Humoral immunity and oxidative stress in black-legged kittiwake (*Rissa tridactyla)* and common eider (*Sommateria mollissima*) from Svalbard – related to bioaccumulated organochlorine contaminants

Lisa Maria Lindsøe

Master thesis in ecotoxicology
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Abstract

Organochlorines can possibly cause toxic effects in birds. In this thesis, levels and relationships between health parameters, organochlorine contaminants (OCs) and body condition index (BCI) in black-legged kittiwakes and common eiders were assessed. Immunoglobulin Y (IgY) levels and Oxidative stress index (OSI) were measured in plasma of birds, and served as health parameters. OCs were measured in pectoral muscle of birds and served as explanatory variables. In addition body condition index, as a measure of the physiological state in individual birds, were examined. Variations in IgY, OSI, OCs and BCI were also assessed between years, months and two locations (Liefdefjorden and Kongsfjorden) on Svalbard. Organochlorine pesticides explained a significant amount of variation in plasma IgY in black-legged kittiwakes, while polychlorinated biphenyls explained a significant amount of variation in plasma IgY in common eiders. OSI was only explained by polychlorinated biphenyls in black-legged kittiwakes. Regarding variations in IgY, OSI, OCs and BCI between years, months and locations no clear conclusions was made.. However, a pattern of no increase in bioavailable OCs was suggested for Kongsfjorden. Also, higher concentrations of some OCs were seen in Kongsfjorden compared to Liefdefjorden.

Abbreviations

AMAP – Arctic monitoring assessment program

BCI – Body condition index

COPOL – Contaminants in Polar Regions

DDE – dichlorodiphenyldichloroethylene

ELISA – Enzyme linked immunosorbent assay

GLM – Generalized Linear Model

HCB – Hexachlorobenzene

HCH – Hexachlorocyclohexane

NILU – Norwegian Institute for Air Research

OCs – Organochlorines

OCP – Organochlorine pesticide

OSI – Oxidative Stress Index

PCA – Principal Component Analysis

PCB – Polychlorinated Biphenyls

POP – Persistent organic pollutant

ROS – Reactive Oxygen Species

TAS – Total Antioxidant Status

TOS – Total Oxidant Status

IgM – Immunoglobuline M

IgY – Immunoglobuline Y

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1 Introduction

1.1 Background

Organochlorines (OCs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are some of the dominating persistent organic pollutants (POPs) in the environment, and are distributed all over the world (Muir et al. 1992; Wright 2002). The main sources of such pollutants are industry and agriculture (Wright 2002; Ørbæk et al. 2007). Low degradability, low water solubility, volatility and lipid solubility are common properties which make OCs persistent and available for long range transport and biomagnification through food chains (AMAP 1998). Long range transport results in contamination of OCs even in areas with few or none local contamination sources, such as the Arctic (AMAP 1998). Svalbard is an arctic archipelago in northern Norway, and PCBs and dichlorodiphenyldichloroethylene (DDE) was reported in seabirds from Svalbard as early as 1972 (Bourne and Bogan 1972). A decline in OCs have been reported in the Arctic during the last decade (Braune et al. 2001; Bustnes et al. 2010), but new POPs with similar properties as OCs are emerging, which makes it important to understand how POPs may be toxic to wildlife, including seabirds (Letcher et al. 2010).

Birds are mainly exposed to OCs through diet (AMAP 1998). Due to biomagnification of OCs in the food chain, birds at higher trophic levels therefore have elevated concentrations of OCs compared to birds at lower trophic levels (Hobson 1993; Gabrielsen et al. 1995; AMAP 1998; Sagerup et al. 2009). Monitoring birds which represent different trophic positions is therefore of interest. Black-legged kittiwakes are piscivores, thus representing a bird strongly linked to the pelagic food chain (Lydersen et al. 1985; Lønne and Gabrielsen 1992; Mehlum and Gabrielsen 1993; Dahl et al. 2003; Lydersen et al. 2009). Common eiders are mainly molluscivores and representing a species strongly linked to the benthic food chain (Dahl et al. 2003; Lydersen et al. 2009). The differences in food preferences indicate that black-legged kittiwakes are on a higher trophic position compared to common eiders, thus containing higher OC concentrations (Hobson 1993; Murvoll et al. 2006).

OCs may cause toxic effects, such as immune alterations and oxidative stress, in birds (Grasman et al. 1996; Oakley et al. 1996; AMAP 1998; Machala et al. 1998; Ercal 2001; Murvoll et al. 2007). For example, a study on immune alterations on glaucous gulls (*Larus*

hyperboreus) on Svalbard showed strong decrease in immune function (heterophile and lymphocytes) with increasing concentrations of OCs such as PCBs and OCPs (Bustnes et al. 2004). In addition, a positive correlation between concentrations of OCs and numbers of parasites in glaucous gulls, suggests that OCs may suppress immune function (Sagerup et al. 2000). Regarding oxidative stress, which is an imbalance between reactive oxygen species (ROS) and antioxidants (Monaghan et al. 2009), a negative relationship have been found between liver vitamin E levels (an antioxidant) and OCPs in Brünnich Guillemots (*Uria lomvia*) from Svalbard (Murvoll et al. 2007).

1.2 Health parameters

When studying toxic effects of OCs, biomarkers related to bird health such as immunoglobulin Y (IgY) and oxidative stress index (OSI) are useful (Grasman et al. 1996; Wayland et al. 2010).

1.2.1 Immunoglobulin Y

The immune system provides a defense against invasion and infection by pathogens, as well as malfunctioning cells. A part of it is trained to discover any substances which are "non-self", such as pathogens, parasites, foreign proteins, and cancerous cells. If the immune systems fails it will result in disease, and reduced health in affected individuals (Fairbrother et al. 2004). The immune system in birds consists of innate or non-specific defense mechanism and specific acquired defense mechanism (Rose 1979). The innate immune system consists of bactericidal enzymes, phagocytes and interferon (Rose 1979). The acquired system consists of humoral immunity which is mediated by B-lymphocytes and cell-mediated immunity which is mediated by T lymphocytes (Rose 1979). T cells cooperate with B cells in the production of antibodies (McArthur et al. 1973). Immunoglobulins are antibodies, and three classes of immunoglobulins have been shown to exist in birds; IgA, IgM and IgY (Warr et al. 1995). IgY is the dominant form in the secondary humoral response (Davidson 2008). Measuring levels of IgY in plasma of birds may therefore be relevant as an indication of humoral immune protection.

1.2.2 Oxidative stress

Oxidative stress occurs when enzymatic and non-enzymatic antioxidants cannot fully neutralize reactive oxygen species (ROS) that are produced, so that free ROS remain for sufficiently long to interact with macromolecules within or outside the cell (Monaghan et al. 2009). Such imbalance have been suggested to be due to e.g. OC exposure, and may lead to mutations, pathologies, cellular ageing and even death in birds (Gracy et al. 1999). ROS is generated when electrons are passed on to molecular oxygen, causing a generation of the highly reactive oxyradicals such as the oxygen radical (O_2), superoxide anion radical (O2-), the hydroxyl radical (OH), the peroxyl radical (ROO), the alkoxyl radical (RO) and the nitric oxide radical (NO) (Kohen and Nyska 2002). An antioxidant (reducing agent) can be classified as a compound capable of preventing the oxidation process, or biological oxidative damage, such as uric acid and a variety of vitamins (Prior and Cao 1999). There are many ways to measure the rate of oxidation. Examples include the measurement of oxidative stress markers such as 8-Hydroxydeoxyguanosine, cyclooxygenase and glutathione-S-transferase (Kohen and Nyska 2002). However, it is, also interesting to measure the total status of antioxidants and oxidants. Such measurements in plasma can be done spectrophotometrically (Miller 1996; Erel 2004; Erel 2005).

1.3 Body condition

To fully understand the health of the bird it is interesting to assess the body condition index (BCI) as disease status have been suggested to be negatively correlated to body condition (Coles 1997; Møller et al. 1998). The body condition is a measure of the birds physiological state, and may be an indicator of the ability to cope with environmental contaminants such as OCs (Jakob et al. 1996). Looking at variation in body condition in relation to health parameters is therefore of interest. Body condition have also been linked to reproductive effort in common eiders (Milne 1976). Using a body condition index calculated as body mass divided by a linear measure of body size, such as wing length, raised to a power of three, is one way to assess the body condition of birds and was originally developed by the fisheries industry (Clark 1979; Jakob et al. 1996). For birds the amount of total reserves are vital, and there is a good correlation between body weight corrected for size and weight of fat (Laughlin 1975).

1.4 Variations between years, months and locations

Even though OCs are declining in most species and in the abiotic environment, there may still be variations in concentrations within species and between species depending on years, months and locations (Braune and Simon 2003). Differences in OCs between years may for example be due to differences in food supply (AMAP 1998). Monthly variations in OCs is often linked to variations in lipid content within birds (Bustnes et al. 2010). For example, female common eiders fast completely during nesting, and rely totally on lipid storages and muscle proteins for energy (Gorman and Milne 1971). Prior to the fasting, common eiders feed heavily and increase their body weight by 20% above winter levels. In this way common eiders obtains a lipid buffer for the fasting period (Gorman and Milne 1971). When fasting starts and lipids are depleted it results in release of different OCs into the blood (Bustnes et al. 2010). Internal variations in OC concentrations between months could cause alterations in health parameters such as oxidative stress and humoral immunity (Bustnes et al. 2004; Hanssen et al. 2004; Hanssen et al. 2005; Bourgeon et al. 2006). For example, a decrease in IgY-levels by 15% in female breeding common eiders have been reported (Bourgeon et al. 2006). Such alterations may be of importance in a greater ecological perspective because plasma IgY levels are positively related to offspring growth, and negatively related to brood reduction (Apanius and Nisbet 2006). In addition, reduced IgY levels may possibly increase susceptibility to infectious diseases, and then again affecting whole populations (Grasman et al. 1996).

Kongsfjorden and Liefdefjorden are two fjords located on the northwest coast and the northern coast of Svalbard, respectively. These two fjords are interesting study sites when assessing the climate change perspective, and differences between locations. While Kongsfjorden is a mix between arctic and Atlantic water (dominated by Atlantic water), Liefdefjorden is dominated by Arctic water masses (Svendsen et al. 2002; Warner et al. 2010). The variation in the different water masses between the two fjords are suggested to vary in the abundance of contaminants, with higher concentrations in Kongsfjorden than in Liefdefjorden (Vieweg et al. 2012). This is expected to be reflected in higher OC concentrations in birds in the Kongsfjorden area. In addition, with an increased influx of Atlantic water in Kongsfjorden, it would change the environment in the fjords towards boreal, which again brings in boreal species (Hop et al. 2002). New introduced species, due to warmer climate, have the potential to alter food web structures (Macdonald et al. 2003). This

may again change the trophic position of different species in the food web, and exposure to different OCs may change because of biomagnification (Hebert and Weseloh 2006).

1.5 Objectives

The main objective of the study was to clarify how IgY, OSI, OCs and BCI in black-legged kittiwakes and common eiders vary between years, months and between two areas. In addition the relationships between the two responses (IgY and OSI) and the presence of organochlorine pollutants in individual birds, as well as body condition, were assessed.

The main objective can be divided into following sub-aims for both species:

- *Was there a relationship between levels of plasma IgY and OSI and muscle concentrations of OCs in black-legged kittiwakes and common eiders?*
- *Was there a relationship between BCI and levels of IgY and OSI in black-legged kittiwakes and common eiders?*
- *Was there a relationship between levels of IgY and OSI in black-legged kittiwakes and common eiders?*

The main objective can be divided into following sub-aim for male black-legged kittiwakes:

- *Were there differences in IgY, OSI, OCs and BCI between birds collected July 2007 and July 2008 in Kongsfjorden?*

The main objective can be divided into following sub-aims for female common eiders:

- *Were there differences in IgY-levels, OSI, OCs and BCI between birds collected July 2007 Kongsfjorden and October 2007 Kongsfjorden?*
- *Were there differences in IgY-levels, OSI, OCs and BCI between birds collected July 2007, July 2008 and July 2009 Kongsfjorden?*
- *Were there differences in IgY-levels, OCs and BCI between birds collected July 2009 Liefdefjorden and July 2009 Kongsfjorden?*

2 Materials and Methods

Detailed lists of solutions and reagents have been included in Appendix C.

2.1 Areas

Sampling of seabirds was conducted as part of the Contaminants in Polar Regions (COPOL) project in Kongsfjorden (79˚ N, 12˚ E) and Liefdefjorden (79˚ N, 13˚ E), Svalbard, Norway (Figure 2.1). The birds were collected in the middle to the inner parts of the two fjords.

Muscle and plasma from common eider and black-legged kittiwake were sampled in different years, months and locations. The number of individuals from each group is given in table 2.1. All seabirds were adults and consisted of male black-legged kittiwakes and female common eiders.

Table 2.1 Number of black-legged kittiwakes and common eiders sampled from each year, month and fjord. Muscle samples for organochlorine analyses and plasma samples for effect analyses were sampled from each bird.

