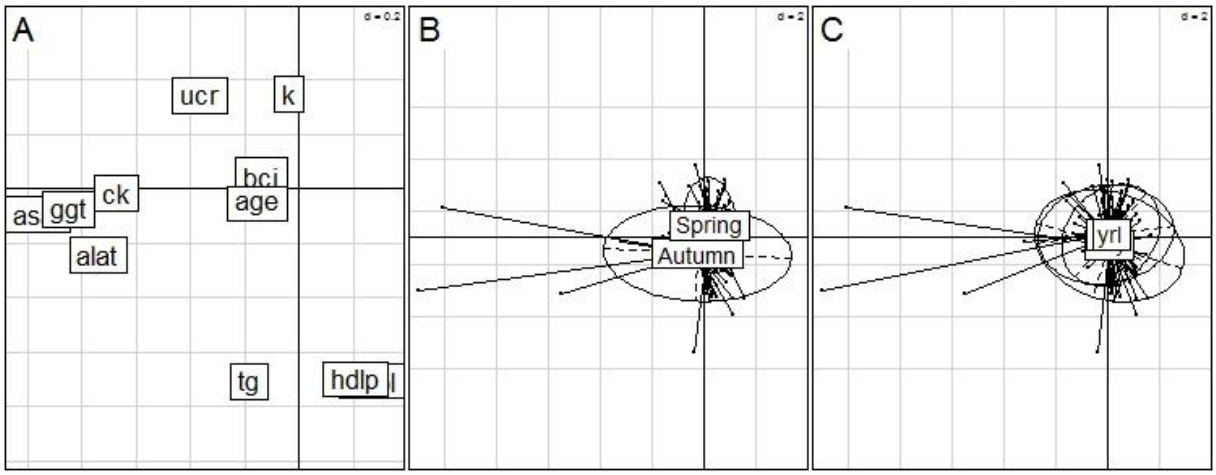

Figure 1: Principal component analysis (PCA) loadings plot for clinical-chemical parameters in female polar bears (n = 111), sampled at Svalbard, Norway during spring (April) and autumn (September), 2012 and 2013. Age and BCI were included as supplementary variables (**A**). Sample scores are grouped by season (**B**) and status (**C**). The first and second principal component explained 33% and 20% of the total variation respectively.

CHOL, HDLP and TG showed a positive correlation along principal component 1 (PC1) (Figure 2A). ASAT, ALAT, CK and GGT also showed a positive correlation. UCR showed a negative correlation with CHOL, HDLP and TG. Sample scores for season does show variations between spring and autumn, but the differences were not large. Scores for bears sampled in autumn showed higher individual variation than those sampled in spring (Figure 2B). This is due to the observed outliers of GGT and CK. No notable variation was found between reproductive statuses (Figure 2C).

3.2 Relationships between CCP and POPs

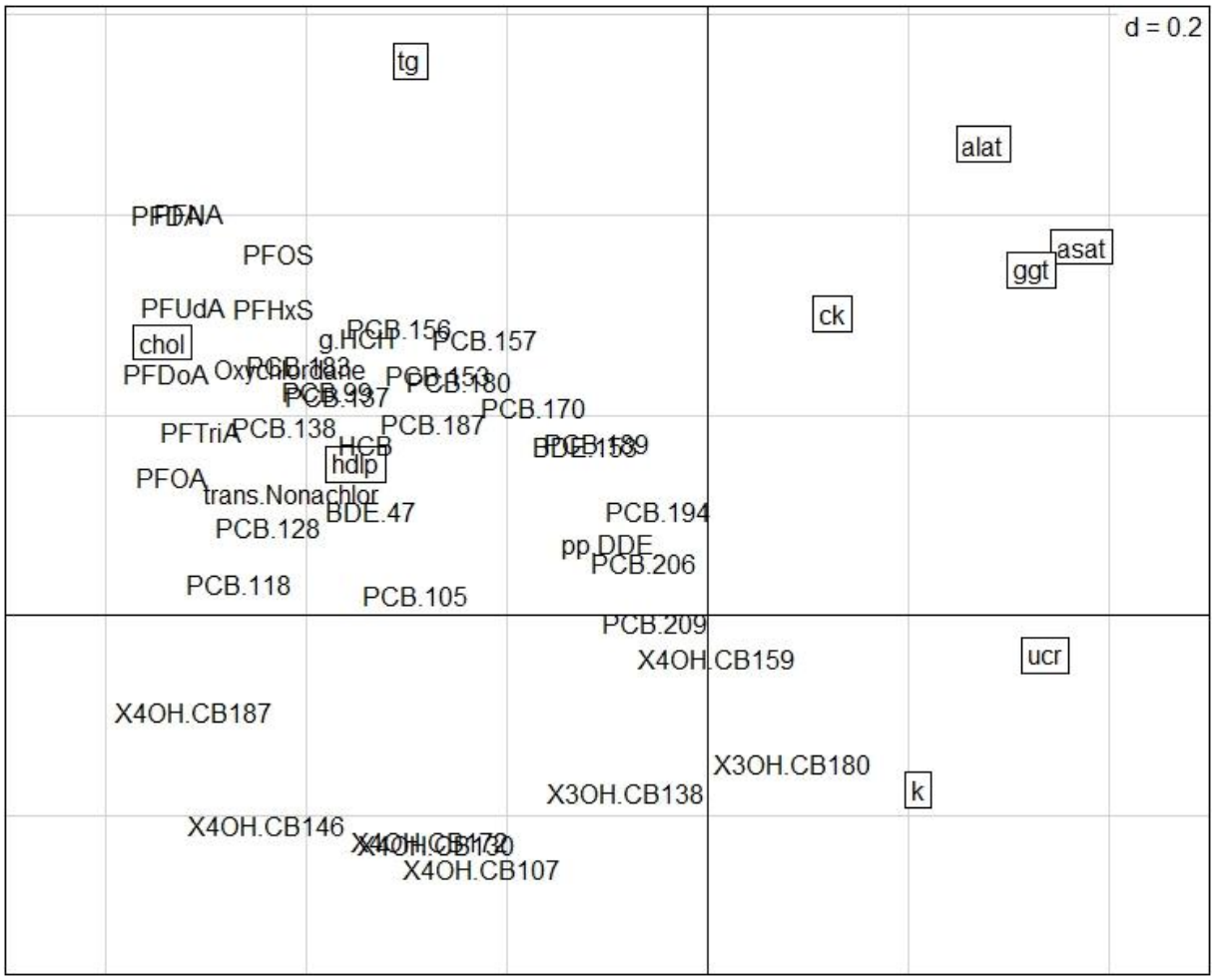

Figure 3: Redundancy analysis (RDA) loadings plot on standardized concentrations of POPs from female polar bear plasma (n = 111), sampled at Svalbard, Norway during spring (April) and autumn (September) 2012 and 2013. The relationships are shown between response variables (CCPs) and explanatory variables (POPs), with BCI and age as supplementary variables. The first and second axis explained 33% and 22 % of the variation, respectively.

From the RDA loadings plot (Figure 3), all POPs except OH-PCBs were situated in the same quadrant of the plot, and were thus positively related. UCR were situated in the opposite quadrant and showed a negative relationship with the POPs. PFASs were summed together, due to high positive inter-correlation and are denoted ∑PFAS in further discussion. 4-OH-CB-197, 4-OH-CB-147, 4-OH-CB-172, 4-OH-CB-130 and 4-OH-CB-107 were also summed together due to high inter-correlation and are denoted ∑OH-PCB in further discussion. It was

not observed any other distribution of POPs in the RDA plot that offered any reason to create other summed groups. These POPs are treated independently in further discussion.

