# The Preen Gland – an Organ for Excretion of Persistent Organic Pollutants in Black-legged Kittiwake (*Rissa tridactyla*)


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**This Master thesis has been a collaboration between**

**The University of Bergen**

**and**

**The Norwegian Polar Institute**


“Can anyone believe it is possible to lay down such a barrage of poisons on the surface of the earth without making it unfit for all life? They should not be called ‘insecticides’, but ‘biocides’.”

-Rachel Carson in Silent spring-

# FØREORD

Med denne oppgåva har eg fullført fem år på universitetet, år der eg har flytta grenser og nådd mange mål. Desse sidene har gitt meg mange bekymringar, mange lange dagar på lesesalen, mange kjekke dagar på lab, mange flotte dagar med feltarbeid, og mange gode dagar saman med kjekke biologar. Ja i grunn har det vore frykteleg artig, ei flott utfordring og ikkje minst lærerikt.

Først av alt skal rettleiarane mine Geir Wing Gabrielsen (NP) og Ingvar Byrkjedal (UiB) ha ein stor takk. Takk for god oppfølging, inspirasjon og gode råd. Takk for at du skjønte eg måtte gjere noko litt annleis Ingvar, og takk Geir for at eg fekk oppleve Svalbard. Takk til Norsk Polarinstitutt som har finansiert dette prosjektet. Godkjenning for gjennomføring av prosjektet vart gitt av sysselmannen på Svalbard og forsøksdyrutvalet.

Stor takk også til alle på NILU i Tromsø for eit fint opphald på labben der. Takk spesielt til Sandra Huber for all hjelp med opparbeiding av prøvene mine, for dei endelege analysane, og gjennomlesing av tekst. Takk til Kjetil Sagerup (NP) som tok så godt imot meg i Tromsø, ordna alt det praktiske, har hjelpt meg mykje med gjennomlesing av tekst og si alltid positive innstilling.

Takk til Claus Bech, Aevar Petersen og Kristin Ólafsdóttir for at eg fekk bruke upubliserte data, Oddveig Øien Ørvoll (NP) for kart, Unilab for lipidanalyser, Knut Helge Jensen (UiB) og John-Arvid Grytnes (UiB) for sine opne dører og store statistikkunnskapar.

Gry, Ida og Tore; takk for hjelp med feltarbeid og ein fantastisk sommar i Ny-Ålesund. Tusen takk til Renate og Eivind som stilte med husrom og hjerterom i Tromsø. Tusen takk kjære medmasterstudentar på lesesalen i Bergen, felles skjebne, felles trøst! De er ein inspirerande gjeng! Spesielt takk til Astrid, du  har betydd meir enn du anar! Til slutt takk for støtte frå familie og venner, som eg ikkje har sett så mykje til i det siste.

Bergen, 1.juni 2010

Silje Aakre Solheim

# Samandrag

Organiske miljøgifter utgjer ei kjemisk divers gruppe av lipofile sambindingar. Desse sambindingane akkumulerar i feittrike vev i levande organismar, ofte med skadelege konsekvensar. Til ei viss grad kan miljøgiftene brytast ned og fjernast via leverfunksjonar. Gumpkjertelen er eit organ hjå dei fleste fuglar, som produserar eit lipidbasert sekret. Sekretet vert overført til fjør gjennom stell av fjørdrakta. På denne måten kan gumpkjertelen tenkjast å vere ei alternativ utskiljingsrute for persistente miljøgifter hjå fugl. Dersom oljen som vert tilført fjøra inneheld miljøgifter, er det mogleg at målbare mengder vil kunne finnast på overflata av fjør. Innsamling av fjør kan difor tenkjast å vere eit alternativ for miljøgiftovervaking, der ein ikkje treng avlive fuglen. Gjennom hekkesesongen i 2009, vart 24 krykkjer *Rissa tridactyla* samla frå ein koloni nær Ny-Ålesund, Svalbard. Prøver frå fjør, lever og gumpkjertel vart analysert for miljøgifter. I tillegg vart lever og gumpkjertelprøver frå 12 krykkjer analysert for lipidklassesamansetnad.