Species	Year	Month	Location	Number
Male black-legged kittiwake	2007	May	Kongsfjorden	2
		July	Kongsfjorden	6
	2008	July	Kongsfjorden	5
			Liefdefjorden	2
Female common eider	2007	July	Kongsfjorden	10
		October	Kongsfjorden	8
	2008	July	Kongsfjorden	8
			Liefdefjorden	2
	2009	July	Kongsfjorden	6
			Liefdefjorden	3

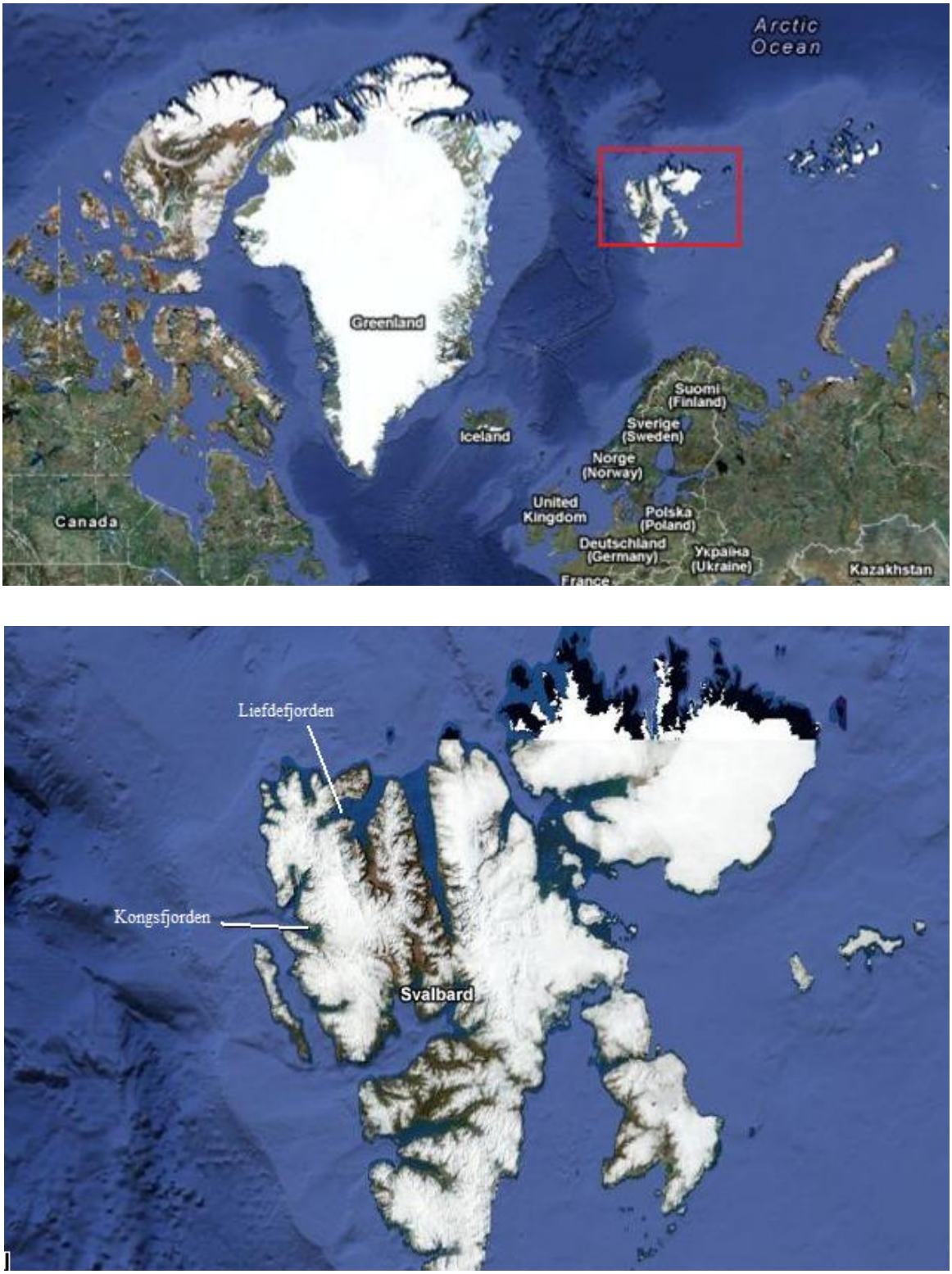

Figure 2.1 The Svalbard archipelago and the two fjords where the collection of black-legged kittiwakes and common eiders was done in 2007, 2008 and 2009. From Google maps with some modifications.

2.2 Sampling

All sampling of birds was approved by the Governor of Svalbard. Blood, for effect analyses, was sampled from the heart of the birds directly after death with a heparinized 20mL syringe and a 21-gauge needle. The syringe was precoated in heparin to avoid coagulation. Samples were kept cold until centrifugation was possible. To separate plasma and platelets, samples were centrifuged at 10000 rpm for 10 minutes. Plasma were then pipetted into tubes and frozen at -80°C until use.

Before any tissue samples were taken, the weight (g) and wing length (cm) was measured, and sex was determined. Weight and wing length was further used to calculate the body condition index (BCI), by using the equation; $BCI = weight/(wing\ length)^3 * 100$. The Pectoral muscle was sampled for organochlorine analyses, wrapped in aluminium foil and stored in marked zip-lock bags. The muscle samples were frozen at -20 °C immediately after sampling.

2.3 Measurement of Immunoglobulin Y-levels

Total Immunoglobulin Y levels was measured in plasma using enzyme-linked immunosorbent assay (ELISA), first described by Engvall et al. (1971) and Van Weemen et al. (1971). The particular method developed especially for avian species is described in Martinez et al. (2003).

2.3.1 Preliminary tests

Before measuring the immunoglobulin Y content of blood plasma it was necessary to determine the appropriate dilution of plasma according to the species being studied. To do so a range of plasma dilutions from 1/500 to 1/64000 were used. 12 plasma samples from each species and a control (carbonate-bicarbonate buffer) were diluted in a dilution series. Elisa plates were coated with 100 μL serial plasma dilutions in carbonate-bicarbonate buffer (0.1 M pH 9.6) and incubated overnight. Next, the plates were read using a Victor^3 multilabel plate reader (PerkinElmer, Finland), and the average quantity of immunoglobulin for the 12 samples of all the dilutions was calculated. The dilution closest to its linear range was selected. In this case it was 1/32000 for the common eider and 1/16000 for the black-legged kittiwake.

2.3.2 Procedure

Plasma samples from black-legged kittiwakes and common eiders were diluted in a 1/16000 and 1/32000 in carbonate-bicarbonate buffer, respectively. To make antibodies in the samples attached to the wells, 96-well ELISA plates were coated with 100 μL diluted plasma samples. Two samples (in two wells) were used for each bird, and two wells were filled with 100 μL carbonate-bicarbonate buffer (control). The ELISA plates were covered with an ELISA plate cover and incubated for one hour at 37°C. Plates were then incubated at 4°C overnight. To prevent unspecific binding, the wells were emptied and then rinsed with 200 μL of PBS-Tween solution in each well. 100 μL of blocking solution was then added to each well in order to block any plastic surface in the well that remained uncoated by the antigen. Plates were covered with a clear sticky plastic ELISA plate cover and incubated for one hour at 37°C. To prevent unspecific binding, wells were then rinsed with 200 μL of PBS-tween solution. Next, 100 μL of antibody solution was added to each well so that the antibodies could bind to the antigens in the well. The antibodies carried an enzyme conjugate that yielded colour when reacting with the colour-development solution. The plates were covered with ELISA plate covers and incubated for one hour at 37°C. After incubation, plates were rinsed three times with 200 μL of PBS-Tween. Finally, 100 μL of the colour development solution (Appendix C) was added to each well. The plates were covered with ELISA plate covers and incubated for one hour at 37°C. During this time a colour reaction happened following which, absorbance was measured at 405 nm using a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). Absorbance was measured to detect and quantify the amount of IgY in the sample.

2.4 Measurement of total oxidant- and total antioxidant-status

This method was used for measurement of total antioxidant status (TAS) and total oxidant status (TOS) and is described in Erel (2004; 2005). TAS and TOS were measured with intent to calculate the OSI. The analysis was performed by using Roche Cobas C111 (Roche Diagnostics, Germany), Total Oxidant Status assay kit (RL0024) and Total Antioxidant Status assay kit (RL0017) (Rel Assay Diagnostics, Germany). All reagents were provided by the kits and described in Appendix C.

2.4.1 Procedure:

A standard curve was made for both TAS and TOS using readymade standards from the kits provided by Rel Assay Diagnostics. The TAS standard curve ranged from 0.0 mmol Trolox equivalents/L (TAS standard) to 1.0 mmol Trolox equivalents/L with intervals of 0.25. The TOS standard curve ranged from 0.0 µmol H_2O_2 equivalents/L (TOS standard) to 20.0 µmol H_2O_2 equivalents/L with intervals of 5.0.

Plasma samples were thawed on ice, and placed directly in the Roche Cobas 111 without any dilutions. The Roche Cobas 111 did automated measurements. The TAS measurement is based on that reduced ABTS molecules being oxidized to $ABTS^{\cdot+}$ using hydrogen peroxide in acidic medium. In the acetate buffer solution (Reagent 2), the concentrate (deep green) $ABTS^{\cdot+}$ molecules stay more stable. While it is diluted with a more concentrated acetate buffer solution at high pH values (Reagent 1), the colour is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching proportional to their concentrations. This reaction is monitored spectrophotometrically and the bleaching rate is inversely related with the TAS of the sample. The reaction rate is calibrated with Trolox, and the assay results are expressed in (mmol Trolox equivalents/L) (Erel 2004).

For TAS measurements the instrument was set as recommended by the kit: 800 µL Reagent 1 was placed in cell and 50 µL standard and 10 µL sample was added. The initial absorbance was read at 660 nm for the first absorbance point. For the second absorbance point 125 µL of Reagent 2 was also added to the cell and incubated for 5 minutes at 37˚C. The absorbance was read a second time at 660 nm.

The results were calculated using this equation provided by the kit:

TAS, µmol Trolox Equivalent/L = (ΔabsStd1 - ΔabsSample) / (ΔabsStd1 - Δabsstd2) x standard 2 value

The TOS principle is based on that oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are present in the reaction medium (Reagent 1). The ferric ion makes a coloured complex with xylenol orange in an acidic medium (Reagent 2). The colour intensity, measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the assay results are expressed in (µmol H_2O_2 Equivalents/L) (Erel 2005).

For the TOS measurements the instrument was set as recommended by the kit: 1000 µL Reagent 1 was placed in a cell and 150 µL of the prepared standard and 21 µL plasma was added. The initial absorbance was read at 530 nm for the first absorbance point. For the second absorbance point 50 µL Reagent 2 was added to the cell and incubated for 5 minutes at 37˚C. The absorbance was read a second time at 530 nm.

The results were calculated using this equation provided by the kit:

TOS, µmol H_2O_2 Equivalent/L = (Δabssample / Δ AbsStd2) x Standard 2 value

The Oxidative Stress Index (OSI) which is used in the statistics was calculated by using this equation:

OSI = (TOS, µmol H_2O_2 Equivalent/L / TAS, µmol Trolox Equivalent/L) x 100

2.5 Organochlorine (OC) pollutants measurements

The OC analyses were performed as part of the COPOL project at the Norwegian institute for Air research`s (NILU) laboratory in Tromsø, Norway. All muscle samples of common eiders and black-legged kittiwakes were analyzed for a range of OCs; PCB 28, 33, 47, 52, 99, 101, 105, 118, 123, 128, 138, 141, 149, 153, 156, 157, 167, 170, 180, 183, 187, 189, 194, p,p`-DDT, o,p`-DDE, p,p`-DDE, o,p`-DDE, o,p`-DDD, α-HCH, β-HCH, γ-HCH, HCB, Heptachlor, *trans*-chlordane, *cis*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor and Mirex. A cold-column extraction technique described in Herzke et al. (2002; 2009) was used.

2.5.1 Procedure

Initially, 2-4 g of bird muscle tissue were homogenized and dried in 20X burnt sodium sulphate (600°C), and stored in the freezer overnight. Next, the dried homogenate was transferred to a glass column and a mixture of ^{13}C-labeled internal standards (2ng) (Appendix C) was added. Then it was extracted three times with 50 mL of 3:1 (v/v) cyclohexane:acetone solvent mixture at 100°C within 15 minutes (10 minutes static and 5 minutes heating time under a pressure of 10 MPa).

Lipid removal from the column extracts was done using a gel permeation chromatography system (GPC) (LATEK, Eppelheim, Germany). A mixture of ^{13}C-labeled PCBs and ^{13}C-labeled OCPs were used as internal standards. The extracts were concentrated and cleaned up using a florisil column (450°C). Before adding 20 µL octachloronapthalene (OCN) as a recovery standard, samples were evaporated to 200 µL. Lipid content (%) was determined gravimetrically for each sample using aliquots of the original organic extracts.

Chromatographic separation and detection was performed using an Agilent 7890A gas chromatograph equipped with a 5975c mass spectrometer (Agilent Technologies, Bøblingen, Germany). As a stationary phase for separation a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness; J&W, Folsom, USA) was used. Helium 6.0 quality was used as a carrier gas at flow rate 1 mL/min with a 1 µL injection volume in splitless mode using a split/splitless injector. The temperature program was set as follows: 70°C was held for 2 minutes, then, 15°C/min to 180°C and 5°C/min to 280°C which was then held for 10 minutes. An ionisation energy of 70 eV was used to ionised sample gas. The electron capture negative

ionisation mode was used for determination and quantification of the OCPs while the electron impact mode was used for determination and quantification of PCBs and DDTs.