As illustrated by the RDA, the lipid-related variables CHOL, HDLP and TG were located close to PFASs, and thus positively related to these compounds. Further analysis on mixed models confirmed positive relationships between the lipid-related CCPs and ∑PFAS. CHOL and HDLP showed positive significant relationships with ∑PFAS: CHOL (β=1.5 [95% CI 0.51, 5.4]), TG (β=0.36 [95% CI 0.13, 0.58]) and HDLP did show a weak significant relationship (β=0.25 [95% CI -0.0086, 0.51]). The, liver enzymes ASAT, ALAT and GGT were located opposite of OH-PCBs, and were thus negatively related to these compounds. This was also confirmed by mixed models. All three variables showed negative significant relationships with ∑OH-PCB: ASAT (β=-0.30 [95% CI -0.45, -0.16]), ALAT (β=-0.30 [95% CI -0.44, -0.16]) and GGT (β=-0.14 [95% CI -0.43, -0.15]).

4. Discussion

4.1 Relationships between CCPs and environmental biological variables

In the present study UCR levels were within the same range as levels reported in female polar bears from Svalbard sampled in April (Tryland et al., 2002). CHOL and TG levels reported in the present study were within the same range as previously reported for the Svalbard population (Tryland et al., 2002), and for female polar bears sampled at Hudson Bay during spring (April and May) (Cattet, 2000). ASAT, ALAT, GGT and CK levels reported in the present study were within the same range as those reported from Svalbard, but with lower upper values for CK reported herein. The mean ASAT and GGT levels were almost twice as high compared to the levels reported in the female polar bears from Hudson Bay. The mean ALAT level was found similar. It is a possibility that different analysis procedures for CCPs can affect the comparisons. It is not known if Reflotron® analysis are severely different from other analytical procedures, but the procedure are simple and cost-effective, and a comprehensive validation of this procedure could be valuable for further research.

Mixed linear effect models revealed that BCI was significantly lower for polar bears sampled in the spring compared to those sampled in the autumn (Table A1, appendix A) (Bourgeon et al., unpublished). This is in accordance with the previous study on female polar bears from Hudson Bay (Cattet, 2000), but it should be noted that the autumn (September) in the present study has been compared to the summer-autumn (July to November) in the Hudson Bay population.

It is proposed that the observed difference in BCI between seasons (Bourgeon et al., unpublished) can be partly explained by the feeding/fasting status of the polar bear. Polar bears start their main feeding period in mid-April in response to the ringed seal reproduction (Lydersen, 1995, Pilfold et al., 2014). After breeding and weaning their cubs, the ringed seals enter their molting period, where they change hair and top skin layer. During this period the ringed seals prefer to stay out of the water, and are more vulnerable for predation. This takes place in May to June (Hammill et al., 1991, Krafft et al., 2006). As the polar bears in this study were sampled during April, it is believed that they may have been feeding recently. This will cause an increase in the UCR for bears sampled during spring. Since they have been sampled in the start of their feeding period, the high UCR combined with the low BCI could be explained by recent feeding, but not continuously feeding for a longer period of time. According to Derocher et al. (1990), the UCR may drop within a time scale of one week after a feeding event, back to levels associated with a fasting status. Thus, UCR may reflect only recent feeding. It is uncertain if continuously feeding over an extended period would cause the UCR to decrease more slowly after feeding stops. In the present study it was found a significant seasonal difference in UCR, with levels being higher in the spring compared to the autumn (Table 1). When calculated from mol/L to mg/dL, UCR showed similar seasonal trend, but with higher levels in the present study compared to the female polar bears from Hudson Bay (Cattet, 2000). No significant difference was found between UCR and BCI. This is similar to what was reported by Tryland et al. (2002). It is suggested that the fasting/feeding status of the female polar bear, reflected in the UCR, may only partly explain variability in BCI, and are vulnerable to confounding factors. According to Bourgeon et al. (unpublished), large inter-individual variations due to random feeding events and a general opportunistic feeding strategy may affect season variability of the BCI. Also, pregnant and lactating females have an incentive to maintain a healthy BCI while fasting, and may thus display lower seasonal variation (Ramsay and Stirling, 1988, Atkinson and Ramsay, 1995, Cattet, 2000).

In the present study CHOL, HDLP and TG was positively correlated (Figure 1A). This can be explained by their relationship in lipid metabolism (Desvergne et al., 2006). All the three variables were negatively correlated with UCR (Figure 1A). It is suggested that the negative relationship between these variables can be explained by a mobilization of the lipid storage for energy when the polar bears are fasting, and thus higher amounts of lipids are released into the blood stream (Atkinson and Ramsay, 1995). TG was the only lipid-related CCP that was significantly different between seasons, with higher levels in the autumn compared to the spring, which also can be explained by lipid mobilization (Tryland et al., 2002). The reason why CHOL and HDLP did not show the same seasonal variability could be due to variability between individuals, and that the parameters may be affected by recent feeding.

All liver enzymes (ASAT, ALAT, GGT and CK) was positively correlated (Figure 1A) in the present study. CK was the only enzymes that were significantly different between seasons, with higher levels in the autumn compared to the spring. Levels of GGT had non-significant differences between seasons, also with higher levels in the autumn compared to the spring, in accordance with what was reported by Cattet (2000). No seasonal difference was observed for ASAT and ALAT. It was observed high outliers for both ASAT, GGT and CK. GGT is a membrane-bound enzyme in a variety of tissues including the liver, but also heart, kidney, skeletal muscles, and tissues of the mammary glands, placenta and fetus (Cattet, 2000). It is possible that GGT may leak into the plasma from damaged liver cells, or cells from any other of the mentioned tissues. According to Tryland et al. (2002), increased levels of CK may be associated with cardiac or skeletal muscle injuries. CK levels have also been associated with capture, struggling and sampling in sea otters (*Enhydra lutris*) (Williams and Pulley, 1983). High levels of ASAT may be due to stress or immobilisation (Aubin et al., 1979, Bossart et al., 2001). The high outliers observed in this study may thus come from recent injuries or events during sampling. No observations or events during sampling was registered that could contribute to explain this.

4.2 Relationships between CCPs and POPs

Cholesterol, HDL-protein, triglycerides and PFAS

Levels of CCPs related to lipid metabolism (CHOL, HDLP and TG) increased with increasing levels of ∑PFAS. The positive relationship between CHOL and PFAS is in agreement with epidemiological studies in humans (Nelson et al., 2010, Eriksen et al., 2013). In contrast, PFAS concentrations were negatively related to CHOL and TG in male Wistar rats and cynomolgus monkeys (Haughom and Spydevold, 1992, Seacat et al., 2002).

PFASs interaction with the peroxisome proliferator-activated receptors (PPARs) may contribute to explain the relationships observed in this study. Three types of PPARs have been identified, α, β and γ (Peraza et al., 2006). These three nuclear receptors are coupled to lipid metabolism. PPARγ has been linked to adipose tissue differentiation and adipogenesis. Mature adipocyte cells are important for TG synthesis and storage. PPARα is involved in lipid catabolism, and has a major role in fatty acid oxidation. Fasting can increase gene expression and activity of PPARα. PPARβ is suggested to have an overlapping role with PPARα in fatty acid oxidation. PPARα and PPARγ are also involved in the CHOL and HDLP metabolism. PPARα upregulates the expression of cholesterol acceptor apolipoprotein apoAI and apoAII, involved in the formation of HDLP particles, which transport CHOL to the liver. PPARα also increases the hepatic expression of scavenger receptor BI (SR-BI/CLA-1), which increases the uptake of HDL-cholesteryl esters from the blood (Desvergne et al., 2006, Casals-Casas and Desvergne, 2011). Studies have also shown that PPARβ is involved in regulating the lipoprotein transport system. A study on rhesus monkeys treated with a PPARβ agonist caused an increase in HDLP and lowering in fasting TG (Oliver et al., 2001). A study on obese and diabetic *db/db* mice treated with PPARβ agonists raised total plasma CHOL (Leibowitz et al., 2000). Based on what have previously been reported, it is a possibility that ∑PFAS positive relationship to lipid-related CCPs can partly be explained by PFAS interaction with PPARs. Studies have shown that both human, mouse and rat PPARα, β and γ can be activated by PFOS and PFOA, suggesting these compounds to at least be partial agonists of the receptor (Heuvel et al., 2006, Wolf et al., 2012, Buhrke et al., 2013). Because of PFAS general analogue structure to fatty acids, it is a possibility that other PFAS than those reported also could exert interactions with one or more of the receptors. However, as mentioned above, previous studies have reported on both positive and negative relationships

between PFAS and lipid-related CCPs. There have also been reported species difference regarding the activation of the receptors, and extrapolating findings to other species should be done with care (Palmer et al., 1998, Cheung et al., 2004, Wolf et al., 2012).

