NTNU – Trondheim
Norwegian University of
Science and Technology

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

Ingunn Ormbostad

Teacher Education with Master of Science

Supervisor: Bjørn Munro Jenssen, IBI
Co-supervisor: Tomasz Maciej Ciesielski, IBI

Norwegian University of Science and Technology
Department of Biology

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Ingunn Ormbostad

Front page: Polar bears (*Ursus maritimus*).
Photo: Tomasz Maciej Ciesielski.

Sammendrag

I denne studien ble det undersøkt om det er en sammenheng mellom klinisk-kjemiske parameter og persistente organiske miljøgifter (POPer) i plasmaprøver fra isbjørn (*Ursus maritimus*) fanget på Svalbard i 2007. De klinisk-kjemiske parameterne undersøkt var hematokrit (HCT), hemoglobin (HB), aspartataminotransferase (ASAT), alaninaminotransferase (ALAT), γ-glutamyltransferase (GGT), kreatin kinase (CK), triglyserid (TG), kolesterol (CHOL), high-density lipoprotein (HDLP), kreatinin (CREA), urea og kalium (K). Endring i homeostasen av klinisk-kjemiske parameter i plasma kan være en indikasjon på påvirkning på lever, hjerte, muskler, skjelett, metabolisme eller endokrinsystemet. Av de tolv klinisk-kjemiske parameterne undersøkt ble det funnet signifikante korrelasjoner til POPer i sju parameter i polarbjørn hunner, og åtte parameter hos hanner. Resultatene indikerer at POPer kan utøve toksisk effekt på ulike organer i isbjørn. Indikasjoner på levertoksisitet var senket nivå av hematologiske parameter (HCT og HB), senket nivå av leverenzym (ASAT og GGT) og økt nivå av metabolitter (TG, CHOL og HDLP) i forhold til konsentrasjonen av POPer. Indikasjon på nyretoksisitet var senket nivå av CREA i forhold til konsentrasjonen av POPer, og muskeltoksisitet av senket nivå av CK i forhold til konsentrasjonen av POPer. Pågående eksponering av POPer kan derfor føre til senket funksjon av lever, nyre og muskler, og det er mulig at dette kan senke isbjørnens tilpasning og overlevelse. Resultatene indikerer også at klinisk-kjemiske parameter i plasma kan anvendes som en ikke-invasiv biomarkør for POP-toksisitet på organer og metabolsk homeostase i isbjørn. Disse biomarkørene vil påvirkes hurtig av miljøfaktorer, inkludert POPer, og kan derfor måle effekter på et tidlig stadium og ved lave eksponeringskonsentrasjoner. Ut ifra denne studien kan vi ikke si noe om forholdet mellom årsak og virkning, og ved tolkning av resultatene er det viktig å ta i betraktning at andre faktorer kan påvirke klinisk-kjemiske parameter.

Abstract

In the present study, clinical-chemical parameters in relationship to persistent organic pollutants (POPs) were investigated in plasma samples from polar bears (*Ursus maritimus*) captured at Svalbard in 2007. The clinical-chemical parameters examined were: hematocrit (HCT), hemoglobin (HB), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT), creatine kinase (CK), triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDLP), creatinine (CREA), urea, and potassium (K). Altered homeostasis of clinical-chemical parameters in plasma may indicate impact on liver, kidney, heart, muscle, bone, metabolism or the endocrine system. Of the twelve clinical-chemical parameters examined in this study, significant association to POPs were found in seven parameters in female polar bears, whereas eight parameters were found in male polar bears. The results indicate that different POPs may exhibit toxic effect to different organs of polar bears. Liver toxicity was indicated by a decrease of hematologic parameters (HCT and HB), a decrease of liver enzymes (ASAT and GGT), and an elevation of metabolites (TG, CHOL, and HDLP) in relation to contaminant concentrations. Further, kidney toxicity was indicated by a decrease of CREA concentrations in relation to contaminant-concentrations, and muscle toxicity by a decrease in CK concentrations in relation to contaminant-concentrations. Continuous exposure to contaminants may therefore result in decreased renal, hepatic, and muscular functions. It is possible that these POP-associated effects may reduce the fitness and survival of polar bears. The results also indicate that clinical-chemical parameters in plasma can be applied as a non-invasive biomarker for toxicity to organs and metabolic homeostasis caused by exposure to POPs in polar bear. Because of the fast response to environmental factors, including POPs, these biomarkers can be used to measure an effect at an early stage, as well as at low exposure concentrations. However, the present study was not designed to evaluate the relationship between cause and effect, and it is important to take into consideration other factors that can affect clinical-chemical parameters when interpreting the results.

Abbreviations

AhR	Aryl hydrocarbon receptor
ALAT	Alanine aminotransferase
AMAP	Arctic Monitoring Assessment Programme
ASAT	Aspartate aminotransferase
ATP	Adenosine triphosphate
BFR	Brominated flame retardants
CYP P450	Cytochrome P450
CHL	Chlordane
CHOL	Cholesterol
CK	Creatine kinase
CREA	Creatinine
CV-ANOVA	Cross-validated analysis of variance
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
GC	Gas chromatography
GGT	γ-glutamyltransferase
HB	Hemoglobin
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexanes
HDLP	High-density lipoprotein
LOD	Limit of detection
LRAT	Long range atmospheric transport
NARA	National Animal Research Authority
NTNU	Norwegian University of Science and Technology
OC	Organochlorine
OH-PCB	Hydroxylated polychlorinated biphenyl
O-PLS	Orthogonal partial least square
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PBDE	Polybrominated diphenyl ether
PL %	Plasma lipid percentage
POP	Persistent organic pollution
Q^2	Predicted variance
R^2X	Explained variance
R^2Y	Goodness of fit
rpm	Revolutions per minutes
TG	Triglyceride
TH	Thyroid hormone
TTR	Transthyretin
TBBPA	Tetrabromobisphenol A
VIP	Variable importance in the projection

Table of content

1. Introduction

1.1. Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are a group of organic compounds of natural or anthropogenic origin. Natural sources of POPs can be volcanic activity and vegetation fires, whereas anthropogenic sources include pesticides, industrial chemicals, and by-product from combustion or chemical processes. Examples of POPs include polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE), hexachlorobenzene (HCB), chlordanes, and DDT (El-Shahawi et al., 2010). Despite the fact that POPs are a structurally diverse group, they share some properties that make them a threat to the environment. Most of them have low water solubility, they are highly lipophilic, and they are resistant to physical, chemical and biochemical degradation (AMAP, 2004; Borgå et al., 2004). Due to their low water solubility they have the capacity to travel long distances in the atmosphere, and can reach remote areas such as the Arctic. Moreover, because of their resistance to degradation, they are stable in the environment, and remain available for uptake by organisms (El-Shahawi et al., 2010). POPs can bioaccumulate in organisms and biomagnify in the food chain. As a consequence, they can reach high concentration in top predators, such as polar bears (Nourizadeh-Lillabadi et al., 2009). Their persistency, and capacity for long-range transport and bioaccumulation, as well as their toxic effects have raised high concern on their environmental impact, and have led to restrictions or even complete ban on the use of these chemicals in many countries (Godduhn and Duffy, 2003; El-Shahawi et al., 2010; Letcher et al., 2010).

1.1.1. Persistent organic pollutants in the Arctic

The Arctic boundary is not clearly defined, but it extends across the northern part of America, Europe and Asia. Eight countries, Canada, Denmark (Greenland and Faroe Islands), Finland, Iceland, Norway, Russia, Sweden, and the United States, are both bordering to and have territories within it (Godduhn and Duffy, 2003; AMAP, 2004).

Despite the fact that only few known sources of POPs are known in the Arctic, considerable levels have been detected in both biotic and abiotic Arctic environments (AMAP, 1998). Transport of POPs into the Arctic occurs via various routes, and the cold Arctic environment

acts as a sink for POPs produced and used elsewhere in the world (Brunström and Halldin, 2000; Haave et al., 2003). Since POPs are semi-volatile and persistent they are able to move long distances in the atmosphere, and according to the Arctic Monitoring Assessment Programme (AMAP), long-range atmospheric transport (LRAT) is the main source of these pollutants to the Arctic region (Halsall, 2004; El-Shahawi et al., 2010). Other sources include drainage from northerly flowing rivers, ocean currents, continental runoff, and drifting ice (Bang et al., 2001). Once in the Arctic environment, the contaminants will persist there for a long time due to the reduced degradation rate at low temperatures (Halsall, 2004). Furthermore, the Arctic ecosystem is characterized by a low annual productivity, few species and short food chains, additionally limiting the opportunities for biodegradation (Andersen et al., 2001).

Both the biological and physical characteristics in Arctic ecosystems differ from most other ecosystems, and there are concerns that these special features make these ecosystems particularly vulnerable to contaminants (Barrie et al., 1992). Because of the cold and fluctuating climate, Arctic animals mostly use lipids as an energy source. Hence, large amounts of lipids are transported in Arctic food webs (Brunström and Halldin, 2000). With most POPs being lipophilic, they will partition into lipids, and species at the highest levels of Arctic food webs may accumulate large amount of POPs through their diet (Routti et al., 2010). In mammals, POPs may be transferred from mothers to their offspring *in utero* and through lactation (Borgå et al., 2004). Low temperatures, limited nutrient availability, and pronounced seasonality with short growing seasons also affect the fate of POPs in these ecosystems (Sobek et al., 2010). Most Arctic animals have adapted to these conditions by going through a seasonal period of fasting, with a preceding period of feeding in which they must obtain sufficient fat reserves. During the fasting period, lipid stores are metabolised. This often leads to mobilization of lipophilic contaminants stored in fat depot, and the blood levels of contaminants will increase. Contaminants in the blood can be distributed throughout the body, and increase the animal's susceptibility for adverse effect (Cherry et al., 2009; Routti et al., 2010).

1.1.2. Polychlorinated biphenyls (PCBs) and their metabolites

In 1966, PCBs were for first time discovered as environmental pollutants (Jensen, 1966). Since then, they can be found throughout the world in water, sediments, air, and animals

(Manahan, 2010). They are of anthropogenic origin, and have been utilised due to their physical properties. The high chemical, thermal, and biological stability, as well as low volatility and high dielectric constant made them suitable for many industrial applications. They have been used in dielectric fluids, heat transformer fluids, lubricants, vacuum pump fluids and as plasticisers (Walker et al., 2006). These properties have also contributed to the worldwide distribution of PCBs. The fact that they are persistent, bioaccumulative, toxic, and have the capacity for long-range transport, has led to banning or strict restrictions in most countries. However, high levels are still detected in the Arctic (Muir and de Wit, 2010).

There are 209 different PCB congeners, featuring different chlorine substitutions on the biphenyl ring (Figure 1). The physiochemical properties of the congeners are dependent on the degree of chlorination (Borja et al., 2005). Generally, as the degree of chlorination increase, the water solubility and vapour pressure decrease and the lipophilicity increase. The toxicity of PCBs is dependent on both the degree of chlorination and the position of the chlorine atoms (Ritter et al., 1995). Polychlorinated Biphenyls without substitution of chlorine atoms in the ortho position are referred to as coplanar, and tend to remain in the same plane. Compounds with a substitution present in the ortho position are referred to as non-coplanar. These PCBs have a more globular structure, and will undergo rotations around the two benzene rings. Coplanar PCBs are regarded as the most toxic, because they can bind to the aryl hydrocarbon receptor (AhR). 2,3,7,8-tetrachlorodibenzo-p-dioxin is one of the most toxic compounds known, and is the compound with the strongest binding affinity for AhR. Because of the structural similarity of coplanar PCBs to this and other dioxins, and the fact that they produce toxic effects similar to dioxins, they are referred to as dioxin-like compounds (Walker et al., 2006). In addition to dioxin-like effects, both the planar and coplanar PCBs may exert AhR independent effects (Ritter et al., 1995). In polar bears, the hormone and vitamin concentrations, organ morphology, as well as reproductive and immune systems are likely to be influenced by PCB exposure (Haave et al., 2003; Oskam et al., 2003; Braathen et al., 2004; Lie et al., 2004; Lie et al., 2005; Letcher et al., 2010; Sonne, 2010).

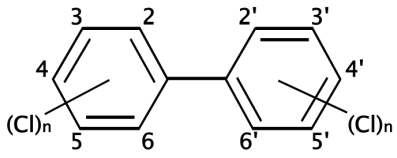

Figure 1. The general structure of PCB.

Hydroxylated PCB metabolites (OH-PCBs) are formed by the oxidative metabolism of PCBs by cytochrome P450 (CYP P450) monooxygenase enzyme systems. These enzymes can

catalyse the formation of OH-PCBs, either by directly inserting an OH-group in the para- or mono-position, or via an arene oxide intermediate. This intermediate may be further metabolized to OH-PCBs by the enzyme epoxide hydroxylase (Sandala et al., 2004; Routti et al., 2008). These metabolites may remain in the blood as a result of competitive binding for circulating transport proteins such as the thyroid hormone (TH) transport protein transthyretin (TTR) (Gebbink et al., 2008a). In mammals, THs are important for physiological functions, such as normal brain development, growth, metabolism and reproduction. Hence, concern has been raised that OH-PCB binding may cause alterations in the levels or function of THs, and in this way disturb physiological and developmental functions (Gabrielsen et al., 2011). *In vitro* studies with OH-PCB have shown endocrine disruption for both thyroid hormones and sex hormones (estrogens) (Moore et al., 1997; Brouwer et al., 1998; Schuur et al., 1998; Kester et al., 2000).

1.1.3. Polybrominated diphenyl ethers (PBDEs)

Polybrominated diphenyl ethers are among the most frequently used brominated flame retardants (BFRs). Because PBDEs do not react with the material, but are mixed directly into the product, they may leak out of the product and be released into the environment (de Wit et al., 2010). Studies have shown that several BFRs have reached the Arctic, with the dominant transport routes assumed to be similar as for PCBs, LRAT, ocean currents, river input, and sea-ice drift (Sørmo et al., 2006). Similar to PCBs, there are 209 structurally different PBDE congeners. In PBDEs, an ether oxygen atom joins the two benzene rings, and bromine atoms can occupy different positions on the rings (Figure 2) (Baird and Cann, 2008). The rate of metabolism depends strongly on the degree of bromination (Sterner, 2010). Due to their environmental persistency and lipophilicity, they may biomagnify in food chains. The fact that BFRs have structural, chemical and physical similarities to PCBs, there are currently concerns that PBDEs might have similar ecotoxicological potential as these compounds (Sørmo et al., 2006). Laboratory studies on rodents suggest that PBDEs have the potential to affect the nervous, thyroid and sex hormone systems and the reproduction (Eriksson et al., 2001; Hallgren and Darnerud, 2002; Birnbaum and Staskal, 2004; Darnerud, 2008).

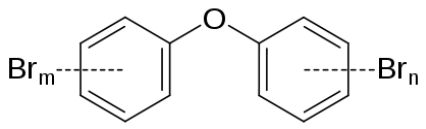

Figure 2. The general structure of PBDE.

1.1.4. Hexachlorobenzene (HCB)

Hexachlorobenzene (Figure 3) was first introduced in 1945 as a fungicide for seed treatment. Today, it is produced as a by-product in the production of many chlorinated compounds. Because of its high lipophilicity, semi-volatility and long half-life in biotic environments HCB has a high potential to bioaccumulate in organisms (AMAP, 1998). Even through its use is banned or highly restricted in most countries, it is still ubiquitous in the environment (Ritter et al., 1995). Exposure of HCB to laboratory animals and humans produces a number of effects, including immunosuppression, hepatic effects, tumor promotion, endocrine disruption, and reproductive impairment (Foster et al., 1993; Schielen et al., 1995; Alvarez et al., 2000; Randi et al., 2006).

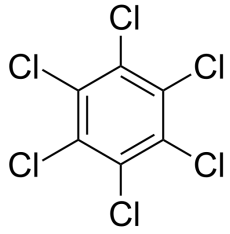

Figure 3. The molecular structure of HCB.

1.1.5. Organochlorine (OC) pesticides

Organochlorine pesticides are a broad group of compounds that share many physico-chemical characteristics. They include pesticides such as chlordane (CHL), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCHs), and mirex (Ritter et al., 1995).