2.5.2 Quality control

For each OC compound, quantification mass and a qualifier mass were necessary to relate the area proportions to the measurements of standard compounds. The quality assurance was carried out by using laboratory blanks and standard certified reference cod liver oil (NIST SRM 1588b, Gaithersburg MD). The limit of detection (LOD) was defined as 3 times signal/noise for each sample and compound (median detection limits for PCBs (22.0 pg/g ww), OCPs (15.0 pg/g ww)). All samples below LOD was reported to be non-detects. Blank samples were analyzed continuously during the experiment (2 blank/10 samples).

2.6 Statistical analyses

All statistical analyses were carried out using JMP 9.0 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). All levels of significance were set to p=0.05 for rejection of H0: “no difference between groups”.

IgY, OSI, OCs and BCI data were divided into different groups in relation to month, year, fjord and species. To decide whether to use parametric or non-parametric tests, the data were analysed for normal distribution (Siegel 1957) using q-q plot, and for homogeneity of variance using Levene`s test (Levene 1960). None of the analysed groups met these requirements, and non-parametric tests were therefore used. OC concentrations (∑PCBs and ∑OCPs), IgY-levels and OSI between groups were evaluated by using Mann-Whitney test and Wilcoxon test.

The PCA and GLM analyses were not divided into month, year and fjord because of low sample size, but one analysis was done separately for each species. Concentrations of OCs is presented in ng/g wet weight (table 3.1 and 3.2), and in pg/g wet weight (Appendix A). All OCs with more than 25% of the samples below the limit of detection (LOD) for the total dataset were excluded from the analysis. The remaining samples with concentration below the LOD were given a value of 0.5 x LOD. OCs not used in further analyses were; PCB 28, 33, 47, 52, 101, 123, 141, 149, 157, 189, 194, p,p`-DDT, o,p`-DDE, o,p`-DDE, o,p`-DDD, α-HCH, β-HCH, γ-HCH, Heptachlor and trans-chlordane. OCs used in further analyses were; PCB 99, 105, 118, 128, 138, 153, 156, 167, 170, 180, 183, 187, p,p`-DDE, HCB, cis-Chlordane, Oxychlordane, cis-Nonachlor, trans-Nonachlor and Mirex. All groups were log-transformed and inspected for outliers. Principal component analyses (PCA) were performed to check for co-variance between OC pollutants, and to reduce the OCs into a more manageable dimensionality. The PCA uses orthogonal transformation to convert possibly correlated values into a set of values that is not correlated. These values are called factors or principal components. Two factors that explained most of the variability in the plot were extracted per PCA, and for both PCA-plots factor one was named “log PCBs” and factor two was named “log OCPs”.

The extracted factors from the PCA were further used as variables in a generalized linear model (GLM) as they are independent and do not correlate. The factors (log PCBs and log OCPs) were used as explanatory variables, and IgY and OSI were used as response variables.

IgY and OSI were log-transformed and inspected for outliers. When variables showed to be insignificant, backwards selection was used to get the most optimal model. To validate the GLM, an inspection of residuals was performed. Only the final model graphs are shown. Linear regression and correlation analyses were all performed on log-transformed values.

3 Results

3.1 Variations between years, months and locations

3.1.1 Health parameters

The median IgY and OSI values when year, month and location were not taken into account, were generally higher in female common eiders compared to male black-legged kittiwakes (IgY; 3x, OSI; 1.3x).

Black-legged kittiwake

The IgY levels in male black-legged kittiwakes from Kongsfjorden were significantly higher in July 2008 compared to July 2007 (Mann-Whitney, Df=1, p=0.006) (Figure 3.1A). OSI in male black-legged kittiwakes from Kongsfjorden did not significantly differ between July 2007 and July 2008 (Mann-Whitney, Df=1, p=0.5) (Figure 3.1B).

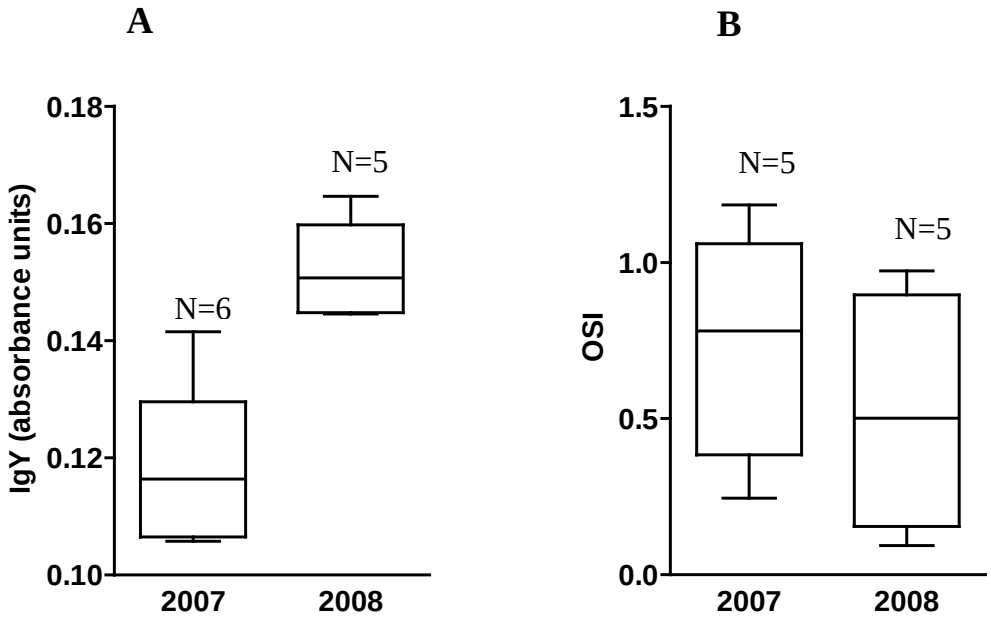

Figure 3.1 A. Immunoglobulin Y (IgY) levels (A) and Oxidative Stress Index (OSI) (B) from male black-legged kittiwakes collected in July 2007 and July 2008 in Kongsfjorden, Svalbard, presented with median, quartiles and 10/90 percentiles. IgY is given as absorbance units. OSI = (TOS, µmol H2O2 Equivalent/L / TAS, µmol Trolox Equivalent/L) x 100). TOS = Total Oxidant Status and, TAS = Total Antioxidant Status.

Common eider

The IgY levels in female common eiders from Kongsfjorden were significantly higher in July 2007 compared to October 2007 (Mann-Whitney, Df=1, p=0.01) (Figure 3.2A). No significant difference was found in OSI between female common eiders collected July 2007 and October 2007 in Kongsfjorden (Mann-Whitney, Df=1, p=0.7). July 2007 had a greater variation between samples compared to October 2007 (Figure 3.2B).

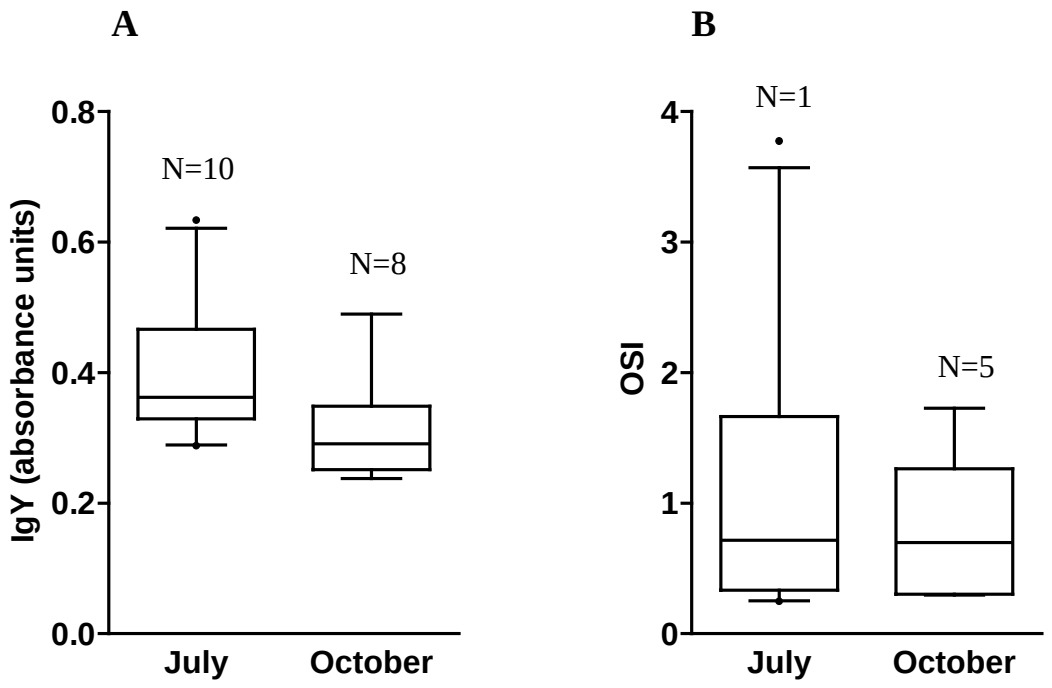

Figure 3.2 Immunoglobulin Y (IgY) levels (A) and Oxidative stress index (OSI) (B) from female common eiders sampled July 2007 and October 2007 in Kongsfjorden, Svalbard, presented with median, quartiles and 10/90 percentiles. IgY-levels are given as absorbance units. OSI = (TOS, µmol H2O2 Equivalent/L / TAS, µmol Trolox Equivalent/L) x 100. TOS = Total Oxidant Status and, TAS = Total Antioxidant Status.

No significant differences were found in IgY levels between female common eiders from Kongsfjorden collected July 2007, July 2008 and July 2009 (Wilcoxon, Df=2, p=0.1) (Figure 3.3A). OSI in female common eiders from Kongsfjorden showed that July 2007 had a significantly higher OSI compared to July 2008 (Wilcoxon, N=17, p=0.01). July 2007 and July 2008 were not significantly different from July 2009 (Wilcoxon, July 2007; N=16, p=0.4, July 2008; N=16, p=0.05) (Figure 3.3B).

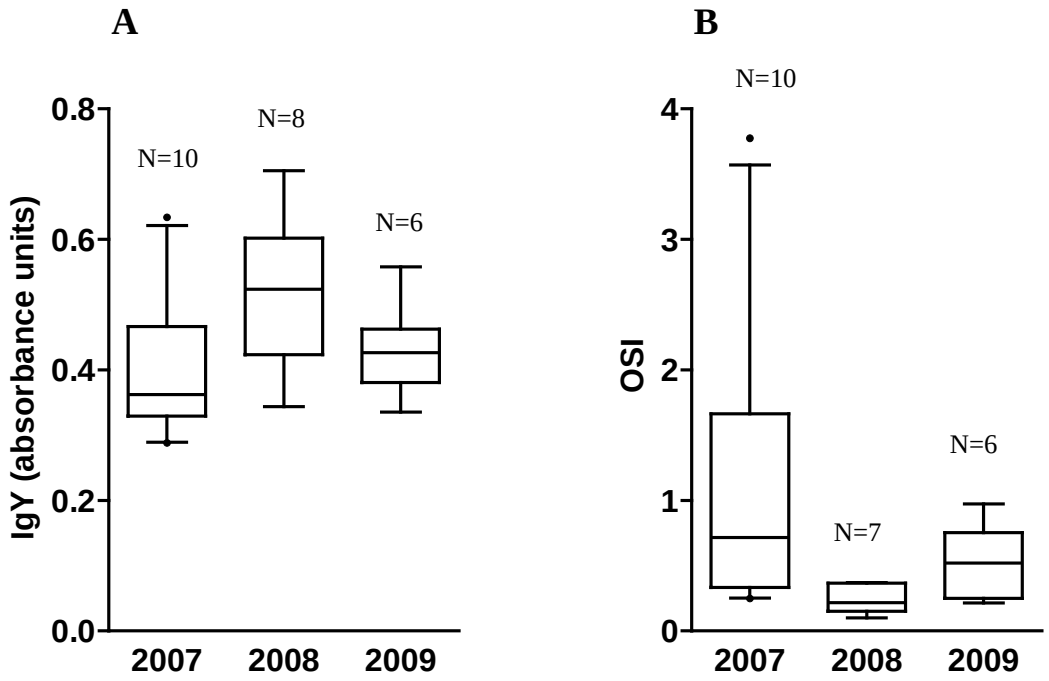

Figure 3.3 Immunoglobulin Y (IgY) levels (A) and oxidative stress index (OSI) (B) from female common eiders collected July 2007, July 2008 and July 2009 in Kongsfjorden, Svalbard, presented with median, quartiles and 10/90 percentiles. IgY levels are given as absorbance units. OSI = (TOS, µmol H2O2 Equivalent/L / TAS, µmol Trolox Equivalent/L) x 100. TOS = Total Oxidant Status and, TAS = Total Antioxidant Status. Levels not connected with the same letters are significantly different (Wilcoxon, p=0.01).

No significant differences were found in IgY-levels between female common eiders from July 2009 in Liefdefjorden and Kongsfjorden for log-transformed data (Mann-Whitney, Df=1, p=0.4). The median IgY-value was somewhat higher in Liefdefjorden (Figure 3.4).