Liver enzymes and OH-PCB metabolites

Liver enzymes ASAT, ALAT and GGT were negatively related to ∑OH-PCB in the present study. PCBs and OCPs were also negatively associated with the liver enzymes along PC1. ASAT and ALAT are important enzymes of amino acid metabolism, and commonly used as biomarkers for liver health. Elevated levels of these enzymes may indicate liver and kidney dysfunction, such as steatosis (Clark et al., 2003, Kon and Marshall, 2008). Previous studies have reported negative relationships between PCBs and ASAT and ALAT and GGT in sledge dogs, male Wistar rats and cynomolgus monkeys (Rao and Banerji, 1990, Arnold et al., 1999, Sonne et al., 2008). A study on liver lesions in polar bears from East Greenland showed that PCBs were negatively associated with steatosis (Sonne et al., 2012b). No information has been found explaining the relationship between OH-PCBs and these enzymes. OH-PCB metabolites are derived from metabolism of PCBs catalysed by CYP enzymes. They are less lipophilic than their parent compound and are known to bind to blood proteins, and shown high affinity to transthyretin (TTR), as a result of structural similarities with the native thyroid hormone T4 (Van den Berg, 1990, Lans et al., 1993, Letcher et al., 2000, Gabrielsen et al., 2015). In the present study OH-PCBs were positively related with PCBs along PC2 in the RDA plot (Figure 2). It is not certain how OH-PCB metabolites interact to affect ASAT, ALAT and GGT levels, but their negative relationship to these enzymes may suggest similar effects as PCBs.

UCR and POPs

UCR and POPs (except OH-PCBs) were negatively associated in the present study. The main reason for this association is suggested to be related to the fasting status of the polar bear. As previously mentioned, when polar bears are fasting they mobilise its lipid storage for energy (Tryland et al., 2002). Lipophilic POPs stored in the lipids will then be released into the blood stream. This also explains why UCR and OH-PCBs did not show the same association, as

OH-PCBs are less lipophilic and more closely related to protein-rich tissue (Van den Berg, 1990, Lans et al., 1993).

Other relationships

CHOL, HDLP and TG were positively related to several other POPs in the present study. BDE-47 was one of the POPs most closely related to HDLP (Figure 2), suggesting a strong positive relationship. This was similar to what was reported in polar bears by Ormbostad (2012), and between PBDE and CHOL in grey seals (*Halichoerus grypus*) reported by Hall et al. (2003). These findings could suggest that the relationship reported is caused by BDE-47 interaction with PPARs. Both BDE-47 and BDE-metabolite 3-OH-BDE-47 has been reported to have a strong affinity to PPARγ, and thus inducing adipogenesis and adipocyte differentiation (Fang et al., 2014, Kamstra et al., 2014, Tung et al., 2014). This is in contrast to what was previously hypothesised by Luthe et al. (2008). Though OH-BDE levels were not analysed in the present study, there are reasons to believe that they also would be correlated to the lipid-related CCPs (Hakk and Letcher, 2003). In general the finding is in accordance with what has previously been reported and may suggests that BDE-47 could affect HDLP levels through PPARγ interactions.

Several PCBs (PCB-99, -118, -128, -138, -156, -183 and -187) were closely related to CHOL and HDLP in the RDA plot (Figure 2). The OCPs oxychlordane, *trans*-nonachlor, HCB and β-HCH were also positively related with these lipid-related CCPs. This is in accordance with previous epidemiological studies on humans (Baker et al., 1980, Goncharov et al., 2008, Lee et al., 2011). These groups of contaminants have also been related to adipogenesis and adipose cell differentiation (Taxvig et al., 2012). Contrary to what was shown for PBDEs, PCBs have been reported to induce antagonistic responses to PPARγ (Ariyoshi et al., 1998, Luthe et al., 2008). The antagonistic behaviour of these contaminants suggests that other mechanisms are more likely to explain the positive relationships reported herein. An important matter should be taken into account is that OCPs and PCBs are lipophilic compounds, and will thus be positively related with lipid-related CCPs on the basis of their lipophilic nature.

5. Conclusion

The present study reports seasonal variability in several plasma CCPs in female polar bear between spring and autumn. The UCR was significantly different between seasons, with lower levels during the autumn, supporting previous studies reporting that the ratio can be used to assess the feeding/fasting status of the polar bear. It was suggested that the seasonal variability in CCPs could be explained by the fasting/feeding status. However, opportunistic behaviour and unobservable events can confounders in evaluating the CCPs. There were found significant positive relationships between lipid-related CCPs (CHOL, HDLP and TG) and ∑PFAS. It was suggested that these relationships could partly be explained by PFAS interaction with PPARs. Significant negative relationships were found between liver enzymes (ASAT, ALAT and GGT) and ∑OH-PCBs. Though no specific mechanism to explain the relationship was suggested, it was suggested that the results may be related to what has previously been reported for PCBs. Moreover, there were observed positive associations between BDE-47 and several OCPs and PCBs and the lipid-related CCPs. It was suggested that interactions with PPARs could contribute to explain some of the observed associations, but as several of the positively related POPs are previously reported to be antagonists of these receptors, other mechanisms could probably contribute to explain these associations. Further research is needed to assess the relationships between POPs and PPAR interactions to understand how these contaminants affect lipid metabolism. Last, many confounding factors exist when studying levels and interactions of compounds derived from polar bear plasma, and measures should be taken to control for these factors. Conclusions should be taken with care.

References

AMAP 1998. *AMAP Assessment Report: Arctic Pollution Issues,* Oslo, Norway, Arctic Monitoring and Assessment Porgramme (AMAP).

ARIYOSHI, N., IWASAKI, M., KATO, H., TSUSAKI, S., HAMAMURA, M., ICHIKI, T. & OGURI, K. 1998. Highly toxic coplanar PCB126 reduces liver peroxisomal enzyme activities in rats. *Environmental toxicology and pharmacology,* 5**,** 219-225.

ARNOLD, D., BRYCE, F., MES, J., TRYPHONAS, H., HAYWARD, S. & MALCOLM, S. 1999. Toxicological consequences of feeding PCB congeners to infant rhesus (Macaca mulatta) and cynomolgus (Macaca fascicularis) monkeys. *Food and chemical toxicology,* 37**,** 153-167.

ATKINSON, S. & RAMSAY, M. 1995. The effects of prolonged fasting of the body composition and reproductive success of female polar bears (Ursus maritimus). *Functional Ecology***,** 559-567.

AUBIN, D. S., AUSTIN, T. & GERACI, J. 1979. Effects of handling stress on plasma enzymes in harp seals, Phoca groenlandica. *Journal of wildlife diseases,* 15**,** 569-572.

BAKER, E. L., LANDRIGAN, P. J., GLUECK, C. J., ZACK, M. M., LIDDLE, J. A., BURSE, V. W., HOUSEWORTH, W. J. & NEEDHAM, L. L. 1980. Metabolic consequences of exposure to polychlorinated biphenyls (PCB) in sewage sludge. *American journal of epidemiology,* 112**,** 553-563.

BARRIE, L., GREGOR, D., HARGRAVE, B., LAKE, R., MUIR, D., SHEARER, R., TRACEY, B. & BIDLEMAN, T. 1992. Arctic contaminants: sources, occurrence and pathways. *Science of the total environment,* 122**,** 1-74.

BEYER, A., MACKAY, D., MATTHIES, M., WANIA, F. & WEBSTER, E. 2000. Assessing long-range transport potential of persistent organic pollutants. *Environmental science & technology,* 34**,** 699-703.

BOAS, M., FELDT-RASMUSSEN, U. & MAIN, K. M. 2012. Thyroid effects of endocrine disrupting chemicals. *Molecular and cellular endocrinology,* 355**,** 240-248.