Chlordane (Figure 4) has been used on agricultural crops and in control of termites. As a consequence of its high persistency, semi-volatility, and lipophilicity, chlordane may undergo LRAT, and reach high concentrations in Arctic organisms (Ritter et al., 1995). It is a mixture of at least 120 compounds, including the two most abundant constituents, *cis*- and *trans*-chlordane, and the metabolite oxychlordane (Tryphonas et al., 2003). Chlordane and its metabolites have caused effects such as immunosuppression, endocrine disruption, reproduction effects, hepatic and renal change, and enhanced tumor formation (AMAP, 1998; Bondy et al., 2000; Tryphonas et al., 2003).

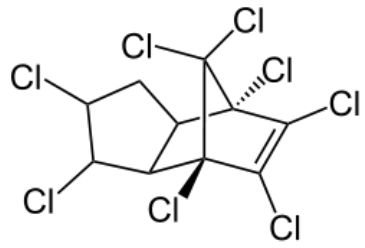

Figure 4. The general structure of chlordane.

During the Second World War, DDT (Figure 5) was widely used to protect the troops and civilians from the spread of vector borne diseases, such as malaria and typhus. After the war,

it was used for agricultural purposes, as well as for control of vector borne diseases. Consequently, its environmental concentration rose rapidly, and concerns on adverse environmental effects led to severe restrictions and bans in the early 1970s. Nevertheless, DDT is still used to control the abundance of mosquito vectors of malaria in numerous countries (Ritter et al., 1995). As with most OC insecticides, DDT and especially its metabolite dichlorodiphenyldichloroethene (DDE) are lipophilic and persistent in the environment. They can therefore bioaccumulate and biomagnify in organisms (Turusov et al., 2002). Dichlorodiphenyltrichloroethane and its metabolites are ubiquitous in the environment, and DDE is almost biologically nondegradable. It is this metabolite that primary are found at higher trophic levels (Baird and Cann, 2008). In laboratory animals, DDT and its metabolites have been found to act as endocrine disruptors, immunosuppressors, and carcinogens, and have been observed to feature reproductive and developmental effects (AMAP, 1998; Turusov et al., 2002; Rogan and Chen, 2005).

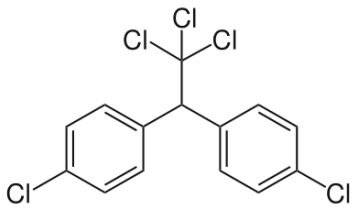

Figure 5. The molecular structure of DDT.

During the Second World War, HCHs (Figure 6) were discovered to be an effective insecticide. Of the eight isomers α-, β-, and γ-HCH are the most abundant (Baird and Cann, 2008). Compared to the other OCs, HCHs feature a lower lipophilicity, and are thus less bioaccumultive. However, HCHs are relatively persistent and volatile, and since 1970 their use are highly restricted or banned (AMAP, 1998). Neurological, renal and hepatic, immunological, reproductive and carcinogen effects have been reported after exposure to HCHs (AMAP, 1998; Willett et al., 1998; Bhatt et al., 2009).

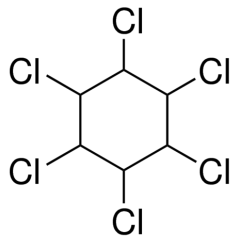

Figure 6. The general structure of HCHs.

Before its ban, mirex (Figure 7) was used as an insectcide and fire retardant. Mirex is considered to be one of the most stable and persistent pesticides. It is lipophilic and has a high potential to bioaccumulate and biomagnify (Puertas et al., 2010). Due to its relatively high volatility, it has the potential to undergo LRAT, and is present in low levels in the Arctic (AMAP, 1998). Studies on the

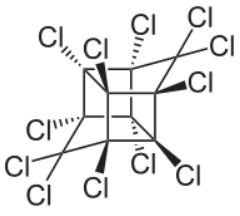

Figure 7. The molecular structure of mirex.

effects of mirex have shown impaired reproduction, enhanced tumor formation, impaired neurodevelopment, and immunosuppression (Moser et al., 1992; AMAP, 1998; Puertas et al., 2010).

1.2. Biotransformation of POPs

Biotransformation processes in the body are conversion of lipophilic chemicals, which are easily absorbed from the gastrointestinal tract and other potential uptake sites, into more hydrophilic chemicals, which can be easily excreted in urine or bile. These processes are catalysed by various enzyme systems in the liver and other tissues. Based on the reaction they catalyse, the enzymes involved can be divided into four categories: hydrolysis, reduction, oxidation, or conjugation. The three first reactions expose or introduce a functional group, which can further be conjugated (Parkinson and Ogilvie, 2008). Factors that influences the biotransformation and hence the toxicokinetics of POPs, include dietary exposure, age, and gender of the respective organism (Routti et al., 2008).

Cytochrome P450 is one of the most important groups of biotransformation enzymes, which generally mediate the initial, oxidative step in the metabolism of halogenated organic compounds, such as PCBs (Helgason et al., 2010). In most cases, biotransformation processes lead to detoxification, which will protect the organisms. However, in some cases biotransformation may lead to bioactivation of compounds. This can make the metabolite more toxic than the parent compound (Walker et al., 2006). Therefore, knowledge about biotransformation processes is crucial for understanding the possible mechanisms behind POP correlated effects (Routti et al., 2008).

1.3. Clinical-chemical parameters in plasma

Analysis of clinical-chemical parameters in plasma can provide valuable information for evaluating the health and physiological status, as well as identify target organs for toxicity in organisms (Castellanos et al., 2010; Firat and Kargin, 2010).

Hematocrit (HCT) is the volume fraction of erythrocytes in whole blood (D´Orazio and Meyerhoff, 2008). Hematocrit measurements provide an evaluation of red blood cell status in an organism, and can indicate anemia, blood loss, or dehydration (Kirk et al., 2010).

Hemoglobin (HB) is the iron-containing pigment of the erythrocytes being responsible for oxygen and CO_2 binding and transport (Nuttal and Klee, 2001).

Aspartate aminotransferase (ASAT) catalyses the reactions of L-aspartate and 2-oxoglutarate to oxaloacetate and L-glutamate, whereas alanine aminotransferase (ALAT) catalyses the reactions of L-alanine and 2-okoglutarate to pyruvate and L-glutamate (Panteghini and Bais, 2008). Both enzymes are widely distributed in animal tissues, and elevated plasma levels are a nonspecific indicator of liver and kidney dysfunction (Franson, 1982; Marshall and Bangert, 2008). Even through these enzymes are good indicators of liver dysfunctions, they are not entirely liver specific. Of these two aminotransferase enzymes, ALAT is the most liver-specific, while ASAT is a useful adjunct to ALAT (Panteghini and Bais, 2008; Evans, 2009).

γ-glutamyltransferase (GGT) catalyses the γ-glutamyl transfers from γ-glutamyl peptides to amino acids, water molecules, and small peptides. In most biological systems, glutathione serves as the γ-glutamyl donor (Krefetz and McMillin, 2005). γ-glutamyltransferase is present in high concentrations in the liver, kidney and pancreas, and elevated plasma levels are a sensitive indicator of hepatobiliary diseases (Marshall and Bangert, 2008).

Creatine kinase (CK) is a dimeric enzyme that catalyses the reversible phosphorylation of creatine by ATP in contractile or transport systems. (Panteghini and Bais, 2008). Creatine kinase is widely distributed in tissues, with the highest activity in skeletal muscles, the heart muscle, and brain tissue (Krefetz and McMillin, 2005). Elevated levels of CK can be related to many diseases, including diseases in skeletal muscle, the heart, the central nervous system, and the thyroid (Panteghini and Bais, 2008).

Triglycerides (TG) and cholesterol (CHOL) are some of the major lipids in the plasma, and the plasma concentrations are a measure of lipid metabolism, transport, and reserves (Van den Steen et al., 2010). Triglycerides are the major lipid in adipose tissue, and its primary function is to provide energy for the cell (Burnett, 2010). Cholesterol has an important role in membrane structures and is the precursor of among others steroid hormones and bile acids (Marshall and Bangert, 2008). These lipids are synthesised in the liver and intestine, and transported in the plasma in macromolecular complexes called lipoproteins (Rifai et al., 2001). Lipoproteins are globular structures with an outer polar coat of proteins, phospholipids, and free cholesterol, and an inner nonpolar coat of triglycerides and

cholesteryl esters (Burnett, 2010). High-density lipoproteins (HDLP) have two important functions, to act as a source of apoproteins for other lipoproteins, and to mediate the reverse cholesterol transport (Marshall and Bangert, 2008).

Creatinine (CREA) is synthesized in the muscle, mainly from the turnover of creatine. Thus, the amount produced every day is a function of total muscle mass, and is relatively constant (Newman and Price, 2001). The kidney secretes CREA, and plasma concentrations are the most reliable indicator of glomerular function (Marshall and Bangert, 2008).

Urea is synthesized in the liver, primarily as a by-product of the deamination of amino acids. It is excreted through the urine, and urea elimination is the major route for nitrogen excretion in mammals. Like creatinine, concentrations of urea in plasma are an indicator of glomerular function (Marshall and Bangert, 2008).

Potassium (K) is the major intracellular cation in the body, with the highest concentrations within the cells. High intracellular K concentrations are essential for many cellular functions, including regulation of neuromuscular excitability, contraction of the heart, intracellular fluid volume, and hydrogen ion concentration (Polancic, 2005). The K homeostasis is highly regulated, and disturbances of this homeostasis have serious consequences (Heusel et al., 2001).

1.4. Polar bear (*Ursus maritimus*)

As the ultimate top predator in Arctic food chains, polar bears are especially at risk of accumulating lipophilic compounds. Currently, East Greenland and Svalbard polar bear are reported to be among the most contaminated animals (Sonne, 2010). Arctic animals, including polar bears, use lipids as energy source, and have a high lipid content that makes them particularly prone to accumulate lipophilic compounds (Brunström and Halldin, 2000). Polar bear feed primarily on ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), consuming large amounts of lipid rich blubber that potentially contain high levels of POPs (Tryland et al., 2002).

Due to an effective CYP P450 system, polar bears can metabolise many of the recalcitrant aromatic compounds, including DDT metabolites and most of the PCB congeners (Bard,

1999). However, due to their large consumption of seal blubber, polar bears can accumulate very high concentrations of the most persistent contaminants (Braathen et al., 2004). Female polar bears usually give birth to two cubs, and when she leaves the nest she has fasted about six month and nursed for 3-4 month. The polar bear milk has a high fat content, and lactating may continue until the cubs reach 2.5 year (Bernhoft et al., 1997; Brunström and Halldin, 2000). During late spring and early summer, polar bears acquire most of the energy reserves that they need for growth and maintenance during the rest of the year. During this period the availability to young and moulting seals is high. The rest of the year they feed little and undergo lengthy fasts (Norstrom and Muir, 1994). This seasonal change in fat storage can lead to release of lipophilic contaminants, and increase the animal's susceptibility for adverse effect (Routti et al., 2010).

1.5. Objectives

By examining clinical-chemical parameters in plasma, previous studies on birds and mammals have reported that different POPs may affect organs and metabolic homeostasis. Measurements of clinical-chemical parameters in plasma may be a non-invasive method to examine such effects in polar bears. To our knowledge there have not been reported any studies on the relationship between POPs and blood clinical-chemical parameters in polar bears. The aim of this study is to examine whether POPs could influence organs and metabolic homeostasis. The study has done so by examining clinical-chemical parameters in relation to POP concentrations in plasma from polar bears. Further, it was examined which POPs, or combinations of POPs that were most relevant for explaining the variations in the clinical-chemical parameters.

2. Method

2.1. Field sampling

Field sampling procedures is described in details in Bytingsvik et al. (2012). Briefly, blood samples were collected from 38 polar bears (20 females and 18 males) captured at Spitsbergen and Edgeøya, Svalbard, Norway (76.7 – 79.8 °N, 11.8 – 21.3 °E, Figure 8) in March/April 2007. Capture day, age and a selection of biometric data were collected. Capture and handling procedures followed standard protocols (Stirling et al., 1989; Derocher and Wiig, 2002), and were approved by the National Animal Research Authority (NARA), Norway. Blood was collected from the femoral vein. Within 8 h after sampling the samples were separated into plasma and blood by centrifugation (3500 rpm, 10 min). Plasma samples were stored at − 20 °C in the field and then at − 70 °C in the lab freezer until analysis.

Figure 8. Sampling site of polar bears (n = 38) captured at Spitsbergen and Edgeøya, Svalbard in 2007. (Norwegian Polar Institute, 2008).

2.2. Clinical-chemistry analysis

The clinical-chemical analyses were performed on fresh material in the field in 2007. The plasma samples were analysed within 12 hours after sampling on a "dry" clinical-chemistry analyzers with test-strip devices (Reflotron®, Boehringer-Mannheim, Mannheim, Germany). Prior to the analysis the samples were kept at ca. 5 °C. The clinical-chemical parameters subjected for analysis were two hematologic parameters (HCT, HB), four enzymes (ASAT, ALAT, GGT and CK), five metabolites (TG, CHOL, HDLP, CREA, and UREA), and one mineral (K). Two or three parallels were analysed for each animal and for each parameter.

2.2.1. Principles of the "dry" clinical-chemistry analyzers (Reflotron®)

The "dry" clinical-chemistry analyzer (Reflotron®) is an analytical instrument for quantitative determination of clinical-chemical parameters from whole blood, serum, plasma or urine. The basic components of the instrument include the reagent carriers and the microprocessor controlled reflectance photometer. The reagent carriers are different for the individual parameters, and include the reagents required for the reactions. With the aid of the optical system, the dye that is formed in the reaction is measured and evaluated by the reflectance photometer (Øvrebust, 1991). The reaction principles for detection of clinical-chemical parameters with the "dry" clinical-chemistry analyser are shown in appendix I.

2.3. Analysis of POPs

The POP analyses were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science, Oslo, Norway. The methods used in the present study were originally described in Brevik (1978) for PCB analysis. The method for analysis of OC pesticides is described in detail in Bernhoft et al. (1997), analysis of PBDEs and HBCDs in Murvoll et al. (2006), and OH-PCBs and OH-BDE in Berg et al. (2010) and Løken (2006). Prior to detection and quantification of POPs, the samples were extracted, plasma lipid percentages (PL %) were determined and the samples were cleaned. Gas chromatography (GC) was applied for detection and quantifications. The following compounds were subjected for analysis in the plasma samples: PCB-28, -47, -52, -66, -74, -99, -101, -105, -114, -118, -123, -128, -137, -138, -141, -149, -151, -153, -156, -157, -167, -170, -180, -183, -187, -189, -194, -206, 4'-OH-CB106, 4-OH-CB107, 4'-OH-CB108, 3-OH-CB118, 4'-OH-CB130, 3´-OH-CB138, 4-OH-CB146, 4´-OH-CB159, 4'-OH-CB172, 3'-OH-

CB180, 4-OH-CB187, BDE-28, -47, -99, -100, -153, -154, -183, -209, 4-OH-BDE42, 3-OH-BDE47, 6-OH-BDE47, 4'-OH-BDE49, 2'-OH-BDE68, HBCD, TBBPA, HCB, oxychlordane, trans-chlordane, trans-nonachlor, cis-nonachlor, *o.p´*-DDT *p.p´*-DDT, *p.p´*-DDE, *o.p´*-DDD, *p.p´*-DDD, α-HCH, β-HCH, γ-HCH, and mirex.

2.3.1. Principles of gas chromatography

Chromatography is a separation technique where the separation of compounds is based upon the distribution of analytes between two phases in a dynamic system. In gas chromatography the two phases include a gaseous mobile phase and a liquid or solid stationary phase (Fowlis, 1995). The technique is used for analysis of both organic and inorganic volatile compounds. It is used for analysis of gases, liquids, and solids, with the latter usually dissolved in volatile solvents. The basic components of the instrument include a carrier gas (mobile phase) source, injector, column (stationary phase) and a detector (McNair and Miller, 2009). The sample is volatilised in the heated injector, transferred through the column, and detected by the detector (Kitson et al., 1996). The analytes separate from one another based on their relative vapour pressure and affinity for the stationary phase (McNair and Miller, 2009). The greater the affinity of the analytes for the stationary phase, the more the analytes will be retained by the column and the longer it will be before it is eluted and detected (Fowlis, 1995).