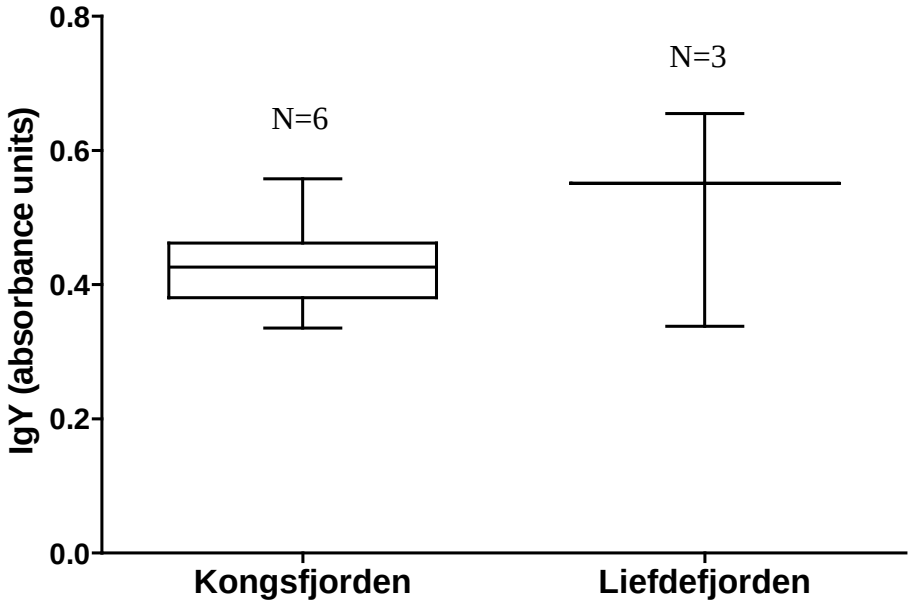

Figure 3.4 Immunoglobulin-Y (IgY) levels from female common eiders sampled July 2009 Kongsfjorden, and July 2009 Liefdefjorden, Svalbard. IgY-levels are given as absorbance units; median, quartiles and 10/90 percentiles.

3.1.2 Organochlorine concentrations

The ΣOCPs and ΣPCBs median concentrations, when year, month and location were not taken into account, were generally higher in male black-legged kittiwakes compared to female common eiders (∑PCBs; 47x, ∑OCPs; 30x) (table 3.1 and 3.2).

Black-legged kittiwake

No significant difference was found in ΣPCBs or ΣOCPs between July 2007 and July 2008 in Kongsfjorden (Mann-Whitney, ΣPCBs; Df=1, p=0.9. ΣOCPs; Df=1, p=0.8). Differences between May 2007 and July 2007 could not be statistically tested due to too few individuals in May 2007 (Table 3.1). The same was true for Kongsfjorden 2008 and Liefdefjorden 2008, with too few individuals in Liefdefjorden (Table 3.1).

Table 3.1 Concentrations of ∑PCBs and ∑OCPs in pectoral muscle of male black-legged kittiwakes from Svalbard. Concentrations given in ng/g wet weight and presented with median, minimum (min) and maximum (max) values.

	ΣPCBs		**ΣOCPs**	
	Median (min – max)	*N*	*Median (mean – max)*	*N*
Kongsfjorden, May 2007	198.8 (174.9 – 222.7)	2	93.9 (86.8 – 101.0)	2
Kongsfjorden, July 2007	404.3 (215.7 – 648.4)	6	65.7 (17.5 – 88.1)	6
Kongsfjorden, July 2008	469.5 (164.5 – 684.3)	5	49.4 (30.4 – 81.0)	5
Liefdefjorden, July 2008	341.9 (251.1 – 432.7)	2	85.1 (61.8 – 108.1)	2
Total	383.4 (164.5 – 684.3)	16	62.5 (17.5 – 108.1)	16

∑PCBs (Polychlorinated Biphenyls) includes; PCB 99, 105, 118, 128, 138, 153, 156, 167, 170, 180, 183, 187.
∑OCPs (Organochlorinated Pesticides) includes; p,p`-DDE (dichlorodiphenyldichloroethylene), HCB (Hexachlorobenzene), *cis*-chlordane, *cis*-Nonachlor, *trans*-Nonachlor.

Common eider

There was a significant higher ΣPCBs concentration in July 2007 compared to October 2007 (Mann-Whitney, Df=1, p=0.0002), but no significant difference in ΣOCPs variation (Mann-Whitney, Df=1, p=0.07). There were no significant differences in ΣPCBs variation between July 2007, July 2008 and July 2009 in Kongsfjorden (Wilcoxon, Df=2, p=0.2). However, there were significantly higher ΣOCPs concentrations in July 2008 compared to July 2009 (Wilcoxon, p=0.0024), and July 2007 compared to July 2009 (Wilcoxon, p=0.0014). There were significantly higher concentrations of ΣPCBs in Kongsfjorden 2009 compared to Liefdefjorden 2009 (Mann-Whitney, Df=1, p=0.04), but not in ΣOCPs (Mann-Whitney, Df=1, p=0.6). It was not possible to test the difference between Liefdefjorden and Kongsfjorden in July 2008 due to low sample size in Liefdefjorden 2008 (Table 3.2).

Table 3.2 Concentrations of ∑PCBs and ∑OCPs in pectoral muscle of female common eiders from Svalbard. Concentrations are given in ng/g (wet weight) and presented with median, minimum (min) and maximum (max) values.

	ΣPCBs		**ΣOCPs**	
	Median (min – max)	*N*	*Median (min – max)*	*N*
Kongsfjorden, July 2007	6.4 (1.5 – 27.6)	10	4.3 (0.8 – 11.6)	10
Kongsfjorden, October 2007	0.9 (0.7 – 1.7)	8	0.8 (0.5 – 5.8)	8
Kongsfjorden, July 2008	12.6 (4.3 – 34.8)	8	4.1 (2.0 – 20.5)	8
Liefdefjorden, July 2008	8.9 (7.4 – 10.3)	2	4.1 (3.7 – 4.4)	2
Kongsfjorden, July 2009	11.3 (8.1 – 49)	6	0.02 (0.01 – 0.09)	6
Liefdefjorden, July 2009	4.4 (4.1 – 8.3)	3	0.06 (0.01 – 0.07)	3
Total	8.1 (0.7 – 49)	37	2.1 (0.01 – 20.5)	37

∑PCBs (Polychlorinated Biphenyls) includes; PCB 99, 105, 118, 128, 138, 153, 156, 167, 170, 180, 183, 187. ∑OCPs (Organochlorinated Pesticides) includes; HCB (Hexachlorobenzene), *cis*-chlordane, Oxychlordane, *cis*-Nonachlor, *trans*-Nonachlor.

3.1.3 Body Condition Index

Black-legged kittiwake

There were no difference in BCI between black-legged kittiwakes from Kongsfjorden in July 2007 and July 2008 (Mann-Whitney, Df=1, p=0.2). Differences between May 2007 and July 2007 could not be tested due to too few individuals in May 2007 (Table 3.3). The same was true for Kongsfjorden 2008 and Liefdefjorden 2008, with too few individuals in Liefdefjorden (Table 3.3).

Table 3.3 Body condition index (BCI) in black-legged kittiwakes presented with median, minimum (min), and maximum (max) values. BCI = weight/(wing length)^3 * 100

	N	**BCI**
		Median (max – min)
Kongsfjorden, May 2007	2	1.34 (1.28 – 1.40)
Kongsfjorden, July 2007	6	1.13 (0.99 – 1.27)
Kongsfjorden, July 2008	5	1.16 (1.11 – 1.27)
Liefdefjorden, July 2008	-	-
Total	13	1.16 (0.99 – 1.40)

Common eider

Common eiders from Kongsfjorden October 2007 had significantly higher BCI compared to common eiders from Kongsfjorden July 2007 (Mann-Whitney, Df=1, p=0.0005) (Table 3.4). No significant difference was found in weight between July 2007, July 2008 and July 2009 in Kongsfjorden (Wilcoxon, weight; Df=2, p=0.7). In addition, no significant difference was found in BCI between Kongsfjorden July 2009 and Liefdefjorden July 2009 (Mann-Whitney, weight; Df=1, p=1).

Table 3.4 Body condition index (BCI) in common eiders presented with median, minimum (min) and maximum (max) values. BCI = weight/(wing length)^3 * 100

	N	**BCI**
		Median (min – max)
Kongsfjorden, July 2007	10	6.6 (4.7 – 8.8)
Kongsfjorden, October 2007	8	9.2 (8.6 – 14.9)
Kongsfjorden, July 2008	8	6.6 (5.1 – 8.2)
Liefdefjorden, July 2008	-	-
Kongsfjorden, July 2009	6	7.2 (5.2 – 7.5)
Liefdefjorden, July 2009	3	6.9 (6.4 – 7.6)
Total	35	3.1 (4.7 – 14.9)

3.2 Relationship between health parameters and organochlorines

3.2.1 Black-legged kittiwake

A PCA was performed to check for co-variation among the OCs, and reduce the dimensionality in the dataset. The first two factors in the PCA totally explained 82% of the variability in the contaminant concentrations among the samples. The different PCB congeners loaded strongest on factor 1(Figure 3.5 and Table 3.5). The OCPs generally loaded strongest on factor 2, except Oxychlordane and Mirex which loaded on factor 1(Figure 3.5 and Table 3.5). Based upon the PCA, factor 1 was further used as a representative for the PCBs (log PCBs) and Factor 2 was used as a representative for the OCPs (log OCPs). Oxychlordane and Mirex was included in the log PCBs group.

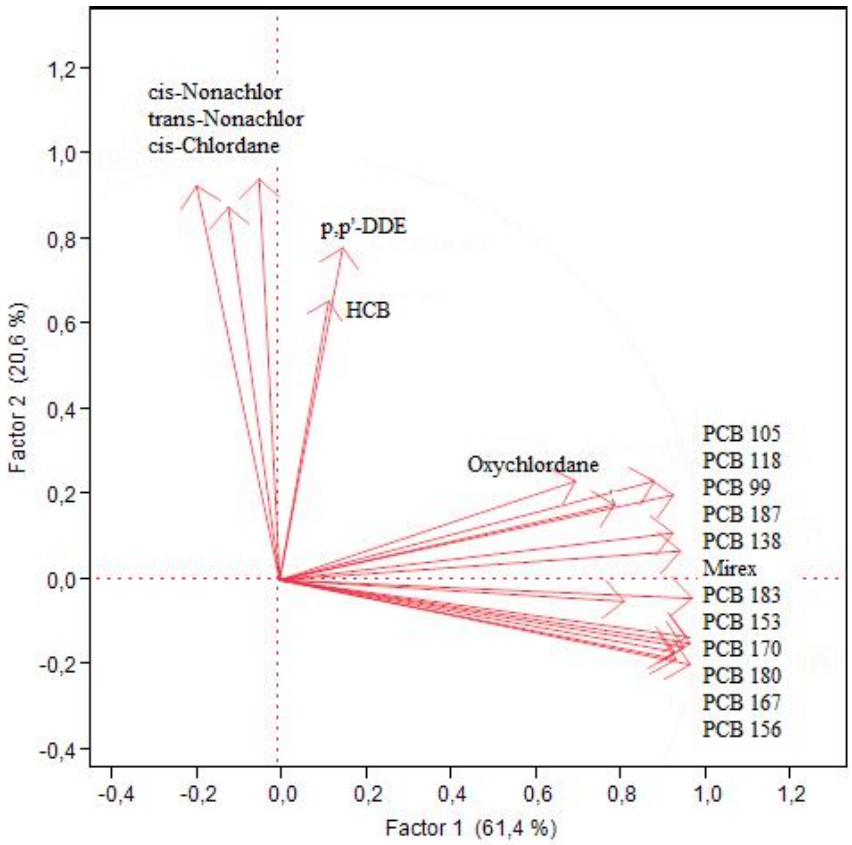

Figure 3.5 Principal component analysis of organochlorine concentrations log(pg/g wet weight) in muscle samples from male black-legged kittiwakes (N=15). Percent variability explained by factor 1 and factor 2 is given in brackets. HCB = Hexachlorobenzene, PCB = polychlorinated biphenyl p,p`-DDE = dichlorodiphenyldichloroethylene.

Table 3.5 Factor loadings of a range of organochlorines based upon a principal component analysis in black-legged kittiwakes. 1 = 100 %. HCB = Hexachlorobenzene, PCB = polychlorinated biphenyl p,p`-DDE = dichlorodiphenyldichloroethylene.

	Factor 1	**Factor 2**
log PCB 99	0.93	< 0.2
log PCB 105	0.88	< 0.2
log PCB 118	0.93	< 0.2
log PCB 128	0.79	<0.2
log PCB 138	0.97	< 0.2
log PCB 153	0.97	< 0.2
log PCB 156	0.97	< 0.2
log PCB 167	0.94	< 0.2
log PCB 170	0.95	< 0.2
log PCB 180	0.93	< 0.2
log PCB 183	0.96	< 0.2
log PCB 187	0.95	< 0.2
log p,p`-DDE	< 0.2	0.78
log HCB	< 0.2	0.66
log *cis*-Chlordane	< 0.2	0.88
log Oxychlordane	0.70	0.3
log *cis*-Nonachlor	< 0.2	0.93
log *trans*-Nonachlor	0.2	0.94
log Mirex	0.81	< 0.2

Relationship between OCs and IgY

With increasing log OCPs, the log IgY decreased (GLM; Table 3.6 and Figure 3.6). PCBs did not significantly affect the IgY levels (GLM; Table 3.6).