BOSSART, G. D., REIDARSON, L. A., DIERAUF, L. A. & DUFFIELD, D. A. 2001. Clinical Pathology. *In:* DIERAUF, L. A. & GULLAND, F. M. D. (eds.) *CRC handbook of marine mammal medicine.* 2nd ed. FL, USA: CRC Press.

BREVIK, E. 1978. Gas chromatographic method for the determination of organochlorine pesticides in human milk. *Bulletin of environmental contamination and toxicology,* 19**,** 281-286.

BUHRKE, T., KIBELLUS, A. & LAMPEN, A. 2013. In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. *Toxicology letters,* 218**,** 97-104.

BUTT, C. M., BERGER, U., BOSSI, R. & TOMY, G. T. 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci Total Environ,* 408**,** 2936-65.

BYTINGSVIK, J., LIE, E., AARS, J., DEROCHER, A. E., WIIG, Ø. & JENSSEN, B. M. 2012a. PCBs and OH-PCBs in polar bear mother–cub pairs: A comparative study based on plasma levels in 1998 and 2008. *Science of the total environment,* 417**,** 117-128.

BYTINGSVIK, J., VAN LEEUWEN, S. P., HAMERS, T., SWART, K., AARS, J., LIE, E., NILSEN, E. M. E., WIIG, Ø., DEROCHER, A. E. & JENSSEN, B. M. 2012b. Perfluoroalkyl substances in polar bear mother–cub pairs: A comparative study based on plasma levels from 1998 and 2008. *Environment international,* 49**,** 92-99.

CASALS-CASAS, C. & DESVERGNE, B. 2011. Endocrine disruptors: from endocrine to metabolic disruption. *Annual review of physiology,* 73**,** 135-162.

CATTET, M. 2000. *Biochemical and physiological aspects of obesity, high fat diet, and prolonged fasting in free-ranging polar bears.* PhD, University of Saskatchewan.

CATTET, M. R., CAULKETT, N. A., OBBARD, M. E. & STENHOUSE, G. B. 2002. A body-condition index for ursids. *Canadian Journal of Zoology,* 80**,** 1156-1161.

CHEUNG, C., AKIYAMA, T. E., WARD, J. M., NICOL, C. J., FEIGENBAUM, L., VINSON, C. & GONZALEZ, F. J. 2004. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor α. *Cancer research,* 64**,** 3849-3854.

CLARK, J. M., BRANCATI, F. L. & DIEHL, A. M. 2003. The prevalence and etiology of elevated aminotransferase levels in the United States. *The American journal of gastroenterology,* 98**,** 960-967.

CONDER, J. M., HOKE, R. A., WOLF, W. D., RUSSELL, M. H. & BUCK, R. C. 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environmental science & technology,* 42**,** 995-1003.

DEROCHER, A. E., NELSON, R. A., STIRLING, I. & RAMSAY, M. A. 1990. Effects of fasting and feeding on serum urea and serum creatinine levels in polar bears. *Marine mammal science,* 6**,** 196-203.

DESVERGNE, B., MICHALIK, L. & WAHLI, W. 2006. Transcriptional regulation of metabolism. *Physiological reviews,* 86**,** 465-514.

DIETZ, R., RIGÉT, F. F., SONNE, C., BORN, E. W., BECHSHØFT, T., MCKINNEY, M. A. & LETCHER, R. J. 2013. Three decades (1983–2010) of contaminant trends in East Greenland polar bears (Ursus maritimus). Part 1: legacy organochlorine contaminants. *Environment international,* 59**,** 485-493.

ERIKSEN, K. T., RAASCHOU-NIELSEN, O., MCLAUGHLIN, J. K., LIPWORTH, L., TJØNNELAND, A., OVERVAD, K. & SØRENSEN, M. 2013. Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. *PloS one,* 8**,** e56969.

FANG, M., WEBSTER, T., FERGUSON, P. & STAPLETON, H. 2014. Characterizing the Peroxisome Proliferator–Activated Receptor (PPARγ) Ligand Binding Potential of Several Major Flame Retardants, Their Metabolites, and Chemical Mixtures in House Dust. *Environmental health perspectives*.

GABRIELSEN, K. M., VILLANGER, G. D., LIE, E., KARIMI, M., LYDERSEN, C., KOVACS, K. M. & JENSSEN, B. M. 2011. Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (Cystophora cristata) mother-pup pairs. *Aquatic toxicology,* 105**,** 482-91.

GABRIELSEN, K. M., KROKSTAD, J. S., VILLANGER, G. D., BLAIR, D. A., OBREGON, M. J., SONNE, C., DIETZ, R., LETCHER, R. J. & JENSSEN, B. M. 2015. Thyroid hormones and deiodinase activity in plasma and tissues in relation to high levels of organohalogen contaminants in East Greenland polar bears (Ursus maritimus). *Environmental research,* 136**,** 413-23.

GONCHAROV, A., HAASE, R. F., SANTIAGO-RIVERA, A., MORSE, G., MCCAFFREY, R. J., REJ, R., CARPENTER, D. O. & ENVIRONMENT, A. T. F. O. T. 2008. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population. *Environmental research,* 106**,** 226-239.

HAKK, H. & LETCHER, R. J. 2003. Metabolism in the toxicokinetics and fate of brominated flame retardants—a review. *Environment international,* 29**,** 801-828.

HALL, A. J., KALANTZI, O. I. & THOMAS, G. O. 2003. Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life—are they thyroid hormone endocrine disrupters? *Environmental pollution,* 126**,** 29-37.

HALLANGER, I. G., WARNER, N. A., RUUS, A., EVENSET, A., CHRISTENSEN, G., HERZKE, D., GABRIELSEN, G. W. & BORGÅ, K. 2011. Seasonality in contaminant accumulation in Arctic marine pelagic food webs using trophic magnification factor as a measure of bioaccumulation. *Environmental toxicology & chemistry,* 30**,** 1026-1035.

HAMMILL, M., LYDERSEN, C., RYG, M. & SMITH, T. 1991. Lactation in the ringed seal (Phoca hispida). *Canadian journal of fisheries and aquatic sciences,* 48**,** 2471-2476.

HAUGHOM, B. & SPYDEVOLD, Ø. 1992. The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. *Biochimica et biophysica acta (BBA)-lipids and lipid metabolism,* 1128**,** 65-72.

HEUVEL, J. P. V., THOMPSON, J. T., FRAME, S. R. & GILLIES, P. J. 2006. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-α,-β, and-γ, liver X receptor-β, and retinoid X receptor-α. *Toxicological sciences,* 92**,** 476-489.

HUNG, H., KALLENBORN, R., BREIVIK, K., SU, Y., BRORSTRÖM-LUNDÉN, E., OLAFSDOTTIR, K., THORLACIUS, J. M., LEPPÄNEN, S., BOSSI, R. & SKOV, H. 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.

JENSSEN, B. M. 2006. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? *Environmental health perspectives,* 114**,** 76-80.

JENSSEN, B. M., VILLANGER, G. D., GABRIELSEN, K. M., BYTINGSVIK, J., BECHSHOFT, T., CIESIELSKI, T. M., SONNE, C. & DIETZ, R. 2015. Anthropogenic flank attack on polar bears: interacting consequences of climate warming and pollutant exposure. *Frontiers in ecology and evolution,* 3**,** 16.

KAMSTRA, J. H., HRUBA, E., BLUMBERG, B., JANESICK, A., MANDRUP, S., HAMERS, T. & LEGLER, J. 2014. Transcriptional and epigenetic mechanisms underlying enhanced in vitro adipocyte differentiation by the brominated flame retardant BDE-47. *Environmental science & technology,* 48**,** 4110-4119.

KON, S. P. & MARSHALL, W. J. 2008. The kidneys, Renal Function and Renal Failure. *In:* MARSHALL, W. J. & BANGERT, S. K. (eds.) *Clinical biochemistry: metabolic and clinical aspects.* Philadelphia, PA, USA: Elsevier health sciences.