2.3.2. Extraction and quantification of POPs

The methods are described in detail in several studies (Brevik, 1978; Bernhoft et al., 1997; Murvoll et al., 2006; Løken, 2006; Berg et al., 2010). Briefly, an internal standard (IS) mixture was added to about 3 g of plasma sample. Homogenization and extraction were performed twice with acetone:cyclohexane (2:3), and PL % were determined gravimetrically using the whole extract. Subsequently, the samples were cleaned with H_2SO_4 (96 %). After reducing the samples to the final volume, the extracts were ready for GC-analyses. The extracts were automatically injected on an HRGC 53 Mega Series gas chromatograph (Carlo Erba Instrumentation, Milan, Italy), or Agilent 6890 Series high-resolution gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). According to requirements of NS-EN ISO/IEC 17025 (TEST 137), the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science is accredited for determination of several POPs in biological matrices. The method is not accredited for determination of OH-metabolites, but it is validated the same way. To ensure adequate quality assurance and control, standard

validation procedures were used for all the samples and for the quantification of all the POPs. The limit of detection (LOD) was determined threefold to the signal to noise level. To avoid missing values in the statistical analysis, the samples with concentration below the detection limit were replaced by random values between zero and the detection limit for the given compound. Compounds that were detected in less than 60 % of the plasma sample were excluded from the data analysis.

2.4. Statistical analysis

The POPs that were detected in the plasma sample of more than 60 % of the individuals, and thus statistically treated were: PCB-47, -74, -99, -101, -105, -114, -118, -128, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -206, 4-OH-CB107, 3´-OH-CB138, 4-OH-CB146, 4´-OH-CB159, 4-OH-CB187, BDE-47, HCB, oxychlordane, trans-nonachlor, *p.p´*-DDT, *p.p´*-DDE, α-HCH, β-HCH, and mirex. Previous studies have shown that levels and toxicokinetics of POPs in polar bears may be affected by capture location (latitude and longitude), age, weight, condition (body condition index [BCI]), and lipid content (Andersen et al., 2001; Borgå et al., 2004; Bernhoft et al., 1997; Lie et al., 2003; Dietz et al., 2004; Verreault et al., 2005b). Therefore, these variables were included in the statistical analysis. Multivariate data analysis (principal component analysis [PCA] and orthogonal partial least squares [O-PLS] regression) was performed using the software Simca-P+ version 12 (Umetrics, Umeå, Sweden). By using multivariate methods, one can investigate the relations between all variables in a single context. Correlation tests were performed using the software STATISTICA version 10 (Statsoft Inc., Tulsa, OK, USA).

2.4.1. Principal component analysis (PCA)

Principal component analysis was used to visualise how variables and individual were grouped and correlated. Principal component analysis can be used to reduce the number of observed variables to a smaller number of orthogonal principal components (PC) that account for most of the variance of the observed variables. The direction in variable space occupied by most data points will define the location of the first PC, and the second PC will coincide with the largest variation orthogonal to the first component. New PCs can be extracted in this way until only minor variation is left unexplained by the model. The explained variation R^2X, tells how much of the variance in the dataset is explained in the model, and how well we are able to mathematically reproduce the data. The predictive ability of a model is explained by the

goodness of prediction, Q^2 (= the predicted variation), and is estimated by using cross-validation. Q^2 indicates the fraction of the total variation of the X´s that can be predicted by a component. The results from the PCA models are presented in score and loading plots. The score plot shows the relationships among the observations (individuals), while the loading plot explains which variables that are influential and how the variables are correlated. Observations and variables close to each other have similar properties, while those that are farther apart carry dissimilar information. Non-influential variables will cluster close to the centre of the co-ordinate system (Eriksson et al., 2006).

In the first PCA analysis, females and males were pooled together. This gave a score plot where females and males were separated (Appendix II). Due to this separation, the statistical analyses were applied for females and males separately. This was done to reveal the effects of contaminants on clinical-chemical parameters, regardless of variations caused by gender.

2.4.2. Orthogonal partial least squares (O-PLS) regression

Orthogonal partial least squares regression was applied to find linear relationships between the Y-matrix (response variables) and the X-matrix (predictor variables). This method is a modification of partial least square (PLS) regression, and was designed to remove systematic variations uncorrelated with the response. O-PLS divides the systematic variation in the X-matrix into two parts: one part that models X related to Y, and one that models X orthogonal to Y (Eriksson et al., 2008). Because organisms come in contact with a large number of different chemicals, and the compounds may interact with each other, it is difficult to establish a connection between individual chemicals and toxic response (Eaton and Gilbert, 2008; Letcher et al., 2010). O-PLS regression can cope with this problem by analyse data with strongly collinear (correlated), noisy, and numerous predictor variables, and also simultaneously model several response variables (Wold et al., 2001). Similar to PCA, values for R^2X and Q^2 are given for the O-PLS model. Here, R^2X reveals how well the predictors are explained by the model. In addition to these, R^2Y (goodness of fit) is also given, and explain how well the response variable is described as a function of the predictor variables (Eriksson et al., 2006).

An O-PLS analysis was performed for each of the clinical-chemical parameters. To approximate normal distribution, the variables were first logarithmically transformed. One

clinical-chemical parameter at the time was set as the response variables, and POPs and biometric variables as the predictor variables. Cross-validated analysis of variance (CV-ANOVA) analysis was applied for significance testing. Statistical significant O-PLS models ($p < 0.05$) indicate that response variables (clinical-chemical parameters) are associated with predictor variables (POPs).

The influence of every predictor variable on the response variable can be computed using variable importance in the projection (VIP). One can compare the VIP values of one predictor variable to the others, and terms with VIP > 1 are the most relevant for explaining the response variables (Eriksson et al., 2006). The predictor variables that were unimportant for explaining the response variables (VIP < 1) were removed from the models. To obtain significant models, predictor variables least important for explaining the response variable were stepwise removed. Despite the significance of these models after stepwise removal of variables, these models were relatively weak in terms of explaining variations of the response variable compared to models without alterations. To assess the relationships (positive or negative) between the response variables and the predictor variables, the variables were plotted in coefficient plots. All models are depictured with jack-knife confidence intervals which indicate the importance of the predictor variables in the models.

2.4.3. Correlation tests

For further analysis of the correlations found in the O-PLS model, correlation tests were performed. Shapiro-Wilk test was used to test if the data were normally distributed. Data that were not normally distributed were $\log_{10}$-transformed before analysis. Depending on whether the variables were normally distributed or not, Pearson product-moment correlation (Pearson correlation) or Spearman´s rank correlation were applied to examine the correlations between the clinical-chemical parameter, contaminants, and the biometric variables. Pearson correlation was applied on the normally distributed variables (with or without $\log_{10}$-transformation), while Spearman´s rank correlation were applied to the variables not normally distributed. The level of significance was set to $p < 0.05$ for all tests.

3. Results

3.1. Biometric variables

Biometrics variables from female (n = 20) and male (n = 18) polar bears are presented in table 1. Individual biometric variables are presented in appendix III.

Table 1. Mean ± standard deviation, median and range (min-max) of capture day, location as latitude and longitude, age, straight length, axillary girth, head length, zygomatic width, weight, total body mass (TBM), body condition index (BCI), and lipid content of polar bear females (n = 20) and males (n =18) captured in Svalbard, Norway, 2007.

	Female (n = 20)			**Male (n = 18)**		
	Mean ± SD	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
Capture day (1 - 365)	90 ± 4	99	85 - 99	90 ± 4	89	85 - 100
Latitude	78.1 ± 1.11	79.7	76.7 - 79.7	78.3 ± 1.09	78.3	76.9 - 79.8
Longitude	16.2 ± 2.52	21.1	12.0 - 21.1	16.7 ± 2.96	16.6	11.8 - 21.3
Age	10 ± 6	24	3 - 24	11 ± 4	12	4 - 17
Straight length (cm)	195 ± 5.97	206	183 - 206	226 ± 12.4	226	203 - 252
Axillary girth (cm)	112 ± 8.92	129	99.0 - 129	152 ± 17.5	157	106 - 174
Head length (mm)	336 ± 13.8	364	313 - 364	393 ± 19.5	396	350 - 424
Zygomatic width (mm)	196 ± 10.2	211	173 - 211	248 ± 25.6	257	195 - 278
Weight (kg)	165 ± 30.7	223	123 - 223	378 ± 85.2	409	171 - 476
TBM (kg)[a]	178 ± 29.1	225	131 - 225	378 ± 85.2	409	171 - 476
BCI[b]	155 ± 12.4	173	133 - 173	209 ± 20.4	218	153 - 229
Lipid (%)	1.54 ± 0.327	2.07	0.825 - 2.07	1.02 ± 0.338	0.990	0.619 - 1.98

[a] Total body mass (TBM) was estimated from the following equation:
$TBM = 0.00003377 \cdot \text{axillary girth}^{1.7515} \cdot \text{body length}^{1.3678}$ (Derocher and Wiig, 2002)
[b] Body condition index (BCI) was estimated from the following equation:
$BCI = (\ln TBM - 3.07 \cdot \ln \text{straight length} + 10.76) \div (0.17 + 0.009 \cdot \ln \text{straight length})$ (Cattet et al., 2002)

3.2. Clinical-chemical parameters

Twelve clinical-chemical parameters were measured in plasma from female (n = 20) and male (n = 18) polar bears from Svalbard. These parameters and concentrations are presented in table 2. Individual concentrations of clinical-chemical parameters are presented in appendix IV.

Table 2. Mean ± standard deviation (SD), median, and range (min-max) of plasma concentrations of hematocrit (HCT), hemoglobin (HB), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT), creatine kinase (CK), triglycerides (TG), cholesterol (CHOL), high-density lipoproteins (HDLP), creatinine (CREA), urea, and potassium (K) in plasma samples of polar bear females (n = 20) and males (n = 18) sampled in Svalbard, Norway, 2007.

	Female (n =20)			Male (n =18)		
	Mean ± SD	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
HCT (%)	43.1 ± 4.26	43,0	36.0 - 52.0	46.9 ± 5.24	47.3	34.0 - 56.0
HB (mmol/L)	8.59 ± 1.10	8.24	6.97 - 10.5	9.09 ± 1.26	8.82	6.37 - 11.4
ASAT (U/L)	54.8 ± 15.7	53.3	34.2 - 101	93.5 ± 31.7	83.3	53.4 - 163
ALAT (U/L)	19.8 ± 9.31	17.3	10.3 - 44.0	42.8 ± 17.3	37.1	16.2 - 88.4
GGT (U/L)	55.0 ± 53.5	35.2	17.5 - 254	134 ± 128	76.1	13.1 - 509
CK (U/L)	126 ± 80.9	89.3	39.9 - 255	129 ± 70.0	114	62.0 - 377
TG (mmol/L)	1.09 ± 0.534	1.18	0.0300 - 2.22	0.973 ± 0.390	0.940	0.0700 - 1.64
CHOL (mmol/L)	8.58 ± 1.31	8.89	5.96 - 10.8	6.61 ± 2.06	6.50	3.90 - 13.1
HDLP (U/L)	1.05 ± 0.193	1.10	0.620 - 1.40	0.963 ± 0.210	1.01	0.550 - 1.30
CREA (µmol/L)	105 ± 15.6	103	82.1 - 136	128 ± 27.0	132	86.8 - 183
UREA (mmol/L)	4.71 ± 2.82	4.17	0.420 - 11.5	4.57 ± 3.88	3.68	0.0900 - 15.1
K (mmol/L)	4.01 ± 0.355	4.00	3.15 - 4.78	4.24 ± 0.296	4.21	3.68 - 4.83

3.3. Concentration and prevalence of POPs

In the POP analysis, the LOD ranged from 0.005-0.150 ng/g ww (Appendix V). Thirty-four compounds were detected in > 60 % of the individuals. Concentrations and prevalence of contaminants are listed in table 3, and are reported in ng/g wet weight (ww). Individual concentrations of POPs are presented in appendix VI. In average, female polar bears had a higher concentration of POPs (175.7 ng/g ww) compared to male polar bears (130.8 ng/g ww). The contaminant profiles were in both sexes dominated by Σ_5OH-PCBs, followed by Σ_{20}PCBs and Σ_7OC pesticides. The PCBs dominating in plasma were PCB-153, PCB-180, PCB-170, PCB-194, PCB-138, and PCB-99. The OH-PCBs dominating were 4-OH-CB187, 4-OH-CB146, and 4-OH-CB107. Of the BFRs, only the PBDE congener BDE-47 was found in detectable levels in > 60 % of the individuals. Of the OC pesticides and HCB, oxychlordane dominated the contaminant profile, while mirex and α-HCH was found in the lowest concentrations. In order of dominance: oxychlordane > HCB > trans-nonachlor > *p,p′*-DDE > β-HCH > *p,p′*-DDT > mirex and α-HCH.

Table 3. Mean ± standard deviation (SD), median, and range (min-max) of persistent organic pollutants in ng/g wet weight (ww) measured in plasma samples of polar bear females (n = 20) and males (n = 18) sampled in Svalbard, Norway, 2007.

	Female (n = 20)			Male (n = 18)		
POP	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
PCB-47	0.20 ± 0.12	0.18	0.068 - 0.46	0.16 ± 0.13	0.13	0.032 - 0.55
PCB-74	0.073 ± 0.029	0.079	0.0030 - 0.12	0.078 ± 0.025	0.071	0.041 - 0.13
PCB-99	3.5 ± 2.1	3.0	1.3 - 9.7	2.4 ± 2.4	1.7	0.59 - 12
PCB-101	0.11 ± 0.10	0.081	0.059 - 0.52	0.071 ± 0.026	0.061	0.040 - 0.14
PCB-105	0.13 ± 0.052	0.13	0.054 - 0.25	0.11 ± 0.038	0.10	0.056 - 0.18
PCB-114	0.019 ± 0.0076	0.018	0.0043 - 0.033	0.013 ± 0.0051	0.014	0.0032 - 0.020
PCB-118	0.38 ± 0.15	0.36	0.17 - 0.80	0.36 ± 0.15	0.32	0.17 - 0.74
PCB-128	0.050 ± 0.023	0.049	0.0062 - 0.10	0.037 ± 0.034	0.031	0.00056 - 0.16
PCB-137	0.37 ± 0.23	0.30	0.17 - 1.1	0.22 ± 0.20	0.16	0.050 - 0.96
PCB-138	3.4 ± 1.6	2.7	1.5 - 6.6	2.7 ± 1.9	2.2	0.60 - 8.8
PCB-153	27 ± 27	16	7.7 - 104	15 ± 18	11	3.4 - 84
PCB-156	0.91 ± 0.52	0.80	0.36 - 2.1	0.63 ± 0.47	0.49	0.25 - 2.3
PCB-157	0.81 ± 0.57	0.63	0.23 - 2.2	0.61 ± 0.42	0.52	0.27 - 2.1
PCB-170	6.6 ± 6.8	4.0	1.5 - 28	4.3 ± 3.6	2.9	1.5 - 18
PCB-180	16 ± 21	7.9	3.6 - 83	8.5 ± 8.4	5.5	2.1 - 40
PCB-183	0.43 ± 0.30	0.32	0.16 - 1.5	0.26 ± 0.26	0.18	0.065 - 1.3
PCB-187	0.073 ± 0.031	0.066	0.024 - 0.14	0.059 ± 0.037	0.047	0.024 - 0.16
PCB-189	0.24 ± 0.17	0.18	0.060 - 0.64	0.18 ± 0.13	0.15	0.051 - 0.65
PCB-194	5.9 ± 7.7	2.9	1.3 - 29	2.9 ± 2.8	2.3	0.93 - 14
PCB-206	1.3 ± 1.8	0.59	0.30 - 6.3	0.52 ± 0.45	0.40	0.21 - 2.2
4-OH-CB107	9.5 ± 6.6	8.3	1.8 - 33	7.4 ± 3.0	6.8	2.2 - 13
3'-OH-CB138	1.3 ± 0.65	1.2	0.50 - 2.6	1.4 ± 0.68	1.3	0.61 - 3.2
4-OH-CB146	34 ± 14	29	15 - 65	19 ± 9.1	20	7.0 - 40
4'-OH-CB159	0.28 ± 0.16	0.28	0.052 - 0.72	0.16 ± 0.10	0.14	0.0048 - 0.33
4-OH-CB187	53 ± 19	56	16.9 - 89	59 ± 32	52	11 - 117
BDE-47	0.14 ± 0.068	0.13	0.037 - 0.31	0.12 ± 0.10	0.072	0.036 - 0.38
HCB	1.5 ± 0.89	1.3	0.40 - 3.8	1.4 ± 0.86	1.4	0.21 - 3.0
Oxychlordane	7.3 ± 4.2	6.4	3.2 - 20	2.4 ± 2.3	1.6	0.51 - 10
Trans-nonachlor	0.52 ± 0.25	0.58	0.037 - 1.0	0.49 ± 0.38	0.37	0.083 - 1.9
p.p'-DDT	0.11 ± 0.090	0.085	0.032 - 0.36	0.077 ± 0.060	0.050	0.0042 - 0.22
p.p'-DDE	0.41 ± 0.25	0.37	0.065 - 1.1	0.32 ± 0.21	0.25	0.12 - 1.0
α-HCH	0.035 ± 0.017	0.037	0.0010 - 0.072	0.036 ± 0.016	0.032	0.014 - 0.075
β-HCH	0.34 ± 0.20	0.33	0.12 - 0.90	0.32 ± 0.24	0.25	0.12 - 1.3
Mirex	0.045 ± 0.029	0.040	0.0045 - 0.12	0.027 ± 0.015	0.029	0.0017 - 0.062

3.4. Associations between POPs and clinical-chemical parameters

3.4.1. Principal component analysis for female polar bears

The PCA for polar bear females, including the biometric variables, POPs and the clinical-chemical parameters gave four significant principal components (eigenvalue > 1). These

accounted for 69.0 % of the variation in the dataset (R^2X = 0.690 Q^2 = 0.248). PC 1 contributed to most of the variability, and explained 30.6 %. PC 2-4 explained 19.1 %, 11.5 %, and 7.8 % respectively. Only the two first components were included in the analysis (R^2X = 0.497, Q^2 = 0.211). Some of the relationships in the PCA plots were identified and supported by significant Pearson or Spearman´s rank correlation tests (Appendix VII, Table AIX).