Table 3.6 Generalized Linear Model with backwards selection. log PCBs and log OCPs as explanatory factors for Immunoglobulin Y-levels in male black-legged kittiwakes sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. Analysis was done for log-transformed values. Significant p-values is marked with a '*'. N = 15, R^2 = 0.31.

	Estimate	S.E	Chi sq.	p-value	95 % C.I
Intercept	-0.89	0.01	85.9	<0.0001*	-0.92 - -0.86
log OCPs	-0.03	0.01	5.5	0.02*	-0.06 – -0.006
Rejected variable					
log PCBs	-	-	2.03	0.2	-

PCBs = Polychlorinated Biphenyls, OCPs = Organochlorinated Pesticides. "log PCBs" and "log OCPs" are samples scores from Factor 1 and Factor 2, respectively, extracted from a principal component analysis on organochlorine concentrations in black-legged kittiwake.

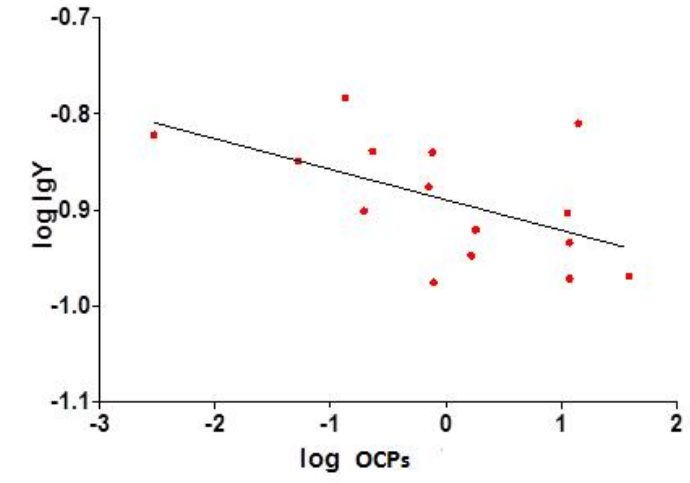

Figure 3.6 Linear Relationship between log IgY (Immunoglobulin Y) and log OCPs (Organochlorinated Pesticides) in male black-legged kittiwakes sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. "log OCPs" is the sample scores from factor 2 extracted from a principal component analysis on organochlorine concentrations in black-legged kittiwake. Model: log IgY = -0.89 – 0.03*log OCPs. (N = 15, R^2 = 0.31 p=0.02).

Relationship between OCs and OSI

With increasing log PCBs, the log OSI increased (GLM; Table 3.7 and Figure 3.7). log OCPs did not significantly affect the log OSI (GLM; Table 3.7). Because there were two OSI values missing, the correlating PCB and pesticide values were excluded from the analysis.

Table 3.7 Generalized linear model with backwards selection. log PCBs and log OCPs as explanatory factors for oxidative stress index in male black-legged kittiwakes sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. Significant p-values is marked with a '*'. Analysis was done for log-transformed values. N = 13, R-square = 0.27

	Estimate	**S.E**	**Chi sq.**	**p-value**	**95 % C.I**
Intercept	-0.45	0.08	14.9	<0.0001*	-0.62 – -0.26
log PCBs	0.2	0.09	4.1	0.04*	0.008 – 0.41
Rejected variable					
log OCPs	-	-	<0.0001	1	-

PCBs = Polychlorinated Biphenyls, OCPs = Organochlorinated Pesticides. "log PCBs" and" log OCPs" are sample scores on Factor 1 and Factor 2, respectively, extracted from a principal component analysis on organochlorine concentrations in black-legged kittiwake.

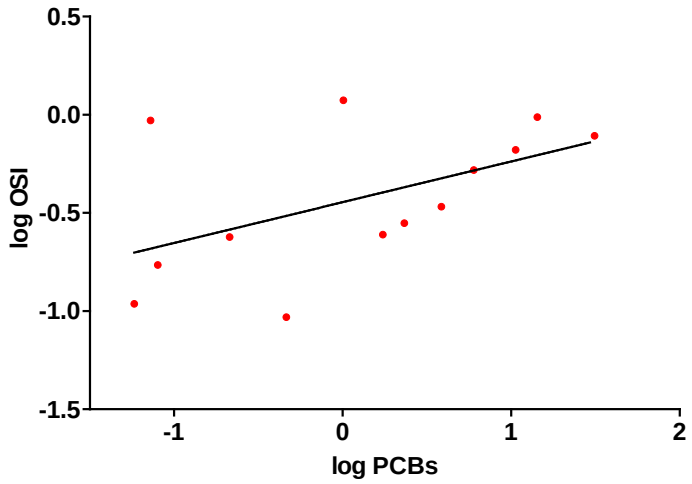

Figure 3.7 Linear Relationship between log OSI (Oxidative Stress index) and log PCBs (Polychlorinated Biphenyls) in male black-legged kittiwakes sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. "log PCBs" are sample scores from Factor 1 extracted from the principal component analysis on organochlorine concentrations in black-legged kittiwake. Model: log OSI = -0.45 + 0.21*log PCBs. (N = 13, R^2 = 0.27 p=0.04).

3.2.2 Common eider

The same method was performed for common eiders as for black-legged kittiwakes to reduce the dimensionality in the dataset. The two factors in the PCA totally explained 89.2% of the variability in organochlorine concentrations among the samples. The PCA indicated that all the PCB congeners loaded strongest on factor 1(Figure 3.8 and Table 3.8). This was also the case for the OCPs *p,p`*-DDE and Mirex, but Mirex also loaded high (0.51) on factor 2. All other OCPs loaded high mainly on factor 2 (Figure 3.8 and Table 3.8). Based upon the PCA, factor 1 was further used as a representative for the PCBs (log PCBs) and Factor 2 was used as a representative for the OCPs (log OCPs) (Figure 3.4 and table 3.4). *p,p`*-DDE and Mirex was included in the "log PCBs" group.

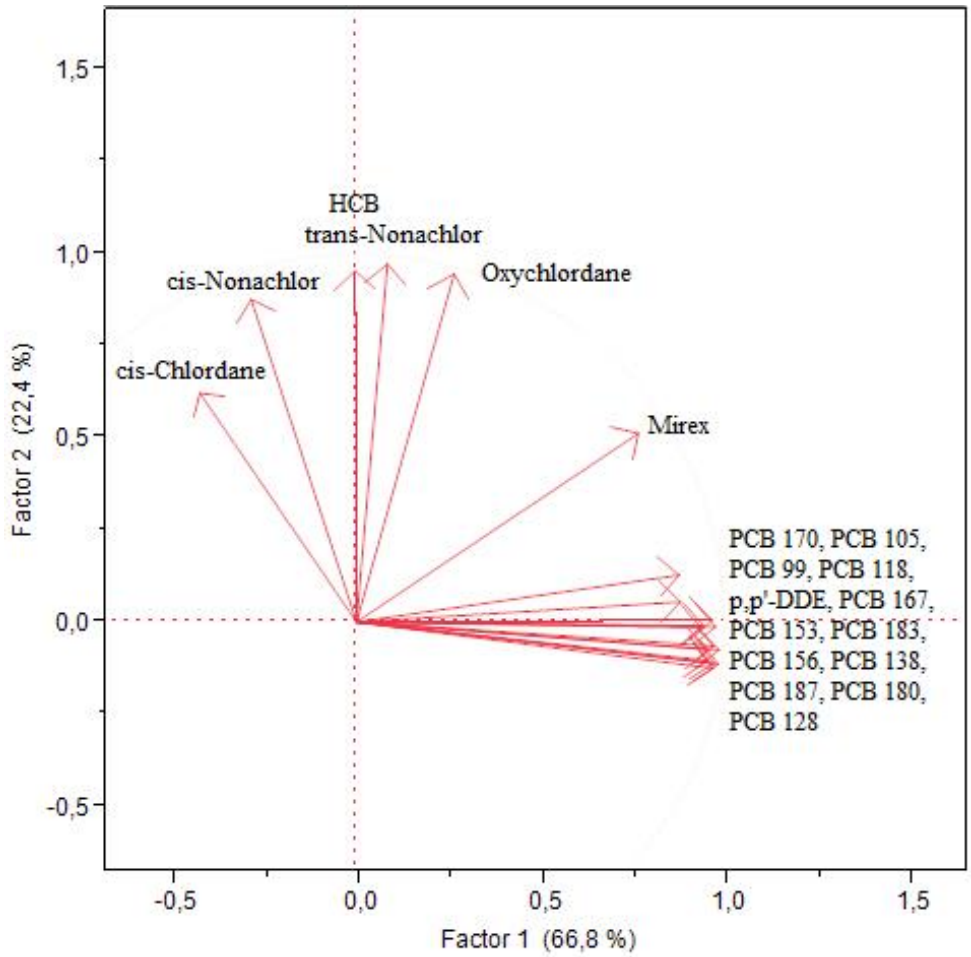

Figure 3.8 Principal component analysis for organochlorine pollutants log(pg/g wet weight) in muscle samples from female common eiders (N=37). Percent variability explained by factor 1 and factor 2 is given in brackets. HCB = Hexachlorobenzene, PCB = polychlorinated biphenyl p,p`-DDE = dichlorodiphenyldichloroethylene.

Table 3.8 Rotated factor loading for different persistent organic pollutants in female common eiders. 1 = 100%. HCB = Hexachlorobenzene, PCB = polychlorinated biphenyl p,p`-DDE = dichlorodiphenyldichloroethylene.

	Factor 1	**Factor 2**
log PCB 99	0.94	< 0.1
log PCB 105	0.88	< 0.1
log PCB 118	0.97	< 0.1
log PCB 128	0.98	< 0.1
log PCB 138	0.98	< 0.1
log PCB 153	0.97	< 0.1
log PCB 156	0.94	< 0.1
log PCB 167	0.87	< 0.1
log PCB 170	0.96	< 0.2
log PCB 180	0.95	< 0.1
log PCB 183	0.97	< 0.1
log PCB 187	0.98	< 0.1
log p,p`-DDE	0.98	< 0.1
log HCB	< 0.1	0.95
log *cis*-Chlordane	-0.43	0.62
log Oxychlordane	0.3	0.95
log *cis*-Nonachlor	< 0.1	0.88
log *trans*-Nonachlor	< 0.1	0.97
log Mirex	0.77	0.51

Relationship between OCs and IgY

With increasing log PCBs, the log IgY increased (GLM; Table 3.9 and Figure 3.9) log OCPs did not contribute to a significant amount of the variation in log IgY (Table 3.9).

Table 3.9 Generalized Linear Model with backwards selection. log PCBs and log OCPs as explanatory factors for Immunoglobulin Y- levels in female common eiders sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. Analysis was done for log-transformed values. Significant p-values is marked with a *. N = 37, R^2 = 0.19

	Estimate	S.E	Chi sq.	p-value	95 % C.I
Intercept	-0.39	0.02	94.4	<0.0001*	-0.42 - -0.35
log PCBs	0.06	0.02	7.8	0.005*	0.02 – 0.09
Rejected variable					
log OCPs	-	-	0.07	0.8	-

PCBs = Polychlorinated Biphenyls, OCPs = Organochlorinated Pesticides. “log PCBs” and” log OCPs” are sample scores on Factor 1 and Factor 2, respectively, extracted from the principal component analysis on organochlorine concentrations in common eider.

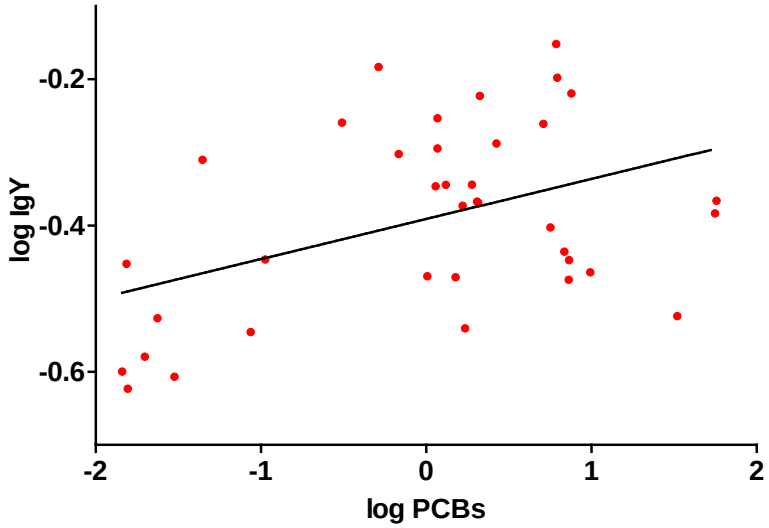

Figure 3.9 Linear Relationship between log IgY (Immunoglobulin Y) and log PCBs (Polychlorinated Biphenyls) in female common eiders sampled in 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. “log PCBs” is sample scores on Factor 1, extracted from the principal component analysis on organochlorine concentrations in common eider. Model: log IgY = -0.39 + 0,05*log PCBs (N = 37, R^2 = 0.19 p=0.005).