KRAFFT, B. A., KOVACS, K. M., ANDERSEN, M., AARS, J., LYDERSEN, C., ERGON, T. & HAUG, T. 2006. Abundance of ringed seals (Pusa hispida) in the fjords of Spitsbergen, Svalbard, during the peak molting period. *Marine mammal science,* 22**,** 394-412.

KREFETZ, R. G. & MCMILLIN, G. A. 2005. Clinical Chemistry: principles, procedures, correlations. *In:* BISHOP, M. L., FODY, E. P. & SCHOEFFS, L. E. (eds.) *Enzymes.* Baltimore, Maryland: Lippincott Williams & Wilkins.

LANS, M. C., KLASSON-WEHLER, E., WILLEMSEN, M., MEUSSEN, E., SAFE, S. & BROUWER, A. 1993. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls,-dibenzo-p-dioxins and-dibenzofurans with human transthyretin. *Chemico-biological interactions,* 88**,** 7-21.

LEE, D.-H., STEFFES, M. W., SJÖDIN, A., JONES, R. S., NEEDHAM, L. L. & JACOBS JR, D. R. 2011. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PloS one,* 6**,** e15977.

LEE, J., RONALD, K. T. & ORITSLAND, N. 1977. Some blood values of wild polar bears. *The journal of wildlife management***,** 520-526.

LEIBOWITZ, M. D., FIÉVET, C., HENNUYER, N., PEINADO-ONSURBE, J., DUEZ, H., BERGER, J., CULLINAN, C. A., SPARROW, C. P., BAFFIC, J. & BERGER, G. D. 2000. Activation of PPARδ alters lipid metabolism in db/db mice. *FEBS letters,* 473**,** 333-336.

LETCHER, R. J., KLASSON-WEHLER, E. & BERGMAN, A. 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. *In:* HURTSINGER, O. (ed.) *The Handbook of Environmental Chemistry.* Berlin, Germany: Springer-Verlag.

LETCHER, R. J., GEBBINK, W. A., SONNE, C., BORN, E. W., MCKINNEY, M. A. & DIETZ, R. 2009. Bioaccumulation and biotransformation of brominated and chlorinated contaminants and their metabolites in ringed seals (Pusa hispida) and polar bears (Ursus maritimus) from East Greenland. *Environmental international,* 35**,** 1118-24.

LETCHER, R. J., BUSTNES, J. O., DIETZ, R., JENSSEN, B. M., JØRGENSEN, E. H., SONNE, C., VERREAULT, J., VIJAYAN, M. M. & GABRIELSEN, G. W. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the total environment,* 408**,** 2995-3043.

LUTHE, G., JACOBUS, J. A. & ROBERTSON, L. W. 2008. Receptor interactions by polybrominated diphenyl ethers versus polychlorinated biphenyls: A theoretical structure–activity assessment. *Environmental toxicology and pharmacology,* 25**,** 202-210.

LYDERSEN, C. 1995. Energetics of pregnancy, lactation and neonatal development in ringed seals (Phoca hispida). *Developments in marine biology,* 4**,** 319-327.

LØKEN, K., LIE, E., LUNDANES, E. & SKAARE, J. 2006. Extension of a multicomponent method to include the determination of OH-PCBs and OH-PBDEs in biological matrices. *Organohalogen compounds,* 68**,** 2430-2433.

MACKAY, D. & WANIA, F. 1995. Transport of contaminants to the Arctic: partitioning, processes and models. *Science of the total environment,* 160**,** 25-38.

MCKINNEY, M. A., LETCHER, R. J., AARS, J., BORN, E. W., BRANIGAN, M., DIETZ, R., EVANS, T. J., GABRIELSEN, G. W., PEACOCK, E. & SONNE, C. 2011. Flame retardants and legacy contaminants in polar bears from Alaska, Canada, East Greenland and Svalbard, 2005–2008. *Environment international,* 37**,** 365-374.

NELSON, J. W., HATCH, E. E. & WEBSTER, T. F. 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general US population. *Environmental health perspectives (Online),* 118**,** 197.

OLIVER, W. R., SHENK, J. L., SNAITH, M. R., RUSSELL, C. S., PLUNKET, K. D., BODKIN, N. L., LEWIS, M. C., WINEGAR, D. A., SZNAIDMAN, M. L. & LAMBERT, M. H. 2001. A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. *Proceedings of the national academy of sciences,* 98**,** 5306-5311.

ORMBOSTAD, I. 2012. *Relationships Between Persistent Organic Pollutants (POPs) and Plasma Clinical-Chemical Parameters in Polar Bears (Ursus maritimus) from Svalbard, Norway.* MSc, Norwegian University of Science and Technology.

PALMER, C. N., HSU, M.-H., GRIFFIN, K. J., RAUCY, J. L. & JOHNSON, E. F. 1998. Peroxisome proliferator activated receptor-α expression in human liver. *Molecular pharmacology,* 53**,** 14-22.

PERAZA, M. A., BURDICK, A. D., MARIN, H. E., GONZALEZ, F. J. & PETERS, J. M. 2006. The toxicology of ligands for peroxisome proliferator-activated receptors (PPAR). *Toxicological sciences,* 90**,** 269-295.

PILFOLD, N. W., DEROCHER, A. E., STIRLING, I., RICHARDSON, E. & ANDRIASHEK, D. 2012. Age and sex composition of seals killed by polar bears in the Eastern Beaufort Sea. *PloS one,* 7**,** e41429.

PILFOLD, N. W., DEROCHER, A. E., STIRLING, I. & RICHARDSON, E. 2014. Polar bear predatory behaviour reveals seascape distribution of ringed seal lairs. *Population ecology,* 56**,** 129-138.

POLANCIC, J. E. 2005. Electrolytes. *In:* SCHOEFFS, L. E., BISHOP, M. L. & FODY, E. P. (eds.) *Clinical chemistry: principles, procedures, correlations.* Baltimore, Maryland: Lippincott, Williams & Wilkins.

POLDER, A., GABRIELSEN, G., ODLAND, J. Ø., SAVINOVA, T., LØKEN, K. & SKAARE, J. 2008. Spatial and temporal changes of chlorinated pesticides, PCBs, dioxins (PCDDs/PCDFs) and brominated flame retardants in human breast milk from Northern Russia. *Science of the total environment,* 391**,** 41-54.

QUINN, G. P. & KEOUGH, M. J. 2002. *Experimental design and data analysis for biologists*, Cambridge University Press.

RAMSAY, M. & STIRLING, I. 1988. Reproductive biology and ecology of female polar bears (Ursus maritimus). *Journal of zoology,* 214**,** 601-633.

RAMSAY, M. A., NELSON, R. A. & STIRLING, I. 1991. Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. *Canadian journal of zoology,* 69**,** 298-302.

RAO, C. & BANERJI, S. 1990. Effect of feeding polychlorinated biphenyl (Aroclor 1260) on hepatic enzymes of rats. *Indian journal of experimental biology,* 28**,** 149-151.

RIGÉT, F., BIGNERT, A., BRAUNE, B., STOW, J. & WILSON, S. 2010. Temporal trends of legacy POPs in Arctic biota, an update. *Science of the total environment,* 408**,** 2874-2884.

RIGÉT, F., BOSSI, R., SONNE, C., VORKAMP, K. & DIETZ, R. 2013. Trends of perfluorochemicals in Greenland ringed seals and polar bears: indications of shifts to decreasing trends. *Chemosphere,* 93**,** 1607-14.

SEACAT, A. M., THOMFORD, P. J., HANSEN, K. J., OLSEN, G. W., CASE, M. T. & BUTENHOFF, J. L. 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicological sciences,* 68**,** 249-264.

SONNE, C., LEIFSSON, P. S., DIETZ, R., KIRKEGAARD, M., JENSEN, A. L., SHAHMIRI, S. & LETCHER, R. J. 2008. Greenland sledge dogs (Canis familiaris) develop liver lesions when exposed to a chronic and dietary low dose of an environmental organohalogen cocktail. *Environmental research,* 106**,** 72-80.