In the PC1-PC2 loading plot for female polar bears (Figure 9), all the POPs, except PCB-74, α-HCH, *p,p´*-DDE, trans-nonachlor, 4-OH-CB107, and 4-OH-CB146 are placed on the right side along PC1 and are positively inter-correlated. Most of the biometric variables and clinical-chemical parameters are placed to the left along PC1. The exceptions were latitude, lipid, age, TG, CHOL, ALAT, and urea, which were placed to the right. The most substituted and recalcitrant POPs are clustered together to the left in the plot, whereas the metabolites and POPs that are easily biotransformed where more spread.

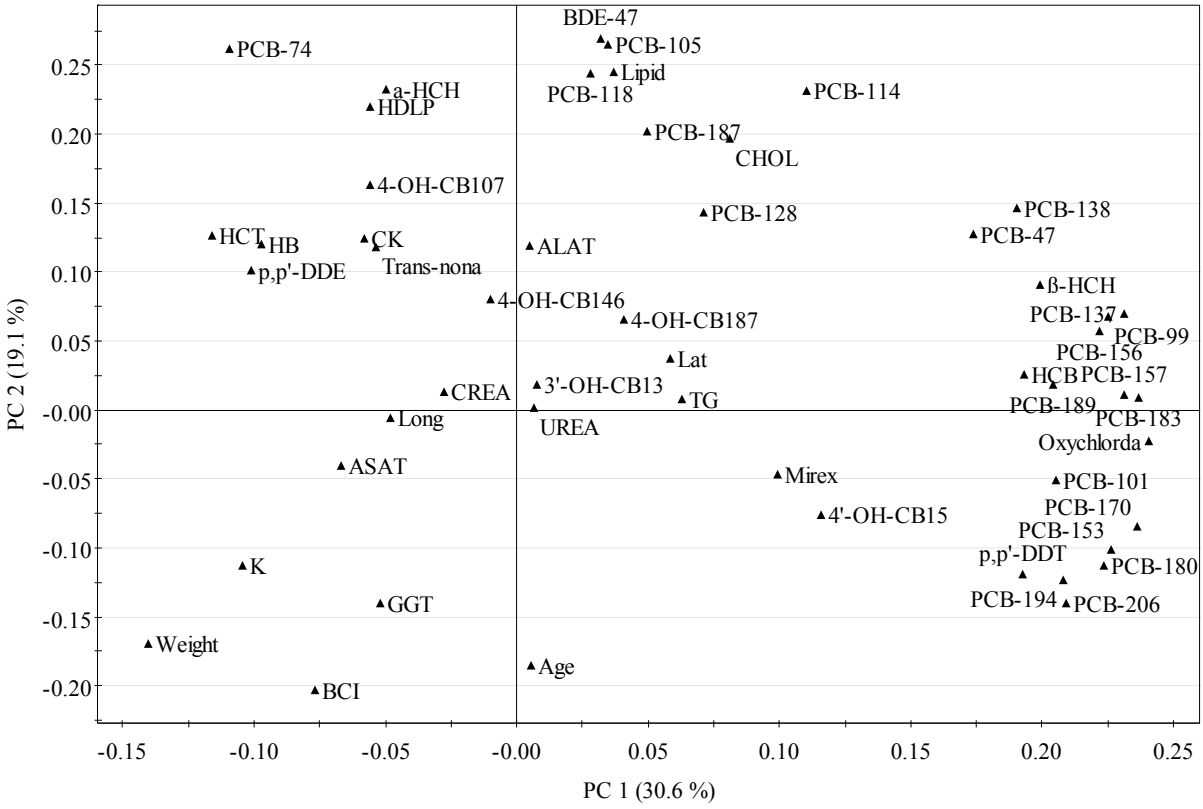

Figure 9. Principal component analysis (PCA) loading plot including biometric variables, clinical-chemical parameters and persistent organic pollutants (POPs) in plasma samples from polar bear females (n = 20) sampled in Svalbard, Norway, 2007.

3.4.2. Principal component analysis for male polar bears

The principal component analysis for male polar bears, including the biometric variables, POPs and the clinical-chemical parameters gave three significant principal components (eigenvalue > 1). These accounted for 66.4 % of the variation in the dataset ($R^2X = 0.664$, $Q^2 = 0.111$). PC 1 contributed to most of the variability, and explained 40.5 %. PC 2 and 3 explained 17.3 % and 8.6 % respectively. Only the two first components were included in the analysis ($R^2X = 0.578$, $Q^2 = 0.127$). Some of the relationships in the PCA plots were identified and supported by significant Pearson or Spearman´s rank correlation tests (Appendix VII, Table AX).

In the PC1-PC2 loading plot for male polar bears (Figure 10), all the POPs, except PCB-74 and 4-OH-CB107, are placed on the right side along PC1 and are positively inter-correlated. Most of the biometric variables and clinical-chemical parameters are placed to the left along PC1. The exceptions were latitude, lipid, TG, CHOL, HDLP, and urea. These are placed to the right, and are negatively correlated to the other biometric variables and clinical-chemical

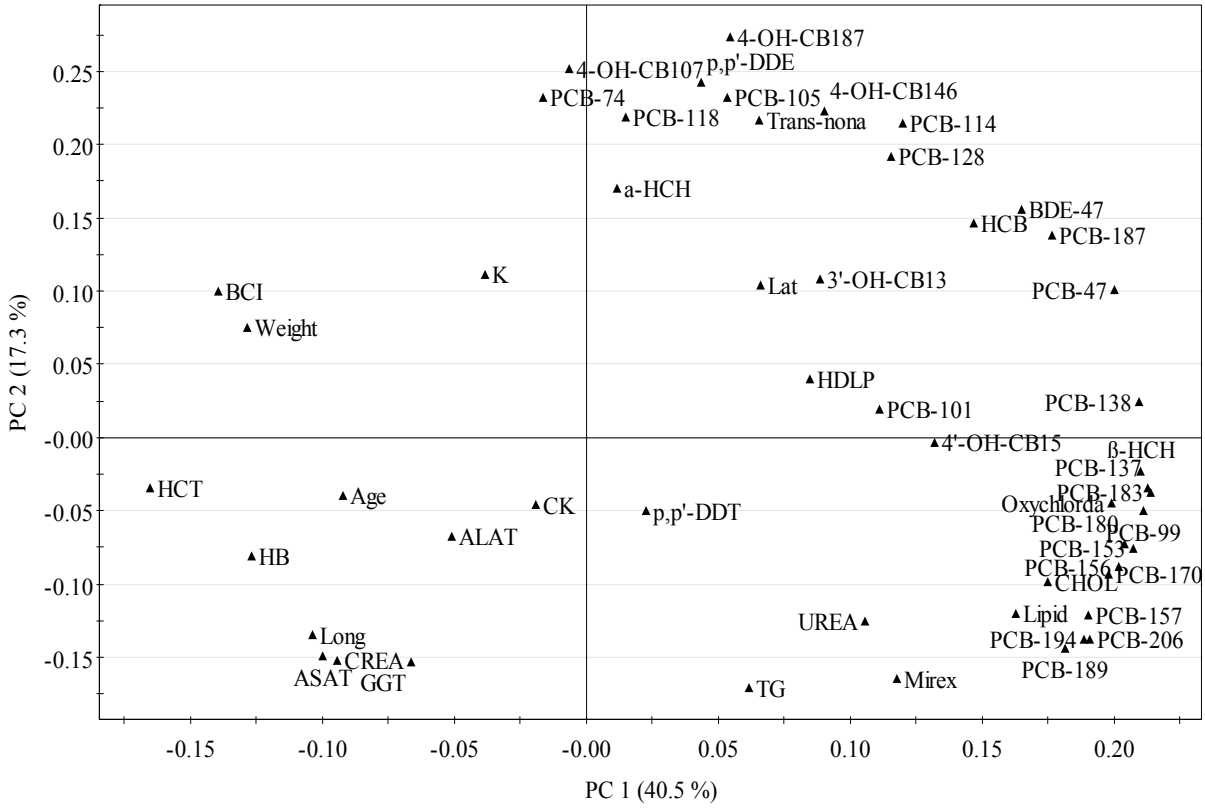

Figure 10. Principal component analysis (PCA) loading plot including biometric variables, clinical-chemical parameters and persistent organic pollutants (POPs) in plasma samples from polar bear males (n = 18) sampled in Svalbard, Norway, 2007.

parameters. However, they correlated positively with the POPs. Along PC2, the most substituted and recalcitrant POPs where placed at the lower part, while the metabolites and POPs that are easily biotransformed at the upper part.

3.4.3. Orthogonal partial least squares regression for female polar bears

Significant O-PLS models were achieved for HCT, HB, GGT, CK, TG, CHOL, and HDLP (Table 4, Figure 11). ASAT, ALAT, CREA, UREA, and K did not give any significant O-PLS models, and were not analysed further. Only the most reliable variables, indicated by jack-knife confidence intervals in the coefficient plots, are included in the text. The POPs that correlated significantly to clinical-chemical parameters are summarized in appendix VIII.

Table 4. Number of components and Y-variables (persistent organic pollutants and biometric variables), goodness of fit (R^2X and R^2Y), goodness of prediction (Q^2), and probability (CV-ANOVA p) in the orthogonal partial least-square (O-PLS) models with the clinical-chemical parameters as X-variables in plasma samples of polar bear females (n = 20) sampled in Svalbard, Norway, 2007.

	Component	Y-variables	R^2X	R^2Y	Q^2	p
HCT	2	18	0.707	0.682	0.454	0.0471
HB	1	15	0.490	0.411	0.313	0.0411
ASAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ALAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GGT	1	13	0.465	0.526	0.367	0.0205
CK	2	9	0.812	0.539	0.460	0.0439
TG	1	11	0.435	0.373	0.313	0.0411
CHOL	2	11	0.600	0.646	0.470	0.0389
HDLP	1	17	0.481	0.503	0.464	0.00502
CREA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
UREA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
K	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. - non significant correlation

The O-PLS coefficient plot for HCT (Figure 11A) in female polar bears showed that most of the predictor variables correlated negatively to HCT. The variables that correlated negatively to HCT included PCB-189 and PCB-206, OH-PCBs, 3´-OH-CB138, and 4´-OH-CB159. The only variable that correlated positively to HCT was PCB-74.

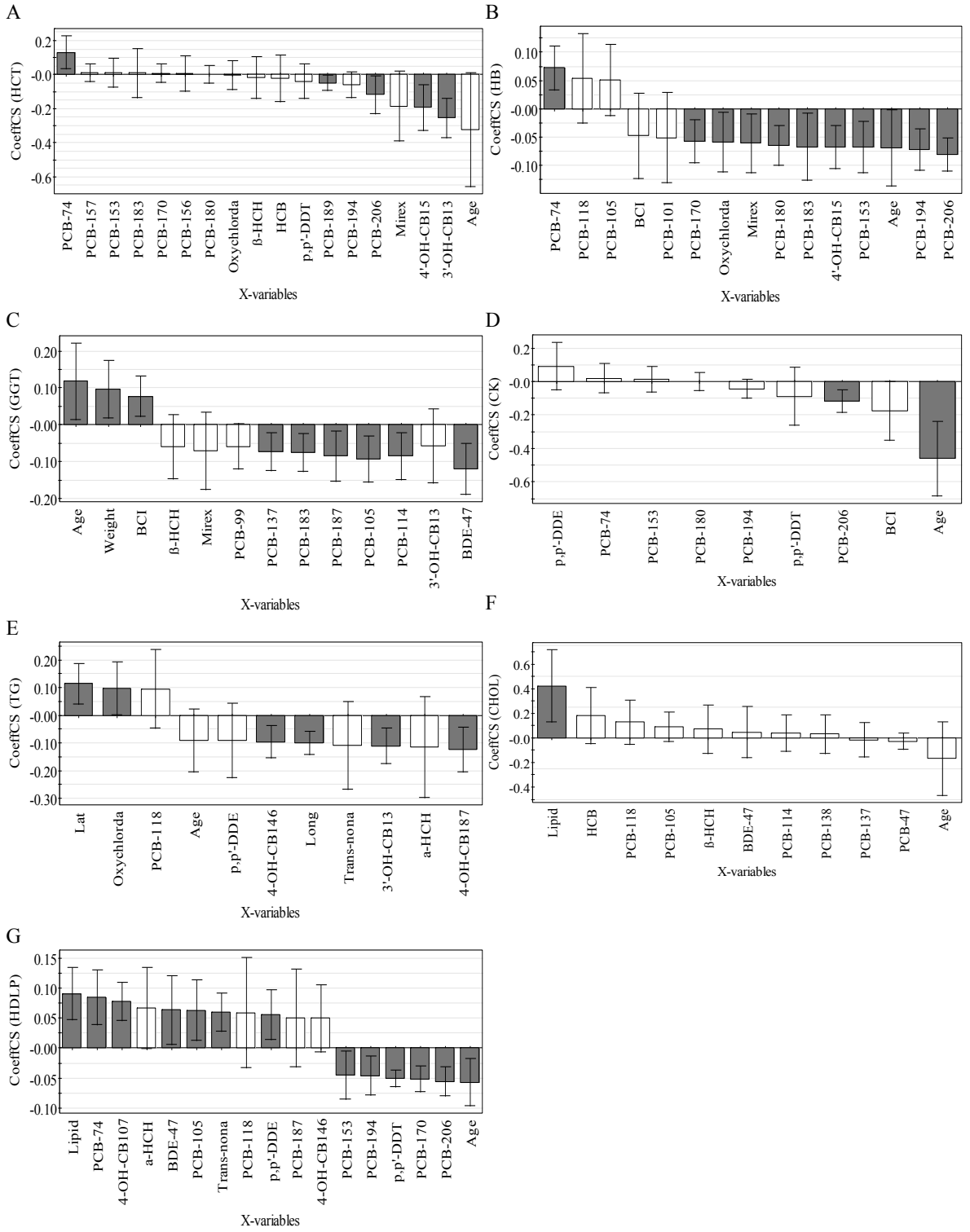

Figure 11. Orthogonal partial least squares (O-PLS) regression coefficient plot showing the relationship between hematocrit (HCT), hemoglobin (HB), γ-glutamyltransferase (GGT), creatine kinase (CK) triglycerides (TG), cholesterol (CHOL), high-density lipoproteins (HDLP) (Y-variables, A-G) and persistent organic pollutants (POPs) and biometric variables (X-variables) in polar bear females (n = 20) sampled in Svalbard, Norway, 2007. All variables are depictured with default jack-knife confidence interval. Open columns represent variables that had jack-knife confidence intervals that crossed 0.