Relationship between OCs and OSI

PCBs or OCPs did not significantly contribute to explaining variation in OSI (GLM; Table 3.10). Because there were six OSI values missing, the correlating PCB and pesticide values were excluded from the analysis.

Table 3.10 Generalized Linear Model with log PCBs (Polychlorinated Biphenyls) and log OCPs (Organochlorinated Pesticides) as explanatory factors for oxidative stress index in female common eiders. Analysis is done for log-transformed values. N = 31

Rejected model	Chi sq.	p-value
Whole model	1.82	0.4
log PCBs	1.27	0.3
log OCPs	0.64	0.4

PCBs = Polychlorinated Biphenyls, OCPs = Organochlorinated Pesticides. “log PCBs” and” log OCPs” are sample scores on Factor 1 and Factor 2, respectively, extracted from the principal component analysis on organochlorine concentrations in common eider.

3.3 Relationship between health parameters and body condition index

To ascertain that variation BCI did not significantly influence the variation in effect parameters (IgY and OSI), linear regression analyses was performed for all black-legged kittiwakes and all common eiders.

There were no significant relationships between log BCI and log IgY, or log BCI and log OSI in either black-legged kittiwakes or common eiders (Table 3.11 and Table 3.12).

Table 3.11 Linear regression analysis for log BCI and log IgY, and log BCI and log OSI in male black-legged kittiwakes from 2007, 2008 and 2009 in Kongsfjorden and Liefdefjorden, Svalbard. Significant p-values marked with a `*`.

X	Y	N	R sq.	F-value	p-value	Relationship
log BCI	**log IgY**	13	0.05	0.7	0.4	none
log BCI	**log OSI**	11	0.2	2.4	0.2	none

BCI = Body condition index, IgY = Immunoglobulin Y, OSI = Oxidative Stress Index,

Table 3.12 Linear regression analysis for log BCI and log IgY, and log BCI and log OSI in female common eiders from 2007, 2008 and 2009 in Liefdefjorden and Kongsfjorden, Svalbard.

X	Y	N	R sq.	F-value	p-value	Relationship
log BCI	**log IgY**	35	0.09	3.3	0.08	none
log BCI	**log OSI**	30	<0.0001	0.002	0.9	none

BCI= Body Condition index, IgY = Immunoglobulin Y, OSI = Oxidative Stress Index.

3.4 Correlation between health parameters

There was no significant correlation between OSI and IgY in black-legged kittiwake using parametric pairwise correlation (Table 3.13). There was however significant negative correlation between OSI and IgY in common eiders (Table 3.13 and Figure 3.10B). Two OSI values were missing for black-legged kittiwakes and six for common eiders. The correlating IgY values were therefore excluded from the analysis.

Table 3.13 Correlation between log OSI and log IgY in black-legged kittiwakes (all males) and common eiders (all females) sampled in 2007, 2008 and 2009 in Liefdefjorden and Kongsfjorden, Svalbard. Significant p-values is marked with a '*'.

Species	N	R	p-value
Black-legged Kittiwake	13	0.05	0.9
Common Eider	31	0.5	0.006*

OSI = Oxidative Stress Index, IgY = Immunoglobulin Y.

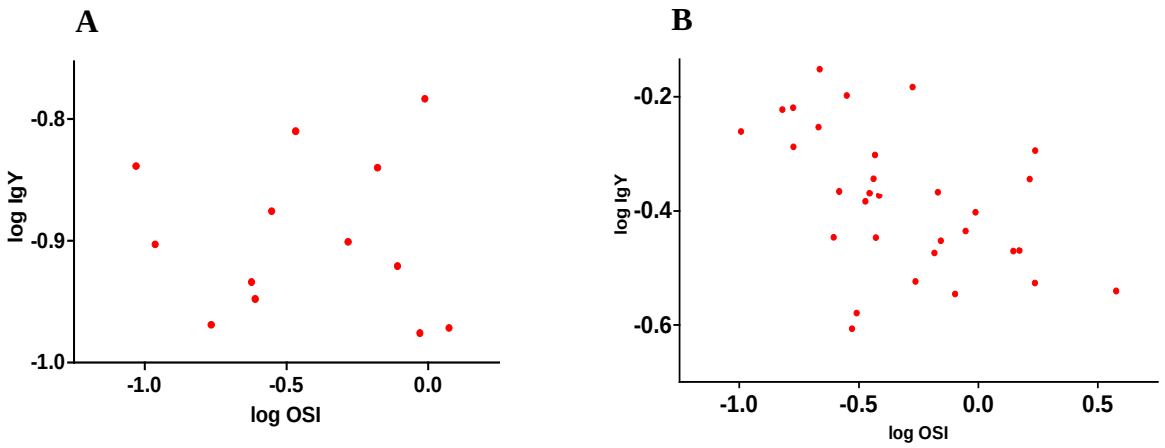

Figure 3.10 Correlation between log IgY and log OSI in plasma samples of male black-legged kittiwakes (A) and female common eiders (B) sampled in 2007, 2008 and 2009, in Kongsfjorden an Liefdefjorden, Svalbard. See Table 3.9 for correlation results.

4 Discussion

4.1 Samples

Samples used for health parameter analyses and OC analyses were plasma and muscle, respectively. Plasma was used because it is a suitable matrix for the measurement of IgY and OSI (Miller 1996; Martinez et al. 2003). Muscle was chosen as study tissue because it has been indicated that there is a correlation between different body tissue concentrations and plasma concentrations of OCs in birds (Henriksen et al. 1998; Olafsdottir et al. 1998). As it is the total amount of OCs using wet weight that is the most appropriate when assessing effects, all OCs were presented in wet weight (Henriksen et al. 1996). However, it is important to note that correlations between concentration of OCs in tissue and plasma have been found using lipid weight (Parham et al. 1997; Henriksen et al. 1998; Olafsdottir et al. 1998). Still, it is reasonable to assume that muscle concentrations of OCs in wet weight gives a good indication of variations in contaminant load within species.

4.2 Variations in health parameters, organochlorines and body condition

4.2.1 Variation between years

When assessing variations in health parameters and OC concentrations between only two or three years it is not possible to conclude on temporal trends. However, it is important to assess such differences to be able to have references when comparing levels and concentrations of health parameters and OCs with future studies assessing the same topics.

Black-legged kittiwake

The consistent muscle concentrations of ∑PCBs or ∑OCPs in black-legged kittiwakes between 2007 and 2008 in Kongsfjorden suggests that there have been no increase of bioavailable OCs to this area. This is consistent with a study by Helgason et al. (2008), showing a decline in ∑PCBs, hexachlorocyclohexane (HCH), oxychlordane and p,p`-DDE in black-legged kittiwakes from 1983-2003 in Northern Norway.

Interestingly, there were significantly higher levels of IgY in plasma in black-legged kittiwakes in 2008 compared to 2007, suggesting better health status in birds sampled in 2008. This may indicate annual variations in food resources, which may affect humoral immunity (in this paper measured as IgY) (Eraud et al. 2008). However, there were no significant variations in BCI between 2007 and 2008, and the reason for the higher IgY- levels in 2008 is therefore unclear. There was no significant difference in OSI in plasma of black-legged kittiwake comparing the two years, which indicated that the OSI status of the birds were stable between 2007 and 2008. To my knowledge, no studies have investigated the variation in IgY and OSI between years, and it is therefore not possible to compare these results to other studies.

Common eider

∑PCBs concentration in muscle in common eider did not vary between 2007, 2008 and 2009 in Kongsfjorden, suggesting the same consistent pattern as in black-legged kittiwakes. However, a decline was observed in ∑OCPs concentrations in muscle in 2009, compared to 2007 and 2008. The relatively low ∑PCBs and ∑OCPs concentrations in muscle in common eider are consistent with earlier findings (Braune et al. 2001; Bustnes et al. 2010).

The IgY-levels in plasma in common eiders did not differ between 2007, 2008 and 2009, and were different from results found in black-legged kittiwakes. This may be because of generally low levels of OCs in this species. Also the BCI in birds from 2007, 2008 and 2008 were similar. OSI in plasma in common eiders from 2007 were higher compared to OSI-levels from 2008, but 2007 and 2008 were not significantly different compared to 2009. One possible explanation for the variations in plasma OSI is exposure to trace metals such as mercury and selenium. These metals have been documented to induce oxidative stress in different bird species such as common loon *(Gavia immer)* and mallard ducks (*Anas platyrhynchos*) (Hoffman and Heinz 1998; Kenow et al. 2008), and are reported to be found in birds from Svalbard (Norheim et al. 1992; Saunes 2011)

4.2.2 Variation between months

It was only possible to test variations in OCs, health parameters and BCI between months in common eiders due to a small sample size of black-legged kittiwakes. However, a study done by Hallanger et al. (2011) on black-legged kittiwakes from Kongsfjorden in 2007 showed a decrease in PCB 52, PCB 99, PCB 101, as well as a range of OCPs, from May to July and October. The same pattern was also observed in little auks (*Alle alle*) (Hallanger et al. 2011). In common eiders, muscle samples from July and October were available, and the ∑PCBs concentrations in muscle showed a significant decrease from July to October, indicating the same pattern as in black-legged kittiwakes and little auks (Hallanger et al. 2011). In July, in Kongsfjorden, the chick-rearing period had started, and the common eiders had started eating after the laying and incubation period (Evenset, pers. Comm.). A study by Bustnes et al. (2010), have earlier showed an increase in blood OC-concentrations in common eiders during incubation. However, because samples in this study is from July, which is soon after egg incubation and in early stages of the chick-rearing period, it is difficult to know if ∑PCBs and ∑OCPs concentrations in July were high or low compared to concentrations in common eider before or during egg-laying and fasting.

Some OCs are known to be potentially immunotoxic (Bustnes et al. 2004), and a decrease in ∑PCBs concentrations in muscle from July to October in common eiders could therefore have caused higher IgY-levels in plasma of common eiders in October compared to July. However, the opposite was seen; there were significantly higher levels of IgY in female common eiders in July compared to October, in Kongsfjorden. Infections like influenza virus and parasites can induce antibody responses in birds, and may be a possible explanation for the variations in IgY-levels between months (Hanssen 2006). As IgY-levels only were measured in absorbance units and not in a specific concentration, it is not possible to give an indication to if the IgY-levels in common eider were high or low compared to other studies.

There were no differences in OSI in plasma in common eider between July and October in Kongsfjorden, suggesting that the OSI responds differently compared to IgY. It is also possible that oxidative stress in common eiders were prevented by a continuous supply of antioxidants through food (Kohen and Nyska 2002). As expected, BCI in common eiders were lower in July, straight after incubation, compared to October. This is suggested to be because of the fasting period when common eiders may loose as much as 40% of their pre-

breeding mass (Hanssen et al. 2003) This is also consistent with findings in Bustnes et al. (2010).

4.2.3 Variation between locations

Only samples from common eiders could be tested for differences in ∑PCBs and ∑OCPs concentrations between Kongsfjorden and Liefdefjorden in 2009. In addition, only plasma IgY-values was tested because of small numbers of OSI values. The higher ∑PCBs concentrations in muscle in common eiders from Kongsfjorden may be a reflection of different water masses, and hence different pollutant status, in the two fjords (Vieweg et al. 2012). While Liefdefjorden is dominated by Arctic water masses, Kongsfjorden is dominated by the more OC contaminated Atlantic water masses (Cottier et al. 2005). Another explanation for the higher ∑PCBs concentrations in common eider in Kongsfjorden may be that the warmer Atlantic water may increase metabolism in lower trophic species such as bivalves and thus increase uptake of contaminants (Vieweg et al. 2012). This may again be reflected in OC concentrations in higher trophic species such as the common eider. The ∑OCPs concentrations in muscle in common eider did not show any differences between the two fjords, and hence showing a different pattern compared to ∑PCBs. However, it is important to note that the sample sizes are small, and natural variations may occur.

The IgY levels in plasma of common eiders did not differ between Kongsfjorden and Liefdefjorden, suggesting similar health status. Also the BCI was similar in birds from the two fjords, supporting the theory about similar health status. In general, common eiders from Svalbard contain low concentrations of OCs (Olafsdottir et al. 1998; Franson et al. 2004; Mallory et al. 2004), which may explain why the IgY-levels in plasma did not reflect the variations in OC-concentrations in muscle.

4.3 Relationship between health parameters and organochlorines

When assessing health parameters in relation to OCs, variables such as year, month or location were not included. One statistical analysis was done for common eider and one for black-legged kittiwake, under the assumption that OCs may directly influence health effect parameters with limited influence of temporally changing factors.

Black-legged kittiwake

Increasing log OCPs values appeared to be related to decreasing log IgY values, suggesting that log OCPs negatively affected the humoral immunity of black-legged kittiwakes. This is consistent with findings in Caspian terns (*Hydroprogne caspia*) (Grasman et al. 1996). log PCBs did not significantly explain any log IgY variation, indicating that the ∑PCB concentrations were below threshold concentrations for avian effects. This suggestion is supported by the fact that the ∑PCBs concentrations in muscle in black-legged kittiwakes in this study were below all threshold levels for avian effects presented in AMAP (1998).