SONNE, C., BUSTNES, J. O., HERZKE, D., JASPERS, V. L., COVACI, A., EULAERS, I., HALLEY, D. J., MOUM, T., BALLESTEROS, M., EENS, M., IMS, R. A., HANSSEN, S. A., ERIKSTAD, K. E., JOHNSEN, T. V., RIGET, F. F., JENSEN, A. L. & KJELGAARD-HANSEN, M. 2012a. Blood plasma clinical-chemical parameters as biomarker endpoints for organohalogen contaminant exposure in Norwegian raptor nestlings. *Ecotoxicology environmental safety,* 80**,** 76-83.

SONNE, C., LETCHER, R. J., LEIFSSON, P. S., RIGÉT, F. F., BECHSHØFT, T. Ø., BOSSI, R., ASMUND, G. & DIETZ, R. 2012b. Temporal monitoring of liver and kidney lesions in contaminated East Greenland polar bears (Ursus maritimus) during 1999–2010. *Environment international,* 48**,** 143-149.

STOCKHOLM CONVENTION. 2001. UNEP/POPS/CONF/4. Stockholm Convention on Persistant Organic Pollutants *In:* STOCKHOLM CONVENTION. (ed.). UNEP.

STOCKHOLM CONVENTION. 2009. C.N.524.2009.TREATIES-4 (Depositary Notification). *In:* STOCKHOLMCONVENTION. (ed.). UNEP.

TAXVIG, C., DREISIG, K., BOBERG, J., NELLEMANN, C., SCHELDE, A. B., PEDERSEN, D., BOERGESEN, M., MANDRUP, S. & VINGGAARD, A. M. 2012. Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation. *Molecular and cellular endocrinology,* 361**,** 106-115.

THIEMANN, G. W., IVERSON, S. J. & STIRLING, I. 2008. Polar bear diets and arctic marine food webs: insights from fatty acid analysis. *Ecological monographs,* 78**,** 591-613.

TRYLAND, M., BRUN, E., DEROCHER, A. E., ARNEMO, J. M., KIERULF, P., OLBERG, R. A. & WIIG, O. 2002. Plasma biochemical values from apparently healthy free-ranging polar bears from Svalbard. *Journal of wildlife diseases,* 38**,** 566-75.

TUNG, E. W., BOUDREAU, A., WADE, M. G. & ATLAS, E. 2014. Induction of Adipocyte Differentiation by Polybrominated Diphenyl Ethers (PBDEs) in 3T3-L1 Cells. *PloS one,* 9**,** e94583.

VAN DEN BERG, K. 1990. Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. *Chemico-biological interactions,* 76**,** 63-75.

WANIA, F. & MACKAY, D. 1996. Peer reviewed: tracking the distribution of persistent organic pollutants. *Environmental science & technology,* 30**,** 390A-396A.

WEISS, J. M., ANDERSSON, P. L., LAMOREE, M. H., LEONARDS, P. E., VAN LEEUWEN, S. P. & HAMERS, T. 2009. Competitive binding of poly-and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicological sciences***,** kfp055.

WILLIAMS, T. D. & PULLEY, L. T. 1983. Hematology and blood chemistry in the sea otter (Enhydra lutris). *Journal of wildlife diseases,* 19**,** 44-47.

WOLF, C. J., SCHMID, J. E., LAU, C. & ABBOTT, B. D. 2012. Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): Further investigation of C4–C12 compounds. *Reproductive toxicology,* 33**,** 546-551.

ZUUR, A. F., IENO, E. N. & ELPHICK, C. S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution,* 1**,** 3-14.

Appendix

A Biological and clinical-chemical parameters

Table A1: Measurements of biological parameters, and concentrations of clinical-chemical parameters from female polar bear plasma (n = 111), captured at Svalbard, Norway during spring (April) and autumn (September) 2012 and 2013. Data of clinical-chemical parameters are presented as mean.

ID	Year	Season	Age	Status	BCI	CHOL	HDLP	CREA	UREA	K	ASAT	ALAT	GGT	CK	TG
7951	2012	Autumn	25	no	-0,896	7,58	2,56	181,67	4,96	3,8	37,25	7,37	81,95	83,15	1,13
23219	2012	Autumn	18	coy	-1,595	8,35	2,31	129,33	1,67	3,63	29	7,65	58,3	143	2,22
23347	2012	Autumn	28	no	-0,162	7,27	2,86	93,55	13,05	3,96	402,5	51	1965	314	2,67
23393	2012	Autumn	20	coy	-0,22	6,12	2,2	94,75	13,8	4,36	281,33	26,3	1845	1160	1,36
23500	2012	Autumn	12	coy	-1,766	9,1	3,07	185	3,41	3,95	52	13,1	152	143,5	2,07
23688	2012	Autumn	17	yrl	-1,391	9,81	3	150,33	1,67	3,5	42	10	45,2	119	1,82
23689	2012	Autumn	10	yrl	-1,809	6,97	2,49	124	1,67	3,99	42,7	7,42	32,85	132	0,95
23703	2012	Autumn	19	coy	-2,468	7,48	2,9	123	1,67	4,66	52,9	9,35	166,33	165,33	1,57
23803	2012	Autumn	8	coy	-1,692	12,15	3,74	104,5	6,72	3,58	64,45	18,55	55,95	150,33	1,58
23881	2012	Autumn	11	no	-2,38	9,46	2,84	98,1	6,02	4,27	59,7	11,85	61,65	116,5	1,53
23906	2012	Autumn	9	coy	-1,454	10,8	3,04	86,85	8,73	3,4	37,9	10,8	63,5	136	1,14
23937	2012	Autumn	7	no	-1,334	8,64	2,55	159	3,63	4,26	54,65	7,7	134	143,67	2,46
23958	2012	Autumn	9	coy	-0,796	6,94	3,12	130	3,64	3,76	76,1	11,4	47,1	283	2,13
26018	2012	Autumn	21	yrl	-0,729	6,65	3,34	70,95	4,9	3,52	63,85	10,85	101,87	192	1,79
26033	2012	Autumn	13	coy	-0,833	10,4	2,84	171,67	1,67	4,07	32,5	6,69	106	124,5	2,66
26084	2012	Autumn	8	no	-0,593	6,04	2,23	133,5	5,57	3,67	174,33	18,3	99,6	616	2,52
26095	2012	Autumn	5	no	-1,245	7,14	2,04	174	4,08	4,08	52,85	7,79	42,2	110	2,25
26102	2012	Autumn	5	no	-0,54	10,65	2,84	188	1,67	3,79	52,3	15,1	192	152,33	2,19
26113	2012	Autumn	NA	coy	-0,647	7,87	2,2	198	4,35	3,95	44,9	10,2	39,65	107,67	1,93
26114	2012	Autumn	7	no	-0,955	7,65	2,87	162	3,72	4,33	48,35	8,96	69,1	145	2,43
26117	2012	Autumn	17	yrl	-1,667	9,54	3,3	187,5	4,31	3,32	61,05	11,65	199	129,5	2,65
26120	2012	Autumn	10	coy	-0,69	9,95	2,33	186	3,56	4,07	65,6	9,36	130,33	198,5	3
26123	2012	Autumn	22	coy	-2,237	11,8	3,95	125	4,3	3,8	34,75	10,8	101,95	130,33	2,66