The O-PLS coefficient plot for HB (Figure 11B) for female polar bears resulted in a coefficient plot that correlated negatively to several of the most persistent contaminants, including PCB-153, -170, -180, -183, -194, and -206, the metabolite 4´-OH-CB159, and the two pesticides oxychlordane and mirex. In addition, HB correlated negatively to age. The only parameter that correlated positively to HB was PCB-74.

The coefficient plot for GGT (Figure 11C) for females correlated inversely to contaminants, and positively to biometric variables. The contaminants contributing the most to the explanation of GGT were PCB-105, -114, -137, -183, -187, 3´-OH-CB138, and BDE-47. The biometric variables that correlated positively to GGT were age, weight, and BCI.

The O-PLS coefficient plot for CK (Figure 11D) for female polar bears correlated negatively to age and PCB-206.

The coefficient plot with TG as the response variable (Figure 11E) correlated negatively to several metabolites, including 3´-OH-CB138, 4-OH-CB146, 4-OH-CB187, trans-nonachlor, and α-HCH, and positively to oxychlordane. Regarding biometric variables, TG correlated negatively to latitude and positively to latitude.

Applying CHOL as the response variables (Figure 11F) resulted in a coefficient plot where all the predictor variables, except lipid, were non-significant.

Applying HDLP as the response variables (Figure 11G) for female polar bears resulted in a coefficient plot that both correlated positively and negatively to contaminants. The variables that correlated negatively were recalcitrant POPs, including PCB-153, -170, -194, -206, and *p,p´*-DDT, and age. The variables that correlated positively included several easily biotransformed POPs, including PCB-74, and PCB-105, metabolites, including 4-OH-CB107, *p,p´*-DDE, and trans-nonachlor, and BDE-47 and lipid.

3.4.4. Orthogonal partial least squares regression for male polar bears

Applying HCT, HB, ASAT, GGT, TG, CHOL, HDLP, and CREA as the response variable resulted in significant O-PLS models in male polar bears (Table 5, Figure 12). Only the most reliable variables (indicated by jack-knife confidence intervals) are included in the text.

ALAT, CK, UREA, and K did not give any significant O-PLS models, and were therefore not analysed further. The POPs that correlated significantly to clinical-chemical parameters are summarized in appendix VIII.

Table 5. Number of components and Y-variables (persistent organic pollutants and biometric variables), goodness of fit (R^2X and R^2Y), goodness of prediction (Q^2), and probability (CV-ANOVA p) in the orthogonal partial least-square (O-PLS) models with the clinical-chemical parameters as X-variables in plasma samples of polar bear males (n = 18) sampled in Svalbard, Norway, 2007.

	Component	**Y-variables**	**R^2X**	**R^2Y**	**Q^2**	**p**
HCT	1	18	0.701	0.463	0.331	0.0493
HB	1	13	0.560	0.472	0.344	0.0421
ASAT	2	18	0.775	0.658	0.550	0.0256
ALAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GGT	1	9	0.552	0.472	0.356	0.0367
CK	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
TG	1	7	0.500	0.509	0.435	0.0137
CHOL	3	20	0.788	0.919	0.668	0.0293
HDLP	1	3	0.511	0.587	0.428	0.0152
CREA	1	11	0.609	0.435	0.345	0.0418
UREA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
K	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. - non significant correlation

The O-PLS coefficient plot for HCT (Figure 12A) in male polar bears showed that all significant predictor variables, except age, correlated negatively to HCT. The variables that correlated negatively to HCT included nine PCBs: PCB-47, 99, -137, -138, -153, -156, -180, -183, -187, two OH-PCBs: 3´OH-CB138 and 4´OH-CB159, BDE-47, and the two pesticides oxychlordane and β-HCH.

Applying HB as the response variable (Figure 12B) for male polar bears resulted in a coefficient plot that correlated negatively to PCB-47, -114, -153, -183, and 4´-OH-CB159.

The coefficient plot with ASAT as the response variable (Figure 12C) correlated inversely to PCB-74, -105, -114, -15, BDE-47, and β-HCH.

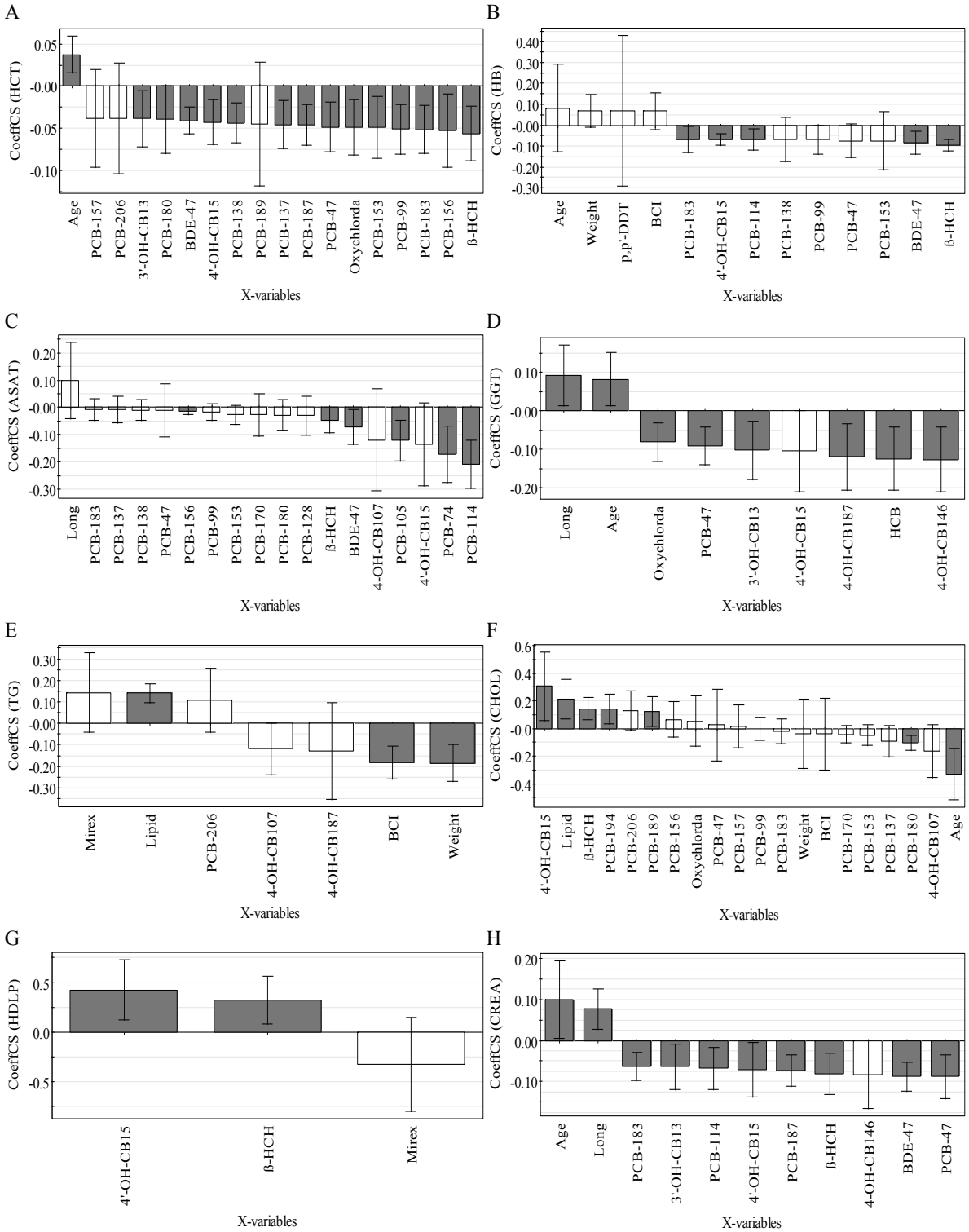

Figure 12. Orthogonal partial least squares (O-PLS) regression coefficient plot showing the relationship between hematocrit (HCT), hemoglobin (HB), aspartate aminotransferase (ASAT), γ-glutamyltransferase (GGT), triglycerides (TG), cholesterol (CHOL), high-density lipoproteins (HDLP), creatinine (CREA) (Y-variables, A-H) and persistent organic pollutants (POPs) and biometric variables (X-variables) in polar bear males (n = 18) sampled in Svalbard, Norway, 2007. All variables are depictured with default jack-knife confidence interval. Open columns represent variables that had jack-knife confidence intervals that crossed 0.

The coefficient plot for GGT (Figure 12D) in males showed that the GGT concentration decreased with increasing concentrations of PCB-47, 3′-OH-CB138, 4-OH-CB146, and 4-OH-CB187, HCB, and oxychlordane. Age and longitude showed the opposite trend.

The coefficient plot for TG (Figure 12E) showed correlations only to the biometric variables weight, BCI and lipid in males. Weight and BCI correlated negatively, and lipid positively to TG.

The O-PLS coefficient plots for CHOL (Figure 12F) and HDLP (Figure 12G) for males were the only clinical-chemical parameters that correlated positively to POPs. CHOL correlated positively to lipid, PCB-189, -194, 4′-OH-CB159, β-HCH, and negatively to age and PCB-180. HDLP correlated positively to 4′-OH-CB159 and β-HCH.

The coefficient plot with CREA as the response variable (Figure 12H) showed that CREA correlated negatively to PCB-47, -114, -183, -187, 3′-OH-CB138, 4′-OH-CB159, BDE-47, and β-HCH, whereas age and longitude showed the opposite correlation.

4. Discussion

Homeostasis is important for organisms to maintain proper physiological functions. Pollutants, such as POPs, may affect this homeostasis, and may thereby reduce the survival and reproduction of animals (Sonne et al., 2010). The POPs profile found in polar bears in this study are comparable with previous findings in polar bears from Alaska, Canada, East Greenland and Svalbard, and confirm the persistence of these contaminants (Dietz et al., 2004; Sandala et al., 2004; Verreault et al., 2005b; Gebbink et al., 2008b; Wolkers et al., 2004; Verreault et al., 2005a; Muir et al., 2006; Verreault et al., 2008; Lie et al., 2003). Persistent organic pollutants may affect clinical-chemical parameters in plasma of polar bears in a way that indicate impact on liver, kidney, heart, muscles, bones, metabolism and the endocrinological system. In the present study, POPs correlated significantly with important clinical-chemical parameters in plasma. This indicates that there is a relatively strong relationship between POPs and these clinical-chemical parameters. In the present study, only the statistically significant results are discussed. Since data regarding relationships between POPs and clinical-chemical parameters in plasma from polar bear is limited, comparisons to data from other mammals are included in the discussion.

4.1. Hepatic toxicity

The liver is the primary organ responsible for metabolism of foreign compounds, and it is also the major target organ for many toxic compounds, including POPs. Earlier studies have shown that POPs may be hepatotoxic (Mayes et al., 1998; Wade et al., 2002; Kutlu et al., 2007; Sonne et al., 2008a; Lu et al., 2010; Sonne et al., 2010). In the present study, clinical-chemical parameters that correlated significantly to POPs and therefore may reflect their effect on the liver include HCT, HB, ASAT, GGT, TG, CHOL, and HDLP.

The hematological indices reflect oxygen transport capacity of blood. Thus, the hematological homeostasis is important for the performance and survival of mammals (Geens et al., 2010). The liver is responsible for the synthesis of heme proteins that are required by hemoglobin. Therefore, it plays a major role in regulation of HCT values and HB concentration in the plasma (Evans, 2009). In this study, polar bears showed a decrease in hematological parameters with increasing POP concentrations, which may seriously affect the breeding capacity and fitness of polar bears (Geens et al., 2010). These results are consistent with

founding of Neale et al. (2005) in harbour seals (*Phoca vitulina*). They observed decreasing levels of HCT and HB with increasing concentrations of PCBs, PBDEs, and DDE. Similar results are also found in Sprague-Dawley rats exposed to mixtures of PCBs, and European starling (*Sturnus vulgaris*) exposed to PBDEs (Mayes et al., 1998; Van den Steen et al., 2010).

Exposure to contaminants may also lead to elevated or decreased levels of liver enzymes (ASAT, ALAT, and GGT) in plasma, which are sensitive indicators of liver injury. In the present study, the plasma levels of ASAT were inversely correlated to POP concentrations in male polar bears. This is in agreement with results reported by Sonne et al. (2008b) in Greenland sledge dogs (*Canis familiaris*) exposed to organohalogen cocktails, and with study of Rao and Banerji (1990) where Wistar rats were exposed to PCBs. Waner and Nyska (1991) suggested that the reason for decreased concentrations of aminotransferase in relation to contaminant exposure is related to the effect of contaminants on pyridoxal 5′-phosphate (vitamin B_6), which is a cofactor necessary for the activity of aminotransferases. We also found a contaminant dependent decrease in the GGT levels in both sexes. This is in accordance with the results reported by Arnold et al. (1999). They found negatively correlations between GGT and PCB congeners in infant rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) monkeys.

While the clinical significance of decreased plasma levels of liver enzymes is not fully understood, the significance of elevated plasma levels is well documented. Kutlu et al. (2007) found a significant increase in serum concentrations of both ASAT and ALAT in adult female Wistar rats exposed to PCBs. Similar results were also shown in experiments by Mayes et al. (1998). They found a sex-related effect in Sprague-Dawley rats, where female showed a dose related increase in ASAT and ALAT when exposed to PCBs. This increase in aminotransferase enzyme concentrations were explained by an increase in cellular enzyme leakage associated with membrane disruption (Mayes et al., 1998). In herring gulls (*Larus argentatus*), Fox et al. (2007) found elevated GGT activity in association with PCB exposure, and Franson (1982) and Mayes et al. (1998) found a positive correlation between GGT concentrations and PCBs levels in black ducks (*Anas rubripes*) and mallards (*Anas platyrhynchos*), and Sprague-Dawley rats respectively. They suggest that these elevated serum levels of GGT may be associated with cholestasis and bile duct damage (Franson, 1982; Mayes et al., 1998).

Since the liver plays an important role in lipid metabolism, altered TG, CHOL, and HDLP homeostasis caused by POPs may indicate an effect on the liver functions. In the present study, we found a negative correlation between TG and OH-PCBs, and a positive correlation between TG and oxychlordane in female polar bears. A negative correlation between TG and POPs are in accordance with the effects found by Fox et al. (2007) in herring gulls, where the TG concentrations were negatively correlated to the PCB levels. Positive correlation have been reported by Bell et al. (1994) in rhesus monkeys exposed to PCBs, by Lu et al. (2010) in male Sprague-Dawley rats exposed to PCBs and TCDD, and by Uemura et al. (2009) among Japan´s general population exposed to dioxins and related compounds.

In the present study, the CHOL concentration increased with increasing level of POPs in male polar bears. Elevated levels of CHOL have also been reported by Mayes et al. (1998) and Wade et al. (2002) in Sprague-Dawley rats exposed to PCB mixtures, and by Arnold et al. (1999) in infant rhesus and cynomolgus monkeys exposed to PCB congeners. Sonne et al. (2012) reported an increase of CHOL levels in Norwegian raptor nestlings exposed to organohalogen contaminants. Mechanism behind the increase in CHOL may be related to altered lipid metabolism, such as increased synthesis or decreased turn over (Bell et al., 1994; Mayes et al., 1998; Sonne et al., 2008a). Other studies have shown no correlations or negative correlations in the CHOL concentrations in relation to levels of POPs in plasma (Van den Steen et al., 2010; Bell et al., 1994; Sonne et al., 2008a).

Compared to the other clinical-chemical parameters analysed herein, few studies have focused on the relationship between HDLP and POPs. In the present study, there were found positive associations between HDLP and different POPs. In accordance with our findings, Ikegami et al. (1991) observed increased levels of HDLP-cholesterol in plasma of male Sprague Dawley rats feed diets containing OC pesticide. In contrast to our findings, Lee et al. (2007) found an inverse correlation between HDLP and OC pesticide among the US human population. These changes in lipid homeostasis may indicate that POPs can cause a change in the fatty acid metabolism.