Interestingly, log PCBs did explain a significant amount of the variation in log OSI, suggesting that PCBs induce oxidative stress in black-legged kittiwakes. One possible explanation is that plasma OSI is more sensitive to PCBs compared to plasma IgY. However, to my knowledge, no study has addressed this topic, and a comparison to other studies is therefore not possible.

Common eider

In common eiders the results showed the opposite of what was expected; there was a significant positive relationship between log PCBs and log IgY. Comparing the ∑PCBs levels in common eider in this study to AMAP (1998) threshold levels for avian species showed ∑PCB concentrations below all threshold levels for reproduction, egg mortality and hatching success. The positive relationship between log PCBs and log IgY may therefore be due to natural variations in plasma IgY-levels, and not a PCB induced effect. Neither log PCBs nor log OCPs explained any of the variation in OSI in common eider. This may be explained by generally low levels of PCBs and OCPs in common eider

4.4 Relationship between health parameters and body condition index

The body condition index did not seem to explain any of the variation in plasma health parameters in either black-legged kittiwake or common eider, suggesting that IgY-levels and OSI does not depend on body condition. This is consistent with findings in Isaksson et al. (2005) where oxidative stress ratio did not correlate with body condition in great tits (*Parus major*). Alonso-Alvarez and Tella (2001) did suggest that body condition in birds had to be

below a specific threshold before experiencing any changes in immune responses explained by body condition. A possible explanation for the lack of relationship between the health parameters and BCI may therefore be that the birds were in good condition and above the threshold suggested by Alonso-Alvarez and Tella (2001). However, it is important to note that the BCI may be biased, as weight (which is one of the parameters in calculating BCI) in birds may vary during the day according to food intake (Owen and Cook 1977; Clark 1979).

4.5 Correlation between health parameters

Only in common eiders were there a significant negative correlation between OSI and IgY. Such correlations have also been found in rats, where increasing oxidative damage is correlated with decreasing immunoglobulin-levels (Ercal et al. 2000). Even though rats and birds are not directly comparable, it could be an indication of a common pattern. Vitamin E levels have been observed to increase antibody-production in broilers, as well as prevent oxidative stress (Miller and Rice-Evans 1997; Leshchinsky and Klasing 2001). As Vitamin E has to be supplied through diet (Karadas et al. 2005), a possible explanation for the negative correlation between OSI and IgY in plasma of common eiders may be that the birds have ingested less Vitamin E than necessary to prevent decreasing IgY-levels and increasing OSI. However, this is just one of many scenarios.

4.6 Conclusions

No firm conclusions could be made regarding variations in IgY, OSI, OCs and BCI between years, months and locations due to small sample sizes. However, a pattern of no increasing bioavailable OCs is suggested for Kongsfjorden. Also, higher concentrations of some OCs were seen in Kongsfjorden compared to Liefdefjorden, possibly due to the influence of different water masses. No clear conclusions could be made regarding IgY and OSI in either species and variations in these health parameters have been speculated to be due to non measured factors such as virus induced responses or exposure to trace metals. There were only differences in BCI between common eiders from July and October, indicating a lower body condition index in July, right after egg incubation.

OCPs seemed to negatively affect IgY levels in black-legged kittiwakes, while PCBs did not, and were below threshold levels for toxic avian effects. PCBs were found to induce OSI. In common eiders, PCBs showed a positive relationship with IgY levels, but because the PCB concentrations were below levels of avian toxic effects, the reason for this relationship is unclear. Neither PCBs nor OCPs was found to influence OSI in common eider.

BCI did not explain any variations in health parameters in either species. This is suggested to be because of a generally good body condition in both species. Only in common eiders were there a correlation between IgY and OSI, but reasons for this are uncertain.

4.7 Future perspectives

For future studies I would recommend to measure concentrations of organochlorines and effect-levels from the same tissue/matrix when assessing relationships. In that way you rule out any uncertainties regarding variations in lipid content in different tissues. In addition, it would be of relevance to collect more samples, and have both sexes represented for each species.

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Appendix A: Analytical data

Organochlorines presented in pg/g wet weight.

ID	Species	Year	Month	Location	Sex	Weight	IgY	OSI
E11	Common eider	2007	July	Kongsfjorden	F	1722	0,29	3,77
E12	Common eider	2007	July	Kongsfjorden	F	1694	0,36	0,37
E13	Common eider	2007	July	Kongsfjorden	F	1409	0,37	0,88
E14	Common eider	2007	July	Kongsfjorden	F	1863	0,36	0,25
E15	Common eider	2007	July	Kongsfjorden	F	1482	0,51	1,73
E16	Common eider	2007	July	Kongsfjorden	F	1792	0,43	0,35
E17	Common eider	2007	July	Kongsfjorden	F	1713	0,45	1,64
E18	Common eider	2007	July	Kongsfjorden	F	1521	0,34	1,48
E19	Common eider	2007	July	Kongsfjorden	F	1280	0,30	0,55
E20	Common eider	2007	July	Kongsfjorden	F	1854	0,63	0,28
E21	Common eider	2007	October	Kongsfjorden	F	2018	0,30	1,73
E22	Common eider	2007	October	Kongsfjorden	F	2303	0,26	0,31
E24	Common eider	2007	October	Kongsfjorden	F	1856	0,24	N.A
E25	Common eider	2007	October	Kongsfjorden	F	1907	0,25	0,30
E26	Common eider	2007	October	Kongsfjorden	F	1609	0,28	0,80
E27	Common eider	2007	October	Kongsfjorden	F	2131	0,49	N.A
E28	Common eider	2007	October	Kongsfjorden	F	2110	0,35	0,70
E29	Common eider	2007	October	Kongsfjorden	F	1777	0,25	N.A
E41	Common eider	2008	July	Kongsfjorden	F	1486	0,60	0,15
E42	Common eider	2008	July	Kongsfjorden	F	1560	0,55	0,10
E43	Common eider	2008	July	Kongsfjorden	F	1440	0,60	0,17
E44	Common eider	2008	July	Kongsfjorden	F	1364	0,41	0,34
E45	Common eider	2008	July	Kongsfjorden	F	1812	0,45	0,37
E47	Common eider	2008	July	Kongsfjorden	F	1732	0,34	N.A
E48	Common eider	2008	July	Kongsfjorden	F	1740	0,70	0,22
E49	Common eider	2008	July	Kongsfjorden	F	1848	0,50	0,37
E53	Common eider	2008	July	Liefdefjorden	F		0,52	0,17
E58	Common eider	2008	July	Liefdefjorden	F		0,45	N.A
E61	Common eider	2009	July	Kongsfjorden	F	1221	0,43	0,26
E64	Common eider	2009	July	Kongsfjorden	F	1791	0,43	0,68
E66	Common eider	2009	July	Kongsfjorden	F	1736	0,34	0,66
E67	Common eider	2009	July	Kongsfjorden	F	1794	0,56	0,21
E68	Common eider	2009	July	Kongsfjorden	F	1788	0,40	0,97
E69	Common eider	2009	July	Kongsfjorden	F	1896	0,42	0,39
E75	Common eider	2009	July	Liefdefjorden	F	1820	0,34	1,40
E76	Common eider	2009	July	Liefdefjorden	F	2030	0,55	N.A
E80	Common eider	2009	July	Liefdefjorden	F	1650	0,66	0,53
K1	Black-legged Kittiwake	2007	May	Kongsfjorden	M	453	0,13	0,11
K6	Black-legged Kittiwake	2007	May	Kongsfjorden	M	482	0,11	0,17
K11	Black-legged Kittiwake	2007	July	Kongsfjorden	M	325	0,12	0,78
K12	Black-legged Kittiwake	2007	July	Kongsfjorden	M	386	0,13	0,52
K13	Black-legged Kittiwake	2007	July	Kongsfjorden	M	373	0,11	0,94
K14	Black-legged Kittiwake	2007	July	Kongsfjorden	M	396	0,11	0,25
K15	Black-legged Kittiwake	2007	July	Kongsfjorden	M	461	0,14	N.A
K16	Black-legged Kittiwake	2007	July	Kongsfjorden	M	396	0,11	1,18
K42	Black-legged Kittiwake	2008	July	Kongsfjorden	M	426	0,15	N.A
K45	Black-legged Kittiwake	2008	July	Kongsfjorden	M	388	0,15	0,34
K47	Black-legged Kittiwake	2008	July	Kongsfjorden	M	398	0,16	0,97
K48	Black-legged Kittiwake	2008	July	Kongsfjorden	M	400	0,14	0,09
K49	Black-legged Kittiwake	2008	July	Kongsfjorden	M	450	0,14	0,66
K55	Black-legged Kittiwake	2008	July	Liefdefjorden	M		0,12	0,24
K59	Black-legged Kittiwake	2008	July	Liefdefjorden	M		0,13	0,28

ID	PCB 99	PCB 105	PCB 118	PCB 128	PCB 138	PCB 153	PCB 156	PCB 167	PCB 170
E11	259,15	215,86	680,87	177,23	1 009,65	2 316,88	80,40	63,95	225,18
E12	485,72	252,43	803,79	313,73	2 070,47	5 516,34	129,77	158,09	663,71
E13	617,20	411,39	1 398,12	367,23	2 439,47	4 721,00	155,96	124,76	281,97
E14	101,38	92,45	118,99	38,35	312,74	395,99	30,63	15,53	69,44
E15	311,36	221,90	758,66	162,62	977,43	1 856,27	62,57	48,53	135,01
E16	327,35	275,73	913,03	216,51	1 185,19	2 331,93	99,06	74,02	175,95
E17	99,00	166,75	640,60	162,98	994,70	2 394,08	82,78	69,80	230,29
E18	140,57	173,08	566,11	149,74	875,73	2 231,87	69,46	64,20	140,21
E19	1 868,91	1 217,41	4 254,63	949,54	5 845,91	8 879,05	426,54	224,63	575,23
E20	524,99	438,22	1 405,23	386,60	2 164,31	3 730,92	180,65	107,09	501,27
E21	59,92	11,14	136,70	18,34	167,50	272,95	12,73	9,16	52,69
E22	65,27	0,94	172,44	40,56	187,73	279,79	12,55	9,75	20,16
E24	39,37	54,58	116,89	27,66	132,24	218,53	8,69	7,99	16,50
E25	64,38	86,50	159,35	36,42	172,35	232,00	24,92	9,93	15,73
E26	55,02	60,17	123,53	36,60	241,45	551,12	22,90	16,70	96,57
E27	79,09	11,44	129,09	18,21	171,73	475,22	14,23	10,34	23,50
E28	58,96	13,13	114,22	19,22	145,11	215,67	12,99	11,54	23,90
E29	51,00	10,15	148,36	18,48	127,35	175,59	12,49	9,60	24,82
E41	411,78	388,93	946,09	255,52	2 110,74	2 352,65	108,83	92,19	107,01
E42	342,45	233,70	915,06	350,65	2 356,64	4 496,82	173,34	92,94	700,18
E43	691,97	348,03	1 439,24	436,36	2 386,58	5 794,09	185,55	117,91	389,88
E44	2 706,33	1 750,49	4 157,47	1 139,64	7 795,43	10 710,78	381,11	284,68	744,99
E45	86,08	229,43	933,02	252,61	1 495,84	3 500,92	114,65	112,53	106,43
E47	691,54	391,74	1 979,59	435,29	3 120,85	5 595,86	210,07	127,57	459,33
E48	579,92	408,08	1 200,87	362,69	2 095,09	4 753,48	117,39	126,91	347,01
E49	323,84	359,70	621,02	76,00	823,52	1 321,00	33,03	37,35	135,67
E53	340,71	265,79	898,58	244,03	3 094,61	3 977,84	56,39	42,49	183,66
E58	196,50	155,16	1 231,61	70,33	1 130,03	2 982,24	49,32	34,02	457,74
E61	1 799,60	1 513,19	4 569,57	1 704,33	9 323,04	20 047,39	554,17	665,34	851,87
E64	323,53	230,16	845,42	264,74	1 608,84	3 720,89	94,32	89,89	201,74
E66	646,16	388,13	1 498,84	520,49	2 883,35	7 561,07	110,45	196,38	611,70
E67	276,90	184,55	808,50	262,75	1 669,25	3 167,30	86,60	24,18	32,77
E68	566,28	606,53	1 352,74	411,20	3 023,76	4 984,27	215,84	147,52	267,88
E69	360,88	192,22	705,14	222,45	1 365,32	3 664,03	90,51	88,61	34,09
E75	334,55	273,28	800,15	147,02	1 305,45	3 439,14	86,46	23,83	269,04
E76	179,00	24,67	453,22	124,74	880,61	1 584,43	54,17	20,73	34,09
E80	171,27	28,32	359,64	147,51	909,75	1 624,77	59,29	21,07	153,45
K1	9 674,49	4 635,87	17 767,81	3 245,83	37 248,41	50 374,38	2 157,07	1 338,13	8 269,82
K6	12 063,36	4 336,19	19 361,26	3 196,99	46 804,20	69 083,38	2 645,71	1 894,79	10 917,53
K11	35 866,35	12 072,05	52 708,98	7 392,96	134 790,58	209 928,43	8 568,13	6 057,29	31 579,41
K12	25 040,17	7 428,89	34 135,67	4 849,94	94 482,72	139 367,89	5 288,11	3 776,89	19 316,28
K13	14 040,06	4 255,69	17 160,83	1 930,02	46 125,74	70 163,98	2 964,37	2 300,58	10 217,99
K14	19 561,74	5 798,44	27 437,48	4 494,67	78 431,78	123 021,34	4 402,90	3 174,80	20 680,40
K15	20 030,77	5 884,56	26 646,08	3 465,94	108 666,99	190 245,86	7 130,84	5 812,54	35 749,35
K16	18 531,57	7 820,30	33 781,69	4 833,60	77 581,54	111 508,84	4 477,30	3 357,12	17 665,99
K42	9 533,74	3 335,93	14 023,70	2 679,00	35 247,30	53 628,41	2 365,00	1 409,00	7 525,93
K45	26 505,78	7 903,05	33 815,78	4 861,45	99 526,39	147 689,60	6 087,67	4 236,79	22 470,04
K47	22 729,93	9 605,60	39 456,84	8 089,16	126 760,40	208 157,56	8 192,11	6 097,96	37 976,40
K48	14 253,55	4 410,05	19 955,52	2 550,96	60 002,43	104 243,58	4 261,20	3 439,13	16 760,14
K49	26 569,89	6 684,60	32 377,43	4 458,15	129 482,94	253 116,26	7 328,94	6 442,15	39 043,74
K55	18 511,12	5 739,35	24 236,49	4 541,69	57 675,87	77 542,53	2 623,15	1 694,27	9 998,20
K59	16 084,56	5 450,07	23 726,41	4 599,15	60 838,61	169 277,08	5 891,28	5 686,26	22 879,86