ID	Year	Season	Age	Status	BCI	CHOL	HDLP	CREA	UREA	K	ASAT	ALAT	GGT	CK	TG
26129	2012	Autumn	12	coy	-1,782	7,67	2,43	170,67	3,67	3,9	42,85	9,53	46,9	160,33	1,95
23222	2013	Autumn	17	coy	-1,225	10,24	3,58	112	4,45	3,27	59,95	11,65	80,05	184	2,3
23343	2013	Autumn	19	yrl	-0,809	5,74	2,75	134	18,7	3,3	50,35	17,8	38,25	122	1,52
23360	2013	Autumn	22	no	-1,443	5,62	1,11	135	1,67	3,95	49,15	2,5	77,95	991,5	1,9
23393	2013	Autumn	14	yrl	-1,594	6,11	2,88	111	1,67	3,89	100,6	15,25	147	117	1,63
23500	2013	Autumn	14	yrl	-2,613	9,77	2,46	79,3	8,01	3,45	214	48,75	207	607,67	2,42
23637	2013	Autumn	13	yrl	-0,63	9,8	3,98	129	4,37	4,66	49,35	8,55	37,2	144,5	1,57
23692	2013	Autumn	18	yrl	-0,075	9,03	3,49	78,35	1,67	4,28	58,4	14,8	159,5	88,9	1,22
23703	2013	Autumn	19	yrl	-0,889	5,83	3,24	59,25	3,72	4,16	51,75	12,85	262	104	1,81
23906	2013	Autumn	12	no	-1,79	10,35	4,04	81,55	4,16	4,19	34,35	13,4	107	92,5	1,41
23937	2013	Autumn	8	coy	-1,701	9,34	3,45	113	3,56	3,83	64,35	11,6	30,1	123,67	1,48
23952	2013	Autumn	5	no	-0,495	4,76	3,28	61,95	8,14	4,1	94,67	20,1	121,5	244,33	1,09
23980	2013	Autumn	6	no	-0,026	7,17	3,37	117	10,35	4,02	65,8	15,25	63,6	145,5	1,41
26010	2013	Autumn	9	no	-1,323	10,35	3,82	122,5	9,14	3,65	68,45	16,4	36,8	165	2,25
26033	2013	Autumn	18	yrl	-1,213	10,03	3,94	178	1,67	3,37	39,8	10,75	125	113	2,06
26095	2013	Autumn	8	no	-0,785	6,43	2,28	177,5	4,95	3,96	51,55	9,61	29,25	131	1,31
26120	2013	Autumn	14	yrl	-0,741	6,08	2,62	221	3,6	3,9	58,85	12,85	33,15	143	1,98
26129	2013	Autumn	16	no	-0,893	8,8	3,82	126	1,67	3,94	38,25	12,55	32,15	104,5	2,12
26137	2013	Autumn	11	no	-2,332	8,38	3,01	136,5	3,79	3,74	35,05	14,5	59,05	131,5	2,28
26179	2013	Autumn	12	no	-0,302	10,15	2,82	200	1,67	3,88	51,4	10,15	70,55	236	2,27
26180	2013	Autumn	17	no	-0,695	7,96	2,57	157,5	1,67	3,78	43,75	7,23	103,5	107	2,09
26183	2013	Autumn	10	no	-0,833	10,03	3,02	182,5	4,285	3,81	43,7	11,95	37	167	2,16
26185	2013	Autumn	26	no	-0,843	11	4,21	141	3,91	4,14	43,6	12	130,5	367,5	2,36
26191	2013	Autumn	8	coy	-1,574	11,2	3,78	122,5	5,39	4,04	47,8	14,8	32,85	117,5	1,8
26193	2013	Autumn	9	coy	-0,786	11,55	3,32	132	10,55	4,17	69,65	11,8	42,55	190,33	2,5
26195	2013	Autumn	7	yrl	-0,824	11,05	3,47	143	4,72	4,16	53,6	12,65	61,4	218,67	2,35
26199	2013	Autumn	8	no	-1,939	7,72	3,39	82,6	7,81	4,29	109	32,45	91,5	115	1,32
7753	2012	Spring	23	yrl	-1,019	6,9	3,12	100,5	8,09	3,76	40,9	8,23	130	105,5	1,6
7951	2012	Spring	25	no	-1,887	6,32	2,14	127,5	3,66	3,5	42,15	10,03	117	79,4	1,51

ID	Year	Season	Age	Status	BCI	CHOL	HDLP	CREA	UREA	K	ASAT	ALAT	GGT	CK	TG
23347	2012	Spring	28	no	-1,199	9,33	3,44	133,5	1,67	3,55	37,05	7,71	218	95	1,77
23361	2012	Spring	16	coy	-0,686	7,62	3,01	77,1	8,75	4,58	45,25	8,81	74,8	73,65	0,4
23393	2012	Spring	20	coy	-1,33	8,5	2,75	77,65	9,99	4,3	57,2	8,39	202,5	79,2	1,31
23479	2012	Spring	14	coy	-1,816	11	3,63	69,63	8,53	4,23	34,47	2,5	54,3	63,8	1,01
23639	2012	Spring	12	yrl	-1,033	8,76	2,61	70,9	9,9	4,04	76,5	15,6	57	88,75	1,59
23688	2012	Spring	17	yrl	-1,708	7,21	2,52	91,1	13,05	3,43	35,85	8,27	40	69,87	0,4
23719	2012	Spring	11	coy	-2,432	8,26	2,2	67,75	16,35	4,36	84,45	22,85	101	114	1,84
23906	2012	Spring	9	coy	-2,812	9,43	2,82	76,35	5,68	4,06	29,75	8,25	36,2	80,2	1,07
23909	2012	Spring	16	no	-1,646	6,16	1,87	81,8	7,33	4,07	49,6	15,3	52,3	114	1,42
23931	2012	Spring	12	coy	-0,503	7,29	3,05	54,9	11,2	4,06	65,4	6,89	59,63	187,67	1,5
23939	2012	Spring	8	no	-1,855	7,85	2,42	87,65	15,5	4,54	75,1	12,25	77,55	139,5	0,4
23952	2012	Spring	4	no	-1,013	6,37	1,92	62,45	1,67	4,28	59,85	15,95	48,5	110	1,39
23980	2012	Spring	6	no	-1,671	6,54	2,79	107,5	4,89	3,86	63,2	16,15	42,35	87,37	1,53
23989	2012	Spring	7	no	-2,375	9,97	2,34	81,5	11,3	3,73	105,73	27,55	76	125,5	1,26
26009	2012	Spring	8	coy	-1,722	10,03	3,34	73,2	1,67	3,79	46	5,89	32,05	101,5	1,33
26018	2012	Spring	21	yrl	-0,857	7,68	1,82	71,55	8,23	3,49	51,4	14,55	31,15	87,5	1,93
26025	2012	Spring	15	no	-0,881	7,34	1,52	77,35	10,12	3,6	43,85	11,9	33,4	93,45	1,07
26066	2012	Spring	14	no	-0,127	7,41	2,8	87,75	1,67	3,92	48,1	6,14	110,5	124,5	1,75
26068	2012	Spring	12	coy	-1,711	8,17	2,93	79,7	10,05	3,85	74,1	7,03	184,5	163	0,97
26072	2012	Spring	14	no	-0,201	6,17	2,18	76,07	13,75	3,93	69,1	13,75	75,5	93,5	1,51
26073	2012	Spring	4	no	-1,057	5,73	3,67	69,4	6,16	3,9	86,97	18,85	54,7	215	2,37
26075	2012	Spring	24	no	-1,06	7,94	2,44	98,95	7,55	3,83	77,9	8,58	113,33	124,5	1,44
26077	2012	Spring	7	yrl	-1,225	10,07	2,54	63,93	14,05	4,29	49,65	10,48	56,2	104	1,05
26084	2012	Spring	8	no	-0,885	9,06	2,82	88,25	4,75	3,78	56,95	17,4	19,4	127	2,19
26088	2012	Spring	7	coy	-0,814	6,81	1,92	147	1,67	3,51	44,83	2,5	157	145,33	1,42
26095	2012	Spring	5	no	-1,226	8,53	1,7	65,6	6,48	3,5	69,65	13,35	17,9	163,5	1,76
26098	2012	Spring	11	no	-0,794	7,36	2,04	83,95	6,59	3,82	41,65	10,75	63,55	94,3	0,95
26099	2012	Spring	11	no	-1,078	10,7	2,5	73,6	13,3	4,2	67,9	9,08	35,4	94,05	1,79