In total, females and males showed significant correlations to approximately the same number of POPs and clinical-chemical parameters that indicate hepatotoxicity (Appendix VIII). However, there were differences between the types of clinical-chemical parameters affected,

and POPs that affected the different parameters. Among the clinical-chemical parameters that may indicate hepatotoxicity, the hematological parameters (HCT and HB) and GGT seem to be the most sensitive in both sexes. In addition, ASAT and CHOL seem to be sensitive indicators in males, and HDLP in females. The POPs that seems to be most hepatotoxic included PCB-74, -180, -206, 4´-OH-CB159, BDE-47, and β-HCH.

4.2. Renal toxicity

Renal toxicity in laboratory animals and wildlife are not as well documented as hepatic toxicity, but some studies have found toxic effect on kidneys after exposure to POPs (Bergman et al., 2001; Ortiz et al., 2003; Sonne et al., 2006; Kutlu et al., 2007; Sonne et al., 2007; Sonne et al., 2010). In the present study, the clinical-chemical parameter that may indicate the occurrence of kidney injury was CREA. In male polar bears, there were a negative correlation between CREA and different POPs. Similar results have also been reported by Sonne et al. (2010) in chicks of raptor bird species exposed to organohalogen contaminants. Since the plasma level of CREA is an indicator of glomerular function, the observed decrease in CREA concentration with increased POP levels in plasma may indicate tubular and glomerular dysfunction, or inflammation in the urinary tract (Sonne et al., 2008a). Other studies have shown increased CREA concentrations with increasing POP levels, and explained this with lower clearance and filtration rates (Sonne et al., 2012; Kutlu et al., 2007).

4.3. Myotoxicity

The clinical-chemical parameter that may indicate myotoxicity was CK. Measurements of CK in plasma are primary used in studies of cardiotoxicity and myotoxicity. Creatine kinase is found free in the cytosol, and muscle damage will lead to leakage of this enzyme to the plasma (Evans, 2009). However, in the present study there was found a significant negative relationship between PCB-206 and CK in female polar bears. The negative correlation found may indicate that this contaminant may affect cardiac- or skeletal muscles via another mechanism. Similar result was found in Greenland sledge dogs exposed to organohalogen pollutants by Sonne et al. (2008a).

4.4. Interpretation

The present study cannot directly evaluate the relationship between cause and effect, but the results indicate that clinical-chemical parameters in plasma may be affected by POPs. However, it is important to remember that clinical-chemical parameters in plasma can be affected by sex, age, geographic location, sampling time, diet, hydration, condition, stress, parasite, disease, captive and laboratory method, reproductive status and other biometric variables (Neale et al., 2005; Dawson and Bortolotti, 1997; Sonne et al., 2010).

In the present study, age was the biometric variable most important in explaining the variation in clinical-chemical parameters, and it correlated both positively and negatively to different parameters. Age differences in clinical-chemical parameters have also been found by Lee et al. (1977) and Tryland et al. (2002) in polar bears from Svalbard and Manitoba respectively, and by Tryland et al. (2006) in ringed seals. This indicates that age should be taken into an account when studying clinical-chemical parameters. The other biometric variables important in explaining the variation in clinical-chemical parameters included weight, BCI, longitude, and latitude.

This study found a significant correlation between POPs and six of the twelve clinical-chemical parameters (HCT, HB, GGT, TG, CHOL, and HDLP) in both female and male polar bears. In addition, significant correlation to CK was found only in females, while significant correlation to ASAT and CREA were found only in males. The sexes also showed differences between various types of POPs that affected the clinical-chemical parameters. Due to this, it is important to take under consideration sex differences when examining clinical-chemical parameters in plasma.

Capture and handling may cause fear, excitement, and apprehension (Vijayan et al., 1997). Such stress effects must be considered when measuring clinical-chemical parameters in plasma. Chapple et al. (1991) studied the effects of restraint and handling on clinical-chemical parameters in chital deer (*Axis axis*), and found significant changes in different parameters. Similar result was also shown by Marco et al. (1998) in mouflon (*Ovis ammon*).

When studying the effect of POPs, it is also important take under consideration that animals are exposed daily to complex mixtures of environmental contaminants, and that these may

interact and cause effects that are different from single compounds alone. Lu et al. (2010) have reported that combined exposure of PCBs and TCDD in Sprague-Dawley rats induced more severe hepatotoxicity than the two compounds alone.

Differences in the response of clinical-chemical parameters in response to POP concentrations indicate that the effects are species and sex specific, and dependent on the individual POP. In addition, exposure time, route of exposure and the duration of the exposure can affect the response of clinical-chemical parameters (Mayes et al., 1998). It is therefore important to interpret the results with great caution when studying clinical-chemical parameters.

5. Conclusion

The statistical relationships between POPs and clinical-chemical parameters in plasma found in this study indicate that exposure to environmental contaminants may affect organs and metabolic homeostasis in polar bears. However it is uncertain if such changes have any health effects. Even though the study indicates a relationship between POPs and clinical-chemical parameters, it is important to emphasise that clinical-chemical parameters in plasma are influenced by multiple factors such as age, diet, hydration, diseases, stress, as well as other environmental factors. The results also indicate that clinical-chemical parameters in plasma can be applied as a non-invasive biomarker for toxicity to organs and metabolic homeostasis caused by POP exposure in polar bears.

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Appendices

Table of content

Appendix I. Reaction principles for detection of clinical-chemical parameters.

Table AI. Reaction principles for detection of clinical-chemical parameters in whole blood, serum, plasma, or urine with the Reflotron® "dry" clinical-chemistry analyser.

Parameter	**Reaction principle**
Hemoglobin	Hemoglobin + $K_3[Fe(CN)_6] \longrightarrow$ methemoglobin Methemoglobin + $Hg(CN)_2 \longrightarrow$ cyanmethemoglobin
Aspartate aminotransferase	α-Ketoglutarate + alaninesulfinate $\xrightarrow{AST}$ pyruvate + glutamate + SO_2 Pyruvate + PO_4^{3-} + $O_2 \xrightarrow[\text{oxidase}]{\text{pyruvate}}$ acetylphosphate + CO_2 + H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ blue dye + 2 H_2O
Alanine aminotransferase	α-Ketoglutarate + alanine $\xrightarrow{ALT}$ pyruvat + glutamate Pyruvate + PO_4^{3-} + $O_2 \xrightarrow[\text{oxidase}]{\text{pyruvate}}$ acetylphosphate + CO_2 + H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ blue dye + 2 H_2O
γ-Glutamyl-transferase	γ-Glutamyl-3-carboxyl-1,4-phenylenediamine + glycylglycine $\xrightarrow{\gamma\text{-GT}}$ 3-carboxyl-1,4-phenylenediamine + g-glutamylglycylglycine 3-Carboxy-1,4-phenylenediamine + 2-N-methylanthranilic acid+ 6 $[Fe(CN)_6]^{3-} \longrightarrow$ green-blue dye + 6 $[Fe(CN)_6]^{4-}$ + 6 H^+
Creatine kinase	Creatine phosphate + ADP $\xrightleftharpoons{\text{creatine kinase}}$ Ceatinine + ATP Glycerol + ATP $\xrightarrow{\text{glycerolkinase}}$ Glycerol-3-phosphate + ADP Glycerol-3-phosphate + $O_2 \xrightarrow{\text{glycerolphosphateoxidase}}$ dihydroxyacetonephosphate + H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ blue dye + 2 H_2O
Triglycerides	Triglycerides + 3 $H_2O \xrightarrow{\text{esterase}}$ glycerol + 3 free fatty acids Glycerol + ATP $\xrightarrow{\text{glycerokinase}}$ glycerol-3-phosphate + ADP Glycerol-3-phosphate + $O_2 \xrightarrow[\text{oxidase}]{\text{glycerophosphate}}$ dihydroxyacetonephosphat+ H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ blue dye + H_2O
Cholesterol	Cholesterol ester + $H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}}$ Cholesterol + RCOOH Cholesterol + $O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}}$ Δ^4-cholestenone + H_2O_2 Indicator + $H_2O_2 \xrightarrow{\text{peroxidase}}$ blue dye + 2 H_2O
High density lipoprotein	1. Precipitation of chylomicrons, VLDL and LDL by dextran sulfate/Mg^{2+} 2. Determination of HDL cholesterol Cholesterolester + $H_2O \xrightarrow[\text{esterase}]{\text{cholesterol-}}$ cholesterol + RCOOH Cholesterol + $O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol-}}$ Δ^4- cholestenone + H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ blue dye + 2 H_2O
Creatinine	Creatinine + $H_2O \xrightarrow{\text{creatininase}}$ creatine Creatine + $H_2O \xrightarrow{\text{creatinase}}$ sarcosine + urea Sarcosine + H_2O + $O_2 \xrightarrow[\text{oxidase}]{\text{sarcosine}}$ glycine + HCHO + H_2O_2 H_2O_2 + indicator $\xrightarrow{\text{peroxidase}}$ violet dye + 2 H_2O
Urea	Urea + $H_2O \xrightarrow{\text{urease}}$ 2 NH_3 + CO_2 NH_3 + indicator $\longrightarrow$ green blue dye
Potassium	K^+ + valinomycin + indicator $\longrightarrow$ green dye

Appendix II. PCA loading and score plots for all polar bears.

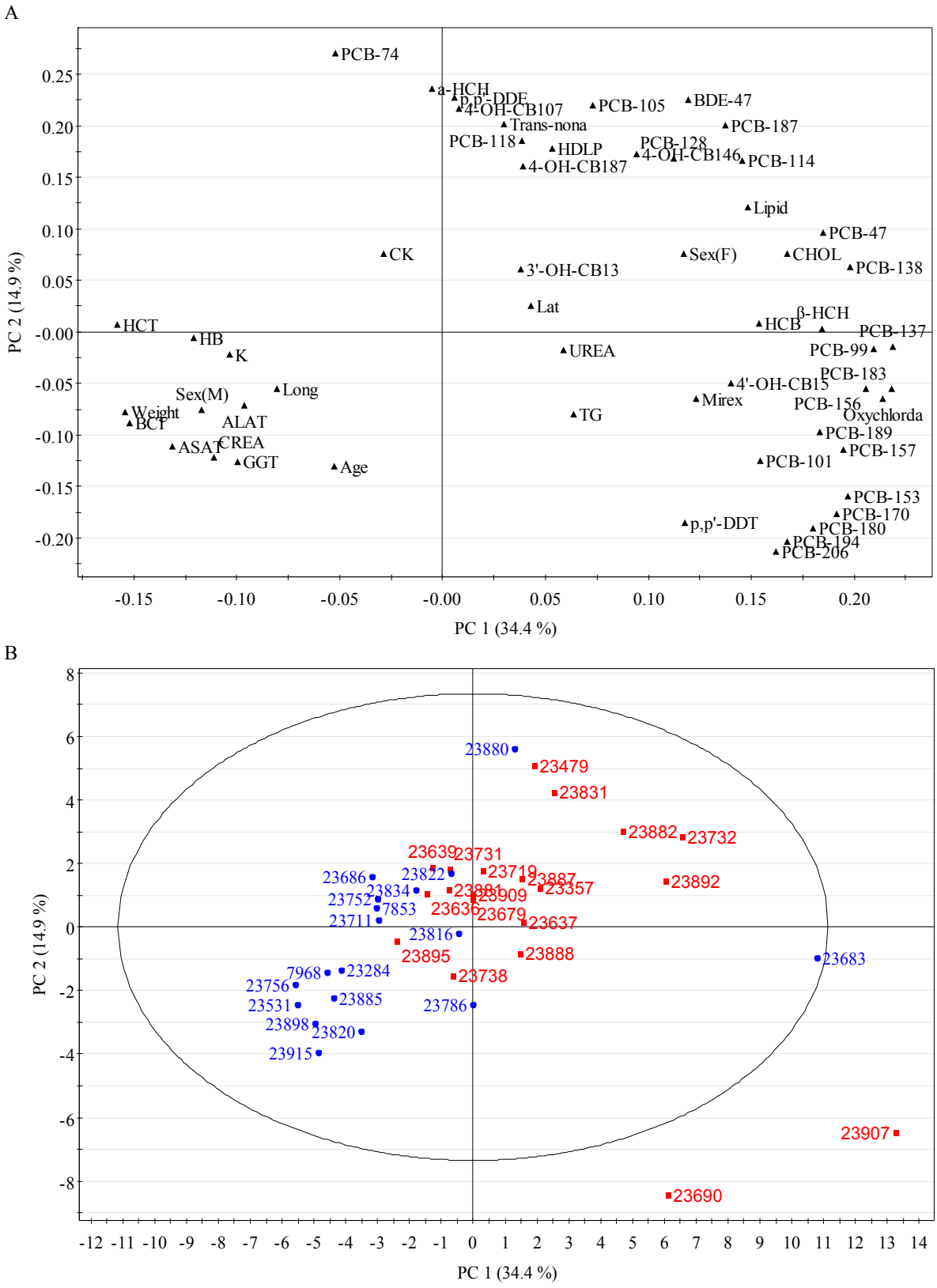

Figure AI. Principal component analysis (PCA) loading (A) and score plots (B) including biometric variables, clinical-chemical parameters and persistent organic pollutants (POPs) in plasma samples from polar bear females (n = 20) and males (n = 18) sampled in Svalbard, Norway, 2007. Females are labelled with red, while males with blue.

Table AII. Individual measurements of biometric variables in female (n = 20) polar bears sampled in Svalbard, Norway, 2007.

ID	Capture day	Lat	Long	Age	Straight length	Axillary girth	Head length	Zygo width	Weight	TBM	BCI	Lipid
23831	85	79.71	12.01	5	195	104	329	185	154	156.2	146.07	1.84
23732	89	76.99	16.44	4	188	103	333	190	144	146.1	141.02	1.90
23639	87	78.34	19.13	5	183	99	319	181	143	131.4	133.12	1.68
23881	85	79.69	12.26	6	189	104	320	180	158	149.7	142.82	1.27
23719	97	78.30	19.28	7	189	108	325	191	141	159.9	147.69	1.74
23479	91	78.34	18.93	7	194	102	332	191	133	149.9	143.03	1.54
23637	93	77.09	15.96	8	206	115	343	202	183	200.8	164.77	1.54
23887	89	76.99	16.65	7	198	120	334	196	195	204.9	166.14	1.60
23357	90	77.55	17.77	11	201	118	340	207	157	203.1	165.54	1.53
23690	97	79.44	14.00	10	199	111	350	196	147	180.0	156.59	0.83
23731	88	77.05	16.54	11	200	114	349	199	173	189.9	160.56	1.59
23636	88	77.09	16.01	15	194	126	334	211	219	217.1	170.31	1.63
23895	91	77.63	21.11	11	200	116	350	199	187	195.8	162.81	1.42
23909	99	79.00	16.21	11	198	114	364	209	176	187.3	159.51	0.88
23907	99	79.13	15.62	13	197	105	342	195	130	161.1	148.36	1.66
23679	86	79.59	12.77	9	200	119	340	200	203	204.7	166.11	1.77
23738	89	76.87	15.54	24	196	121	361	208	223	205.1	166.15	1.04
23892	90	77.58	18.35	17	191	100	313	197	123	141.8	138.85	2.07
23888	90	76.70	17.12	21	193	129	327	200	187	224.6	172.81	1.52
23882	86	79.59	12.74	3	184	103	322	173	123	141.9	138.79	1.85

Table AIII. Individual measurements of biometric variables in male (n = 18) polar bears sampled in Svalbard, Norway, 2007.