ID	PCB 180	PCB 183	PCB 187	ppDDE	HCB	*cis*-chlordane	oxychlordane
E11	629,20	137,59	320,50	4 244,47	3 182,39	5,74	1 186,43
E12	1 834,20	356,11	937,21	8 767,86	5 094,60	6,34	1 466,40
E13	848,73	297,81	830,72	9 960,25	5 703,71	6,73	1 554,18
E14	193,57	32,26	119,93	1 221,00	892,84	9,27	346,94
E15	371,77	107,94	323,21	4 442,85	3 036,74	6,24	702,19
E16	497,05	130,66	410,25	4 737,07	2 958,55	6,32	982,16
E17	648,11	163,83	412,56	4 060,87	451,73	4,67	247,90
E18	393,16	115,23	340,65	2 741,99	174,72	4,59	463,73
E19	1 484,17	483,02	1 333,22	12 987,41	8 868,99	6,62	2 190,52
E20	1 304,91	227,74	567,71	8 219,00	2 266,09	6,55	1 005,36
E21	122,58	19,39	41,44	282,42	356,20	15,64	92,98
E22	68,31	15,03	39,41	354,61	380,98	15,90	134,31
E24	79,18	12,32	10,19	403,63	533,45	7,05	82,34
E25	89,62	11,72	40,73	362,96	474,00	12,73	98,73
E26	339,64	54,36	82,81	667,19	248,61	5,41	99,27
E27	157,86	46,32	78,13	454,66	481,25	8,68	173,10
E28	84,40	17,89	20,25	270,60	471,27	16,49	138,89
E29	53,55	18,50	31,03	133,38	549,93	9,14	118,90
E41	647,51	116,94	539,37	2 652,90	1 240,22	6,23	471,69
E42	1 799,84	398,44	1 099,13	4 313,74	2 760,91	38,82	849,20
E43	1 245,91	273,58	1 042,73	7 314,88	5 640,94	7,21	1 561,73
E44	2 221,52	754,08	2 165,79	18 789,04	11 875,32	1,53	3 404,21
E45	453,23	230,60	677,31	4 394,24	1 091,85	11,36	610,90
E47	1 413,84	365,64	1 023,58	7 181,97	1 907,77	4,40	1 594,36
E48	1 143,66	304,68	777,08	8 434,99	5 446,70	6,52	1 605,35
E49	331,78	126,13	128,26	4 025,16	1 951,23	21,69	675,14
E53	558,92	144,50	532,53	4 410,89	2 321,39	1,26	770,55
E58	752,26	40,60	327,71	4 959,58	2 536,58	14,45	1 087,24
E61	3 037,48	1 296,53	3 606,13	26 662,55	0,31	2,46	9,49
E64	707,00	194,95	612,16	4 329,00	0,22	1,94	9,00
E66	1 882,05	295,18	1 375,45	8 869,24	0,35	2,99	15,14
E67	824,95	130,20	624,15	2 520,70	0,35	2,22	15,28
E68	884,67	370,77	785,77	5 176,75	0,24	1,46	15,10
E69	1 021,20	251,57	669,26	4 460,97	69,86	2,66	10,82
E75	928,38	167,83	549,19	4 791,52	45,15	4,18	12,46
E76	403,61	25,25	244,91	1 980,39	44,26	2,78	16,10
E80	457,36	110,96	306,14	2 566,55	0,27	1,71	10,00
K1	23 299,89	4 935,58	12 013,50	58 789,43	20 867,92	160,81	9 858,55
K6	32 884,77	6 913,24	12 585,64	68 237,40	22 176,60	288,11	10 300,48
K11	95 569,23	18 996,62	34 855,49	88 111,48	N.A	N.A	N.A
K12	57 787,28	12 858,67	20 900,11	38 237,39	15 336,61	76,33	21 420,17
K13	31 163,32	6 046,38	9 312,97	21 452,46	N.A	N.A	N.A
K14	65 354,08	12 117,32	18 953,61	58 909,00	15 268,07	96,92	14 326,88
K15	117 150,06	20 941,75	25 655,26	17 458,19	N.A	N.A	N.A
K16	53 913,90	11 015,69	18 374,49	62 815,62	15 576,85	129,21	19 815,84
K42	21 713,67	4 535,11	8 457,78	22 827,96	7 325,63	10,48	10 307,67
K45	68 817,55	14 097,43	33 438,43	57 040,48	20 260,52	65,70	30 141,89
K47	103 353,56	20 140,22	39 603,49	52 255,93	9 445,09	38,69	15 653,56
K48	55 230,49	10 281,01	19 114,59	19 752,13	11 697,90	156,20	11 184,45
K49	125 165,62	24 665,13	29 004,68	32 722,38	15 394,30	51,81	18 540,49
K55	26 286,07	5 986,69	16 219,92	87 159,24	16 443,20	226,69	22 029,69
K59	86 647,47	13 665,16	17 949,64	22 596,16	37 115,73	59,61	16 717,65

ID	*cis* -Nonachlor	*trans* -Nonachlor	Mirex	% Lipid
E11	33,08	325,50	300,33	1,69
E12	84,44	147,94	832,82	2,23
E13	38,76	381,73	603,80	2,20
E14	88,28	67,16	26,97	2,61
E15	151,77	391,93	140,98	2,69
E16	34,73	256,77	324,40	2,98
E17	45,91	29,94	146,37	1,50
E18	70,75	34,04	252,44	1,52
E19	35,91	461,23	1 623,02	1,40
E20	37,72	371,44	334,39	1,45
E21	1 942,82	90,74	13,54	2,89
E22	40,98	104,99	21,55	4,11
E24	609,08	51,34	7,81	2,69
E25	185,62	83,00	9,55	3,75
E26	87,06	33,53	8,92	1,26
E27	41,83	88,46	51,80	2,47
E28	5 066,22	64,98	3,88	3,59
E29	28,68	54,12	4,38	1,71
E41	113,31	216,69	123,50	2,04
E42	124,81	266,33	453,65	1,27
E43	228,76	404,36	462,18	1,33
E44	779,56	4 468,63	688,04	2,33
E45	99,86	147,09	258,34	2,30
E47	140,46	529,80	446,66	1,59
E48	149,13	638,29	386,55	2,07
E49	145,86	305,16	164,42	1,95
E53	180,45	408,47	391,82	2,67
E58	227,97	576,29	210,11	2,89
E61	0,60	0,97	30,97	1,14
E64	0,56	0,90	26,34	2,25
E66	0,86	1,38	34,36	2,39
E67	0,76	1,22	56,99	1,95
E68	0,64	1,02	52,90	2,15
E69	1,06	1,06	33,01	2,18
E75	1,09	1,09	29,81	3,13
E76	1,24	1,24	42,50	2,10
E80	0,58	0,93	33,15	1,72
K1	4 470,00	2 510,99	4 778,55	4,42
K6	5 031,69	5 268,24	5 667,81	6,51
K11	N.A	N.A	N.A	5,98
K12	1 014,89	547,17	9 562,83	6,44
K13	N.A	N.A	N.A	7,83
K14	952,62	877,13	9 839,81	7,22
K15	N.A	N.A	N.A	4,55
K16	1 303,90	1 073,93	9 117,94	5,92
K42	234,67	43,26	3 578,81	4,52
K45	2 712,05	904,70	10 346,95	4,54
K47	436,36	329,27	7 708,11	3,41
K48	828,50	829,64	7 585,25	6,10
K49	728,49	516,57	14 754,19	5,76
K55	1 938,06	2 352,61	3 666,83	3,68
K59	1 021,92	1 006,65	14 184,63	5,17

IgY= Immunoglobulin Y, OSI= Oxidative Stress Index, PCB = Polychlorinated biphenyls, HCB= Hexachlorobenzene, p,p`-DDE = dichlorodiphenyldichloroethylene

Appendix B: Chemicals

Product	Producer Numb.	Producer	Country
2,2-azino-di-3-ethylbenzalonine sulfonic acid (ABTS)	A1227	Sigma Aldrich	Norway
Acetone	320110	Sigma Aldrich	Norway
Anti-chicken IgY peroxidise conjugate	A9046	Sigma Aldrich	Norway
Citric acid	251275	Sigma Aldrich	Norway
Cyclohexane	442725	Sigma Aldrich	Norway
Fat free powdered milk	M7409	Sigma Aldrich	Norway
Helium 6.0	-	Hydrogas	Norway
Hydrogen peroxide 31.3 %	H3410	Sigma Aldrich	Norway
Isotope labelled internal standards	-	Cambridge Isotope Laboratories	USA
Potassium phosphate, monobasic	P9791	Sigma Aldrich	Norway
Sodium bicarbonate	S8875	Sigma Aldrich	Norway
Sodium carbonate, monohydrate	S4132	Fisher Scientific	UK
Sodium chloride	S7653	Sigma Aldrich	Norway
Sodium phosphate, dibasic	S7909	Sigma Aldrich	Norway
Sodium sulphate	S6547	Sigma Aldrich	Norway
Total antioxidant status assay kit	RL0017	Rel Assay Diagnostics	Turkey
Total oxidant status assay kit	RL0024	Rel Assay Diagnostics	Turkey
Tween	P1379	Sigma Aldrich	Norway
Octachloronapthalene	36935	36935	Norway

Appendix C: Solutions and reagents

Measurement of IgY-levels

Carbonate-bicarbonate buffer

0.25 L of 0.1 M Na_2CO_3 (made with deionized water) was added to 0.5 L of 0.1 M $NaHCO_3$ until a pH of 9.6 was reached. The solution was stored at 4°C.

PBS-Tween

35.06 g of NaCl was added to 4 L of deionized water, 19.59 g PO_4H_2K was added to 0.96 L of deionized water, and 64.73 g of PO_4HNa_2 was added to 3.04 L of deionized water. All the above solutions were mixed and 4 mL of Tween added. The solution was stored at 4°C until use.

Blocking Solution

For one plate, 0.75 g of % non-fat dry milk was mixed in 15 mL of PBS-Tween solution.

Anti-body Solution

For one plate, 40 μL of chicken anti-body (Sigma Ref A-9046) was mixed with 10 mL of PBS-Tween. Pre-prepared tubes were stored in -80°C freezer and thawed when needed.

Colour development Solution

0.1 M citric acid (9.607 g in 0.5 L of deionized water) was added to 0.5 L of 0.1 M Na_2HPO_4 (17.907 g in 0.5 L of deionized water) until pH of 5.0. 0.05 g of ABTS (2,2-azino-di-3-ethylbenzalonine sulfonic acid) was added to 100 mL of the above solution. Frozen tubes were prepared by measuring out 10 mL of the mixture containing the ABTS in tubes stored in the freezer at -20°C until use. For one plate, one tube (10 mL) was thawed and 10 μL of hydrogen peroxide 31.3% was added to the tube. The solution was vortexed before use.

Measurement of TAS and TOS

Reagents in Total Antioxidant Status kit (Appendix B):

Reagent 1	Acetate buffer solution 0.4 mol/L (pH 5.8).
Reagent 2	$ABTS^{\cdot+}$ in Acetate buffer solution 30 mmol/L (pH 3.6).

Reagents in Total Oxidant Status Kit (Appendix B):

Reagent 1	150 µM xylenol orange, 140 mM NaCl and 1.35 M glycerol in 25 mM H_2SO_4 solution (pH 1.75)
Reagent 2	5 mM ferrous ammonium sulphate and 10 mM o-dianisidine dihydrochloride in 25 mM H_2SO_4 solution.

Measurement of total oxidant and total antioxidant status

Isotope labelled internal standards	
^{13}C-labelled PCBs	^{13}C-labelled PCBs
PCB 28	α-HCH
PCB 52	β-HCH
PCB 101	γ-HCH
PCB 118	*trans*-chlordane
PCB 138	*trans*-Nonachlor
PCB 153	HCB
PCB 180	*p,p`*-DDE
	p,p`-DDT