ID	Year	Season	Age	Status	BCI	CHOL	HDLP	CREA	UREA	K	ASAT	ALAT	GGT	CK	TG
26102	2012	Spring	5	no	-0,995	7,98	2,93	80,23	6,84	4,66	52,6	14,1	41,6	100,85	1,44
26103	2012	Spring	15	no	-0,917	6,42	2,26	76,05	4,51	4,06	93,9	24,85	105	116	2,05
26106	2012	Spring	17	yrl	0,078	6,03	2,22	69,3	5,97	4,13	58,4	13,05	34,1	140	0,94
7951	2013	Spring	26	no	-1,37	6,2	2,5	110	1,67	3,94	55,95	10,06	50,6	85,15	1,57
23360	2013	Spring	21	coy	-2,396	8,75	2,79	159	7,72	3,82	73,55	20,6	108	91	1,73
23637	2013	Spring	12	no	-1,469	8,35	3,13	89,5	1,67	3,59	62,6	11,25	32,55	85,55	1,84
23639	2013	Spring	13	no	-1,241	10,2	4,15	91,3	3,38	4,12	53,85	26,25	48,75	104	1,73
23688	2013	Spring	18	yrl	-2,33	8,63	2,56	82,3	8,92	4,04	48,75	12,95	64	89,85	0,97
23689	2013	Spring	11	no	-1,57	6,41	2,76	84,5	6,14	3,7	78,7	16,95	25,45	82,9	1,61
23692	2013	Spring	20	yrl	-1,939	11,25	3,64	103,33	6,86	3,84	47,2	15,1	77,7	98,37	1,25
23881	2013	Spring	12	no	-2,214	7,38	2,94	85,5	6,72	4,4	50,4	10,4	54,35	113	0,99
23882	2013	Spring	9	yrl	-2,103	7,64	2,26	113	4,64	3,81	51,5	14,3	49,4	77,15	1,07
23909	2013	Spring	17	coy	-2,461	5,25	2,68	96,7	9,87	3,4	65,6	14,5	458,5	125,5	1,68
23937	2013	Spring	8	coy	-2,932	7,6	2,61	81,8	4,81	3,7	59,7	11,25	33,5	89,85	0,96
23980	2013	Spring	7	no	-1,47	8,62	3,22	83,25	1,67	4,3	33,5	15,1	34,5	80,45	1,39
26068	2013	Spring	13	no	-0,332	6,93	3,41	87,43	16,3	3,65	83,2	12,3	111,5	145	2,65
26095	2013	Spring	6	no	-1,865	7,33	3,13	66,37	4,83	4,21	76,3	19,13	21,1	108	1,31
26131	2013	Spring	17	no	-1,858	10,95	3,66	105	1,67	3,74	59,4	15,7	49,85	93,3	1,13
26132	2013	Spring	19	yrl	-1,641	7,92	2,83	106,33	4,02	4,18	46	14,65	351	99,13	1,12
26135	2013	Spring	13	no	-1,13	8,38	2,45	100,4	1,67	4,45	36,15	10,65	45,65	109	0,98
26137	2013	Spring	6	coy	-3,095	9,22	3,82	76,75	8,79	3,62	102,5	21,7	97,35	141,5	1,54
26141	2013	Spring	10	no	-0,889	7,02	2,56	89,25	1,67	3,91	39,2	8,59	25,7	115,5	1,63
26143	2013	Spring	8	no	-0,672	6,42	3,3	68,45	1,67	4,1	59,8	15,05	58,5	88,1	0,85
26147	2013	Spring	18	coy	-1,023	7,49	3,3	95,4	5,28	3,93	52,2	13,2	118,5	47,8	1,11
26153	2013	Spring	14	coy	-1,722	11,65	4,38	96,45	10,6	3,46	69,5	22,35	142,33	85,25	3,59
26157	2013	Spring	9	coy	-2,296	8,69	2,55	69,95	7,55	3,88	70,2	10,75	31,05	114,5	1,01
26160	2013	Spring	19	coy	-3,075	7,9	4,17	67,1	4,09	3,24	49,9	11,9	158,5	93,5	1,4
26163	2013	Spring	21	yrl	-1,63	7,56	3,19	72	3,71	4,31	58,45	11,05	72,1	115	1,5

ID	Year	Season	Age	Status	BCI	CHOL	HDLP	CREA	UREA	K	ASAT	ALAT	GGT	CK	TG
26165	2013	Spring	12	no	-2,065	8,01	2,69	91,2	3,96	4,12	69,67	22,7	56,87	95,87	1,64
26168	2013	Spring	12	coy	-1,707	6,71	3,83	85,5	7,09	3,62	60,9	21,3	45,65	122,5	1,23
26172	2013	Spring	10	no	-0,639	10,8	4,59	71,5	1,67	3,75	36,55	8,85	60,2	111,5	1,74

B Reaction principle of the Reflotron® analysis

Table B1: Reaction principles for Reflotron® analysis

Parameter	Reaction principle
Cholesterol	$cholesterol\ esters + H_2O \xrightarrow{cholesterol\ etserase} cholesterol + RCOOH$ $cholesterol + O_2 \xrightarrow{cholesterol\ esterase} cholestenone + H_2O_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} dye + H_2O$
High-density lipoprotein	Dissolution of cyclomichrons, VLDL and LDL by dextran sulfate/Mg^{2+}, then same test principle as for cholesterol
Creatinine	$creatinine + H_2O \xrightarrow{creatinine\ iminohydrolase} N-methylhydantoin + NH_3$ $N-methylhydantoin + 2H_2O + ATP \xrightarrow{N-methylhydantoinase} N-carbamoyl\ sarcosine + ADP + P_i$ $N-carbamoyl\ sarcosine + H_2O \xrightarrow{N-carbamoyl\ sarcosine\ hydrolase} sarcosine + H_2O + NH_3$ $sarcosine + H_2O + O_2 \xrightarrow{sarcosine\ oxidase} glycine\ HCHO + H_2O_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} dye + H_2O$
Urea	$(N H_2)_2CO + H_2O \xrightarrow{urease} 2NH_3 + CO_2$ $NH_3 + indicator \longrightarrow NH_4^+ + dye$
Potassium	$K^+ + valinomycin + indicator - H \rightleftharpoons [valinomycin - K]^+ [indicator]^- + H^+$ $K^+ + valinomycin + A - H \rightleftharpoons [valinomycin - K]^+ [A]^- + H^+$
Aspartate aminotransferase	$\alpha - ketoglutarate + alaninesulfinate \xrightarrow{yieaspartate\ aminotransferaselds} glutamate + pyruvate + SO_3^{2-}$ $pyruvate + PO_4^{3-} + O_2 + H_2O \xrightarrow{pyruvate\ oxidase} acetylphosphate + H_2O_2 + CO_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} indicator + H_2O$
Alanine aminotransferase	$\alpha - ketoglutarate + alanine \xrightarrow{alanine\ aminotransferase} glutamate + pyruvate$ $pyruvate + PO_4^{3-} + O_2 + H_2O \xrightarrow{pyruvate\ oxidase} acetylphosphate + H_2O_2 + CO_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} indicator + H_2O$

γ-glutamyltranferase	$\gamma - glutamyl - 3 - carboxy - 1{,}4 - phenylenediamine + glycyleglycine \xrightarrow{\gamma - glutamyltransferase} 3 - carboxy - 1{,}4 - phenylenediamine + \gamma - glutamyglycyleglycine$ $3 - carboxy - 1{,}4 - phenylenediamine + 2 - methylantranilic\ acid + 6[Fe(CN)_6]^{3-} \longrightarrow dye + 6[Fe(CN)_6]^{4-}$
Creatine kinase	$creatinephosphate + ADP \xrightarrow{creatinine\ kinase} creatine + ATP$ $glycerol + ATP \xrightarrow{glycerol\ kinase} glycerol - 3 - P + ADP$ $glycerol - 3 - P + O_2 \xrightarrow{glycerol\ peroxidase} duhydroxyacetonephosphate + H_2O_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} indicator + H_2O$
Triglycerides	$triglycerides + 3H_2O \xrightarrow{esterase} glycerol + 3\ RCOOH$ $glycerol + ATP \xrightarrow{glycerol\ kinase} glycerol - 3 - P + ADP$ $glycerol - 3 - P + O_2 \xrightarrow{glycerol\ peroxidase} duhydroxyacetonephosphate + H_2O_2$ $H_2O_2 + indicator \xrightarrow{peroxidase} indicator + H_2O$