ID	Ordinal date	Latitude	Longitude	Age	Straight length	Axillary girth	Head length	Zygomatic width	Weight	TBM	BCI	Lipid
23816	93	76.99	16.27	7	221	140	396	226	312.0	312.0	197.59	1.19
23683	95	79.32	13.96	6	203	106	350	197	170.6	170.6	152.71	1.98
23711	90	77.58	18.35	10	230	159	378	241	411.8	411.8	218.28	1.10
23686	85	79.67	12.32	7	239	153	395	240	405.7	405.7	217.36	0.69
23284	92	77.04	17.02	10	231	162	403	259	428.0	428.0	221.16	0.86
23834	99	79.00	16.21	8	220	174	376	258	453.8	453.8	225.26	0.83
23752	87	78.37	19.13	16	229	164	388	274	432.1	432.1	221.84	0.64
23786	89	76.87	15.54	12	239	159	406	278	434.0	434.0	222.34	1.05
23756	88	77.00	16.54	10	221	168	403	258	429.4	429.4	221.20	0.62
7968	89	76.99	16.65	13	222	146	387	236	337.9	337.9	203.50	1.02
23820	85	79.76	12.16	15	252	159	424	275	466.6	466.6	227.96	1.07
23915	100	79.30	19.58	14	203	118	374	195	205.9	205.9	166.57	1.44
23898	91	77.36	21.26	17	245	163	413	278	468.9	468.9	228.19	0.95
23531	91	78.14	20.87	11	215	154	367	255	355.0	355.0	207.03	0.66
23885	87	78.30	19.28	14	228	174	420	267	476.5	476.5	229.04	0.90
7853	87	78.68	20.50	16	223	155	410	271	377.5	377.5	211.72	0.79
23880	85	79.79	11.77	14	221	142	372	223	319.8	319.8	199.43	1.05
23822	86	79.79	13.91	4	231	137	407	225	319.1	319.1	199.44	1.50

Appendix IV. Individual concentrations of clinical-chemical parameters.

Table AIV. Individual measurements of clinical-chemical parameters in female (n = 20) polar bears sampled in Svalbard, Norway, 2007.

ID	HCT	HB	ASAT	ALAT	GGT	CK	TG	CHOL	HDLP	CREA	UREA	K
23831	45.5	10.1	49.6	23.8	31.6	195.0	1.7	10.6	1.3	105.0	4.3	4.1
23732	40.5	9.9	56.4	28.8	17.5	166.5	1.0	10.8	1.1	113.0	5.4	4.0
23639	50.0	9.3	48.8	23.3	37.5	226.0	1.0	7.8	1.1	102.0	8.3	4.4
23881	45.5	10.2	70.3	21.1	33.0	188.5	1.2	6.7	1.0	94.7	1.0	3.9
23719	49.0	8.9	37.0	16.3	23.0	197.5	1.2	9.3	1.1	95.6	0.4	4.0
23479	43.0	8.3	59.0	11.7	27.1	42.8	1.2	9.4	1.3	126.5	3.2	3.2
23637	43.0	8.2	101.1	42.3	27.1	255.0	1.4	8.9	1.4	113.5	10.3	4.2
23887	38.0	7.9	53.9	11.3	32.0	253.5	1.1	9.5	1.2	91.3	3.7	4.1
23357	41.0	7.4	46.1	15.0	37.4	64.0	1.2	9.0	1.1	135.5	11.5	4.0
23690	39.3	7.8	59.2	10.3	51.3	51.1	2.2	7.5	0.6	128.0	5.1	3.7
23731	43.0	10.2	57.3	17.2	44.3	46.7	0.1	7.8	0.9	102.9	5.3	4.0
23636	44.0	8.5	66.6	18.7	50.9	87.6	1.4	9.2	1.2	128.5	1.5	4.1
23895	46.5	10.5	55.5	12.8	102.3	127.0	1.1	7.6	0.9	120.5	3.1	4.8
23909	38.0	7.0	77.3	22.8	25.2	91.0	0.1	6.5	0.8	82.1	7.0	4.2
23907	36.0	7.0	39.8	12.3	22.7	39.9	1.2	9.7	0.9	85.8	3.6	3.8
23679	46.5	7.9	50.0	18.4	104.2	82.6	1.5	9.8	1.2	103.5	5.0	3.6
23738	40.0	7.9	45.0	15.6	254.0	52.3	1.1	6.0	0.7	95.1	2.7	4.3
23892	40.0	7.8	52.7	44.0	84.4	51.4	0.8	8.4	1.1	104.0	5.2	3.7
23888	42.0	8.1	34.2	13.6	68.6	57.1	0.0	8.3	0.9	88.3	4.0	4.4
23882	52.0	8.9	36.2	17.5	26.0	251.0	1.4	8.8	1.1	95.1	3.6	3.8

Table AV. Individual measurements of clinical-chemical parameters in male (n = 18) polar bears sampled in Svalbard, Norway, 2007.

ID	HCT	HB	ASAT	ALAT	GGT	CK	TG	CHOL	HDLP	CREA	UREA	K
23816	40.0	8.1	108.8	28.7	29.4	105.2	0.9	8.3	0.9	100.7	0.2	4.1
23683	34.0	6.4	67.6	32.2	75.5	119.6	1.6	13.1	1.2	98.5	15.1	3.7
23711	50.0	8.5	79.4	28.0	64.9	77.3	0.9	7.0	1.3	145.5	0.7	4.5
23686	44.0	7.6	79.6	38.4	65.6	161.5	0.9	4.4	0.8	113.0	2.0	4.3
23284	48.0	8.8	93.6	26.2	76.6	105.0	0.9	6.8	1.3	114.0	3.4	4.1
23834	46.0	8.9	85.9	63.1	46.1	114.0	0.1	7.4	1.2	87.6	8.9	4.6
23752	49.5	9.8	116.3	44.6	51.5	77.7	0.9	3.9	0.7	135.5	3.2	3.9
23786	40.0	10.1	67.9	48.4	69.9	87.1	1.2	6.9	1.0	162.0	3.2	4.8
23756	50.5	10.6	154.0	53.6	50.9	114.0	0.9	4.1	0.6	155.5	4.6	4.1
7968	45.0	7.9	87.5	71.2	309.5	115.0	1.1	7.6	1.0	130.5	3.9	4.4
23820	53.0	10.9	80.8	88.4	78.5	104.0	1.1	6.2	0.8	132.5	0.6	4.6
23915	56.0	10.6	163.0	51.4	509.0	121.5	1.6	5.4	0.5	183.0	10.4	4.0
23898	46.5	9.2	141.0	34.1	107.0	377.0	1.3	6.8	1.1	133.5	4.7	3.9
23531	47.0	9.4	109.5	35.8	283.0	136.0	1.2	5.3	0.9	120.0	9.6	4.1
23885	53.5	11.4	58.8	44.8	309.5	75.1	0.1	6.2	1.1	164.0	0.1	4.3
7853	50.0	8.4	78.8	33.4	148.5	62.0	1.0	5.0	0.9	144.5	3.9	4.4
23880	43.0	8.6	57.3	33.0	116.0	152.0	0.9	6.0	1.1	100.5	4.4	4.6
23822	47.5	8.5	53.4	16.2	13.1	218.0	1.2	8.5	1.0	86.8	3.5	4.0

Appendix V. Limit of detection for POPs.

Table AVI. Limit of detection for persistent organic pollutants (POPs) in plasma samples of polar bears (n = 38) sampled in Svalbard, Norway, 2007. The limit of detection was determined as threefold to the signal to noise level.

POPs	Detection limits (ng/g)
PCB-47	0.025
PCB-74	0.025
PCB-99	0.025
PCB-101	0.025
PCB-105	0.015
PCB-114	0.010
PCB-118	0.010
PCB-128	0.020
PCB-137	0.015
PCB-138	0.020
PCB-153	0.025
PCB-156	0.020
PCB-157	0.020
PCB-167	0.040
PCB-170	0.020
PCB-180	0.020
PCB-183	0.015
PCB-187	0.015
PCB-189	0.015
PCB-194	0.020
PCB-206	0.020
4-OH-CB107	0.025
3'-OH-CB138	0.060
4-OH-CB146	0.020
4'-OH-CB159	0.120
4-OH-CB187	0.020
BDE-47	0.025
HCB	0.010
Oxychlordane	0.015
Trans-nonachlor	0.015
p.p'-DDT	0.040
p.p'-DDE	0.025
α-HCH	0.015
β-HCH	0.025
Mirex	0.018

Appendix VI. Individual concentrations of POPs.

Table AVII. Individual measurements of persistent organic pollutants (POPs) in female (n = 20) polar bears sampled in Svalbard, Norway, 2007.

ID	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
23831	0.175	0.101	3.017	0.092	0.252	0.030	0.803	0.091	0.393	4.732	17.780	0.818	0.508
23732	0.360	0.086	6.965	0.097	0.210	0.033	0.547	0.056	0.668	5.883	35.535	1.993	1.937
23639	0.094	0.099	1.637	0.080	0.148	0.016	0.383	0.024	0.210	2.013	10.054	0.430	0.365
23881	0.069	0.100	1.269	0.111	0.164	0.018	0.405	0.030	0.168	1.462	10.774	0.616	0.711
23719	0.117	0.102	2.797	0.067	0.195	0.027	0.479	0.022	0.308	2.326	16.046	1.110	0.935
23479	0.209	0.120	3.028	0.070	0.171	0.025	0.472	0.046	0.375	3.373	14.724	0.611	0.452
23637	0.184	0.060	2.776	0.076	0.085	0.014	0.248	0.036	0.265	2.340	15.606	0.906	0.807
23887	0.200	0.056	3.114	0.065	0.092	0.013	0.268	0.051	0.299	3.019	16.099	0.715	0.543
23357	0.215	0.066	3.851	0.081	0.134	0.019	0.346	0.050	0.358	4.544	21.152	0.784	0.587
23690	0.068	0.003	3.373	0.164	0.083	0.020	0.315	0.021	0.374	2.681	104.165	1.213	1.532
23731	0.161	0.078	2.012	0.082	0.126	0.018	0.368	0.060	0.237	2.444	9.354	0.497	0.299
23636	0.114	0.084	1.395	0.071	0.113	0.013	0.300	0.045	0.178	1.911	7.921	0.365	0.232
23895	0.134	0.059	1.701	0.065	0.054	0.004	0.202	0.046	0.166	1.948	7.728	0.402	0.264
23909	0.113	0.058	2.104	0.119	0.104	0.013	0.220	0.006	0.291	2.480	14.012	0.498	0.400
23907	0.445	0.028	9.695	0.521	0.132	0.020	0.291	0.060	1.121	6.570	99.398	2.123	2.207
23679	0.102	0.088	1.698	0.089	0.149	0.021	0.510	0.048	0.221	2.267	14.974	0.584	0.366
23738	0.201	0.051	2.955	0.073	0.073	0.011	0.242	0.063	0.251	2.670	17.523	0.862	0.676
23892	0.350	0.080	4.921	0.095	0.162	0.032	0.563	0.080	0.504	4.565	35.551	1.823	1.464
23888	0.232	0.043	4.239	0.059	0.054	0.011	0.172	0.059	0.367	3.371	26.029	0.885	0.974
23882	0.455	0.108	6.478	0.167	0.160	0.022	0.428	0.099	0.633	6.511	35.841	0.965	1.005

Table AVII. Continues.

ID	PCB-170	PCB-180	PCB-183	PCB-187	PCB-189	PCB-194	PCB-206	4-OH-CB107	3'-OH-CB138	4-OH-CB146	4'-OH-CB159	4-OH-CB187	BDE-47
23831	4.621	9.623	0.412	0.109	0.197	2.521	0.369	7.142	0.814	18.957	0.077	31.823	0.199
23732	9.705	16.787	0.580	0.109	0.540	5.140	0.680	11.124	1.675	42.689	0.334	54.666	0.307
23639	2.715	6.211	0.277	0.067	0.096	1.782	0.363	7.923	0.498	25.317	0.122	39.330	0.153
23881	4.486	7.787	0.228	0.066	0.176	2.829	0.473	10.374	0.719	26.629	0.284	39.661	0.115
23719	3.767	7.197	0.264	0.041	0.139	2.059	0.298	7.925	0.644	17.896	0.192	22.665	0.191
23479	3.292	6.948	0.447	0.095	0.117	2.274	0.414	32.770	1.247	65.191	0.282	89.256	0.237
23637	5.315	9.133	0.265	0.052	0.330	4.006	0.753	11.603	2.560	59.311	0.723	68.402	0.085
23887	3.875	7.793	0.313	0.083	0.189	2.401	0.593	10.139	2.566	43.922	0.293	75.151	0.106
23357	3.925	8.664	0.522	0.069	0.171	3.264	0.603	15.355	1.181	57.021	0.351	40.964	0.165
23690	22.349	70.500	0.721	0.024	0.433	29.460	6.195	2.048	0.725	15.142	0.388	16.933	0.037
23731	1.987	4.662	0.243	0.063	0.126	2.355	0.545	12.366	1.459	31.140	0.124	51.174	0.151
23636	1.699	4.771	0.252	0.060	0.066	1.635	0.543	8.715	1.215	25.540	0.147	56.896	0.087
23895	1.541	3.546	0.161	0.042	0.060	1.315	0.414	6.553	1.122	36.346	0.139	57.972	0.052
23909	3.492	7.086	0.375	0.138	0.131	3.006	0.592	9.637	2.393	31.840	0.299	67.966	0.161
23907	27.733	83.440	1.477	0.081	0.513	23.985	6.334	1.829	1.098	27.627	0.470	75.543	0.123
23679	3.140	7.882	0.319	0.050	0.143	3.999	1.050	4.596	0.573	20.652	0.052	34.258	0.089
23738	3.981	7.956	0.213	0.041	0.211	2.437	0.594	3.406	0.618	25.714	0.246	41.469	0.060
23892	10.165	21.645	0.569	0.113	0.643	14.430	2.630	14.414	1.765	43.418	0.368	68.104	0.127
23888	6.374	12.227	0.308	0.049	0.355	4.971	1.250	5.832	1.617	41.411	0.450	64.468	0.082
23882	7.463	15.128	0.734	0.116	0.235	3.395	0.509	6.729	1.322	27.833	0.193	68.247	0.200

Table AVII. Continues.

ID	HCB	*p,p'*-DDT	*p,p'*-DDE	Oxychlordane	Trans-nonachlor	α-HCH	β-HCH	Mirex
23831	2.129	0.142	0.866	5.470	0.760	0.039	0.284	0.059
23732	2.420	0.093	0.357	12.060	0.600	0.061	0.900	0.021
23639	0.399	0.048	0.565	4.701	0.261	0.039	0.138	0.028
23881	0.527	0.078	0.505	3.405	0.315	0.023	0.115	0.038
23719	0.856	0.032	0.237	6.155	0.209	0.036	0.352	0.005
23479	0.630	0.075	0.425	7.170	0.576	0.072	0.364	0.046
23637	1.911	0.125	0.407	7.970	0.502	0.016	0.351	0.049
23887	1.504	0.033	0.377	6.501	0.738	0.035	0.329	0.118
23357	0.598	0.061	0.254	8.771	0.414	0.041	0.464	0.060
23690	1.318	0.341	0.068	14.107	0.037	0.001	0.423	0.072
23731	1.199	0.071	0.335	3.598	0.823	0.038	0.192	0.038
23636	1.100	0.093	0.517	3.368	0.638	0.040	0.115	0.041
23895	1.099	0.173	0.306	3.996	0.614	0.038	0.219	0.024
23909	0.428	0.091	1.136	3.206	0.248	0.025	0.130	0.076
23907	3.809	0.357	0.065	20.169	0.177	0.013	0.701	0.097
23679	1.340	0.059	0.218	4.989	0.429	0.027	0.164	0.018
23738	1.810	0.052	0.264	6.248	0.623	0.032	0.323	0.009
23892	2.877	0.187	0.409	8.383	1.022	0.061	0.472	0.030
23888	2.117	0.098	0.333	6.695	0.761	0.033	0.333	0.020
23882	1.877	0.077	0.515	9.589	0.576	0.040	0.461	0.048

Table AVIII. Individual measurements of persistent organic pollutants (POPs) in male (n = 18) polar bears sampled in Svalbard, Norway, 2007.

ID	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
23816	0.191	0.058	2.955	0.062	0.080	0.013	0.255	0.044	0.275	3.003	14.716	0.870	0.620
23683	0.554	0.059	11.687	0.094	0.124	0.019	0.337	0.060	0.963	8.792	83.842	2.281	2.077
23711	0.124	0.093	2.179	0.059	0.117	0.015	0.401	0.029	0.177	2.166	10.589	0.587	0.503
23686	0.131	0.126	1.105	0.049	0.179	0.018	0.679	0.033	0.109	1.566	7.015	0.353	0.270
23284	0.100	0.059	1.326	0.056	0.057	0.008	0.191	0.020	0.093	1.211	6.631	0.384	0.317
23834	0.220	0.078	2.276	0.050	0.097	0.014	0.268	0.033	0.231	2.451	10.908	0.595	0.545
23752	0.217	0.083	1.845	0.072	0.173	0.019	0.737	0.046	0.151	2.618	8.037	0.488	0.460
23786	0.162	0.050	3.853	0.144	0.094	0.014	0.302	0.066	0.353	4.689	25.537	1.326	1.222
23756	0.096	0.066	1.240	0.040	0.076	0.003	0.288	0.020	0.108	1.597	6.597	0.308	0.308
7968	0.032	0.123	0.854	0.069	0.148	0.014	0.358	0.001	0.085	1.018	5.056	0.415	0.547
23820	0.068	0.065	1.472	0.071	0.099	0.012	0.287	0.011	0.192	2.275	13.239	0.681	0.870
23915	0.037	0.049	0.947	0.088	0.083	0.005	0.259	0.027	0.119	1.331	6.183	0.396	0.551
23898	0.074	0.041	1.150	0.048	0.056	0.003	0.173	0.006	0.098	1.304	6.743	0.396	0.425
23531	0.033	0.075	0.593	0.053	0.084	0.010	0.263	0.013	0.050	0.596	3.416	0.254	0.291
23885	0.067	0.067	1.468	0.061	0.107	0.013	0.344	0.029	0.126	1.886	10.803	0.485	0.597
7853	0.134	0.105	2.877	0.073	0.135	0.016	0.360	0.037	0.301	3.177	14.583	0.456	0.498
23880	0.394	0.113	2.967	0.125	0.181	0.020	0.476	0.158	0.318	5.255	18.334	0.536	0.531
23822	0.236	0.093	2.584	0.057	0.140	0.017	0.422	0.037	0.263	2.704	11.153	0.556	0.418

Table AVIII. Continues.

ID	PCB-170	PCB-180	PCB-183	PCB-187	PCB-189	PCB-194	PCB-206	4-OH-CB107	3'-OH-CB138	4-OH-CB146	4'-OH-CB159	4-OH-CB187	BDE-47
23816	3.646	7.860	0.377	0.067	0.162	2.183	0.471	8.239	2.408	39.868	0.331	83.610	0.092
23683	17.507	40.458	1.254	0.140	0.650	13.542	2.196	3.300	1.816	22.629	0.302	55.118	0.331
23711	2.806	5.225	0.194	0.040	0.155	1.754	0.360	5.378	1.574	23.205	0.152	85.337	0.069
23686	1.488	3.585	0.120	0.046	0.051	0.930	0.213	11.993	1.641	22.755	0.124	89.857	0.140
23284	2.003	3.403	0.108	0.032	0.095	1.314	0.294	6.634	1.384	17.786	0.212	48.420	0.046
23834	3.752	6.665	0.291	0.089	0.154	2.461	0.423	12.429	3.241	32.898	0.251	105.766	0.208
23752	2.655	5.194	0.167	0.068	0.121	1.317	0.229	12.868	1.784	25.793	0.098	82.230	0.074
23786	8.360	14.323	0.371	0.070	0.348	4.742	1.005	8.295	2.370	15.971	0.326	47.181	0.077
23756	2.011	4.271	0.126	0.033	0.086	1.046	0.215	2.242	0.614	17.226	0.005	78.041	0.040
7968	2.710	4.153	0.088	0.024	0.149	2.530	0.374	7.090	0.617	7.798	0.138	15.796	0.053
23820	6.146	11.011	0.190	0.030	0.235	4.587	0.737	5.516	1.222	11.297	0.177	24.193	0.063
23915	2.939	4.475	0.110	0.048	0.160	2.692	0.501	5.364	0.846	10.022	0.024	17.277	0.061
23898	2.649	4.054	0.108	0.025	0.158	2.417	0.486	5.307	1.205	10.102	0.185	23.127	0.036
23531	1.639	2.141	0.065	0.035	0.104	1.568	0.236	4.542	0.728	6.949	0.020	10.687	0.047
23885	4.475	8.242	0.140	0.029	0.237	3.303	0.599	7.056	1.242	9.868	0.100	39.845	0.064
7853	4.998	10.937	0.269	0.060	0.088	1.603	0.238	6.356	0.695	21.405	0.074	80.917	0.176
23880	5.100	11.368	0.379	0.158	0.146	2.578	0.480	9.923	1.474	31.912	0.134	117.148	0.384
23822	2.809	5.832	0.300	0.064	0.087	1.636	0.347	10.042	1.130	22.133	0.145	48.988	0.147

Table AVIII. Continues.

ID	HCB	*p,p'*-DDT	*p,p'*-DDE	Oxychlordane	Trans-nonachlor	α-HCH	β-HCH	Mirex
23816	1.991	0.030	0.213	5.361	0.622	0.024	0.327	0.047
23683	2.995	0.112	0.255	10.268	0.471	0.036	1.250	0.062
23711	1.788	0.036	0.248	3.009	0.485	0.040	0.309	0.002
23686	1.755	0.095	0.252	1.013	0.339	0.037	0.259	0.019
23284	1.181	0.038	0.167	1.703	0.385	0.030	0.195	0.007
23834	1.613	0.108	0.525	1.264	0.346	0.032	0.405	0.003
23752	2.842	0.111	0.525	1.740	0.844	0.036	0.240	0.018
23786	2.054	0.056	0.128	3.038	0.487	0.014	0.368	0.046
23756	1.225	0.022	0.178	2.290	0.590	0.062	0.170	0.031
7968	0.206	0.004	0.260	0.587	0.083	0.027	0.202	0.022
23820	0.690	0.225	0.233	1.452	0.305	0.022	0.205	0.032
23915	0.362	0.175	0.302	0.702	0.345	0.017	0.165	0.040
23898	0.605	0.043	0.120	1.405	0.240	0.032	0.195	0.018
23531	0.335	0.031	0.258	0.515	0.125	0.025	0.123	0.030
23885	1.211	0.164	0.250	1.175	0.308	0.030	0.211	0.033
7853	0.233	0.019	0.415	1.266	0.618	0.075	0.290	0.036
23880	2.342	0.077	1.042	2.910	1.848	0.064	0.386	0.020
23822	2.099	0.038	0.307	3.238	0.362	0.042	0.482	0.027

Appendix VII. Correlation analysis.

Table AIX. Correlation analysis between clinical-chemical parameters and biometric variables and POPs, using Pearson´s product-moment correlation and Spearman´s rank correlation, in plasma samples of polar bear females (n = 20) sampled in Svalbard, Norway, 2007.

Correlated variables		r-value	p-value	Correlation test
HCT	Age	-0.459	0.042	Pearson
	PCB-74	0.681	0.001	Pearson
	PCB-189	-0.478	0.033	Pearson
	PCB-194	-0.505	0.023	Spearman´s rank
	PCB-206	-0.689	0.001	Spearman´s rank
	3´-OH-CB138	-0.466	0.038	Spearman´s rank
	4´-OH-CB159	-0.527	0.017	Pearson
	Mirex	-0.535	0.015	Pearson
HB	PCB-74	0.495	0.026	Pearson
	PCB-153	-0.499	0.025	Spearman´s rank
	PCB-180	-0.468	0.038	Spearman´s rank
	PCB-183	-0.457	0.043	Pearson
	PCB-194	-0.55	0.012	Spearman´s rank
	PCB-206	-0.699	0.001	Spearman´s rank
	4´-OH-CB159	-0.474	0.035	Pearson
ASAT	PCB-99	-0.469	0.037	Pearson
	PCB-128	-0.449	0.047	Pearson
	PCB-138	-0.469	0.037	Pearson
	PCB-153	-0.457	0.043	Spearman´s rank
ALAT	*p.p´*-DDE	0.57	0.009	Spearman´s rank
GGT	Age	0.554	0.011	Spearman´s rank
	Weight	0.531	0.016	Spearman´s rank
	BCI	0.454	0.044	Spearman´s rank
	PCB-105	-0.498	0.025	Spearman´s rank
	PCB-114	-0.455	0.044	Spearman´s rank
	PCB-137	-0.511	0.021	Spearman´s rank
	PCB-187	-0.517	0.019	Spearman´s rank
	BDE-47	-0.693	0.001	Spearman´s rank
CK	Age	-0.632	0.003	Spearman´s rank
	PCB-206	-0.465	0.039	Spearman´s rank
TG	Lat	0.619	0.004	Spearman´s rank
	Long	-0.553	0.011	Spearman´s rank
	4-OH-CB146	-0.457	0.043	Spearman´s rank
CHOL	Age	-0.449	0.047	Pearson
	Lipid	0.736	<0.001	Pearson
	PCB-105	0.574	0.008	Pearson
	PCB-114	0.585	0.007	Pearson

Table AIX. Continues.

	Correlated variables	r-value	p-value	Correlation test
CHOL	PCB-118	0.563	0.01	Pearson
	PCB-137	0.47	0.036	Pearson
	PCB-138	0.532	0.016	Pearson
	BDE-47	0.52	0.019	Pearson
	β-HCH	0.463	0.04	Pearson
HDLP	Age	-0.479	0.033	Pearson
	Lipid	0.671	0.001	Pearson
	PCB-74	0.652	0.002	Pearson
	PCB-105	0.488	0.029	Pearson
	PCB-118	0.461	0.041	Pearson
	4-OH-CB107	0.606	0.005	Pearson
CREA	n.s			
UREA	4-OH-CB146	0.459	0.042	Pearson
K	Lat	-0.476	0.034	Pearson
	Weight	0.486	0.03	Pearson
	PCB-101	-0.502	0.024	Spearman´s rank
	PCB-105	-0.449	0.047	Pearson
	PCB-114	-0.65	0.002	Pearson
	PCB-118	-0.455	0.044	Pearson
	PCB-183	-0.576	0.008	Pearson

Table AX. Correlation analysis between clinical-chemical parameters and biometric variables and POPs, using Pearson´s product-moment correlation and Spearman´s rank correlation, in plasma samples of polar bear males (n = 18) sampled in Svalbard, Norway, 2007.

Correlated variables		r-value	p-value	Correlation test
HCT	Age	0.483	0.042	Pearson
	PCB-47	-0.604	0.008	Pearson
	PCB-99	-0.635	0.005	Pearson
	PCB-128	-0.471	0.048	Spearman´s rank
	PCB-137	-0.566	0.014	Pearson
	PCB-138	-0.541	0.021	Pearson
	PCB-153	-0.61	0.007	Pearson
	PCB-180	-0.491	0.038	Pearson
	PCB-183	-0.64	0.004	Pearson
	PCB-187	-0.569	0.014	Pearson
	PCB-206	-0.471	0.048	Pearson
	3´-OH-CB138	-0.481	0.043	Pearson
	4´-OH-CB159	-0.714	0.001	Pearson
	BDE-47	-0.504	0.033	Pearson
	HCB	-0.563	0.015	Pearson
	Oxychlordane	-0.606	0.008	Pearson
	β-HCH	-0.696	0.001	Pearson
HCB	Age	0.528	0.024	Pearson
	Weight	0.602	0.008	Spearman´s rank
	BCI	0.598	0.009	Spearman´s rank
	PCB-47	-0.489	0.039	Pearson
	PCB-114	-0.505	0.033	Pearson
	BDE-47	-0.559	0.016	Pearson
	β-HCH	-0.626	0.005	Pearson
ASAT	Long	0.514	0.029	Pearson
	PCB-99	-0.498	0.035	Pearson
	PCB-105	-0.502	0.034	Pearson
	PCB-114	-0.783	<0.001	Pearson
	PCB-128	-0.552	0.018	Spearman´s rank
	PCB-137	-0.496	0.036	Pearson
	PCB-138	-0.501	0.034	Pearson
	PCB-153	-0.515	0.029	Pearson
	PCB-156	-0.519	0.027	Spearman´s rank
	PCB-180	-0.496	0.036	Pearson
	PCB-183	-0.495	0.037	Pearson
	BDE-47	-0.598	0.009	Pearson
	β-HCH	-0.571	0.013	Pearson
ALAT	n.s			

Table AX. Continues.

Correlated variables		r-value	p-value	Correlation test
GGT	Age	0.593	0.009	Pearson
	PCB-47	-0.666	0.003	Pearson
	3´-OH-CB138	-0.487	0.041	Pearson
	4-OH-CB146	-0.651	0.003	Pearson
	4-OH-CB187	-0.504	0.033	Pearson
	HCB	-0.628	0.005	Pearson
	Oxychlordane	-0.649	0.004	Pearson
	β-HCH	-0.483	0.042	Pearson
CK	n.s.			
TG	PCB-74	-0.544	0.02	Spearman´s rank
	4-OH-CB107	-0.491	0.038	Spearman´s rank
	4-OH-CB146	-0.488	0.04	Spearman´s rank
	4-OH-CB187	-0.653	0.003	Spearman´s rank
	Mirex	0.483	0.042	Spearman´s rank
CHOL	Age	-0.475	0.047	Pearson
	Lipid	0.817	<0.001	Pearson
	PCB-99	0.597	0.009	Pearson
	PCB-137	0.57	0.013	Pearson
	PCB-153	0.608	0.007	Pearson
	PCB-156	0.637	0.004	Spearman´s rank
	PCB-157	0.505	0.033	Spearman´s rank
	PCB-170	0.597	0.009	Pearson
	PCB-180	0.544	0.02	Pearson
	PCB-183	0.613	0.007	Pearson
	PCB-189	0.655	0.003	Pearson
	PCB-194	0.711	0.001	Pearson
	PCB-206	0.742	<0.001	Pearson
	4´-OH-CB159	0.72	0.001	Pearson
	Oxychlordane	0.558	0.016	Pearson
	β-HCH	0.693	0.001	Pearson
HDLP	4´-OH-CB159	0.551	0.018	Pearson
	β-HCH	0.48	0.044	Pearson
CREA	Long	0.496	0.036	Pearson
	Age	0.605	0.008	Pearson
	PCB-47	-0.566	0.014	Pearson
	4-OH-CB146	-0.587	0.01	Pearson
	BDE-47	-0.564	0.015	Pearson
	β-HCH	-0.52	0.027	Pearson
UREA	n.s.			
K	n.s.			

Appendix VIII. POPs significantly correlated to clinical-chemical parameters.

Table XI. Summary of the persistent organic pollutants (POPs) that correlated significantly to clinical-chemical parameters (indicated by default jack-knife confidence intervals) in the orthogonal partial least squares (O-PLS) regression plots for female (n = 20) and male (n=18) polar bears captured in Svalbard, Norway, 2007.

	HCT		HB		ASAT		GGT		CK		TG		CHOL		HDLP		CREA		Σ Hep[1]		Σ Ren[2]		Σ Myo[3]		Σ POPs	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
PCB-47		x						x										x		2		1				3
PCB-74	x		x			x									x				3	1					3	1
PCB-99		x																		1						1
PCB-105						x	x								x				2	1					2	1
PCB-114				x		x	x											x	1	2		1			1	3
PCB-137		x					x												1	1					1	1
PCB-138		x																		1						1
PCB-153		x	x												x				2	1					2	1
PCB-156		x				x														2						2
PCB-170			x												x				2						2	
PCB-180		x	x											x					1	3					1	3
PCB-183		x	x	x			x											x	2	2		1			2	3
PCB-187		x					x											x	1	1		1			1	2
PCB-189	x													x					1	1					1	1
PCB-194			x											x	x				2	1					2	1
PCB-206	x		x						x						x				3				1		4	
4´-OH-CB107															x				1						1	
3´-OH-CB138		x						x				x						x	2	2		1			2	3
4-OH-CB146								x				x							1	1					1	1
4´-OH-CB159	x	x	x	x										x		x		x	2	4		1			2	5
4-OH-CB187								x				x							1	1					1	1
BDE-47		x		x		x	x								x			x	2	3		1			2	4
HCB								x												1						1
Oxychlordane		x	x					x				x							2	2					2	2
Trans-nonachlor															x				1						1	
p,p´-DDT															x				1						1	
p,p´-DDE															x				1						1	
β-HCH		x		x		x								x		x		x		5		1				6
Mirex			x																1						1	
Σ POPs	6	14	10	5		6	6	6	1		4			5	11	2		8	36	37		8	1		37	45

[1] Sum of POPs indicating hepatotoxicity
[2] Sum of POPs indicating renal toxicity
[3] Sum of POPs indicating myotoxicity