Fjør frå krykkjer inneheldt målbare mengder av miljøgifter, og miljøgiftprofilen i fjør var mest lik den frå gumpkjertel samanlikna med lever. Dette indikerar at det skjer ei overføring av miljøgifter frå gumpkjertel til fjør. Det var målt ein relativt høg konsentrasjon av miljøgifter i gumpkjertelen. Dette skaper eit godt utganspunkt for utskiljing av miljøgifter gjennom gumpkjertelen. Miljøgiftkonsentrasjonane i både lever- og gumpkjertel auka frå rugeperiode til ungeperioden. Dette kan føre til auka utskiljing av miljøgifter frå gumpkjertelen i energikrevjande periodar der miljøgiftene er mest konsentrert. På ei anna side, er det funne ein trend for lågare fjørkonsentrasjonar i ungeperioden samanlikna med rugeperioden. Dette kan indikere at produksjonen av gumpkjertelolje er lågare i ungeperioden, og at fuglen dermed ikkje kan utnytte denne ekstra fordelen. På trass av store skilnadar i lipidklassesamansetnad i lever og gumpkjertel, ser det ikkje ut for å vere spesielle restriksjonar med omsyn på kva miljøgifter som faktisk kan skiljast ut frå gumpkjertelen.

Utanom ein PCB vart alle aktuelle miljøgifter, funne i målbare mengder i fjør. Det er funne ein manglande korrelasjon mellom konsentrasjonane i fjør og i dei indre organa, og i tillegg ein avvikande sesongrespons for miljøgifter i fjør. Dette kan ein kanskje sjå i samanheng med sjøfugl, som krykkjer, sin stadige kontakt med saltvatn som kan vaske vekk miljøgiftene. I så tilfelle kan dette vere ein indikasjon på at fjør frå sjøfugl ikkje er like anvendelege til miljøgiftovervaking som ein kan tenkje seg at fjør frå terrestre fuglar kan vere.

# Abstract

Persistent organic pollutants (POPs) constitute a chemically diverse group of lipophilic substances that may accumulate in living organisms, often with harmful effects. To some extent these substances may be expelled via the liver functions. The preen gland is an organ in most birds that produces a lipid-based secrete which is applied to the bird's feathers by preening. The preen gland may thus be an alternative way for birds to excrete POPs. Furthermore, if the preen oil applied to the feathers contain POPs, traceable amounts of the pollutants might be left on the feathers. Sampling of feathers may therefore be a non-destructive alternative for monitoring POPs in birds. During the breeding season 2009, 24 black-legged kittiwakes *Rissa tridactyla* were sampled in a colony near Ny-Ålesund, Svalbard in order to analyse feather, liver and preen gland samples for POPs. In addition liver and preen gland samples from 12 kittiwakes were analysed for lipid class composition.

Kittiwake feathers were found to be contaminated by POPs and the POP profiles in feather and preen gland were found to be more similar than the feather and liver profiles. This indicates a transfer of POPs from the preen gland to the feathers. High levels of contaminants were measured in the preen gland. This creates a basis for the kittiwake to excrete POPs through the preen gland. Similar to liver, the POP concentrations in the preen gland increased from the incubation- to the chick rearing period. This could give the kittiwake an even greater opportunity to excrete POPs in energy demanding periods where POPs are most concentrated. However, a trend for lower feather contamination in chick rearing- compared to incubation period, may indicate lower preen oil production and thereby no increase in the excretion of POPs in energy demanding periods. Despite a very different lipid composition in liver and preen gland samples, only small magnitude differences appeared in the POP profiles and there seem to be no severe restrictions to which POPs could possibly be excreted through the preen gland.

With the exception of one PCB congener, all POP compounds detected in this study where found in traceable amounts in feathers. A lack of correlation between feather- and the inner organ concentration and a deviating response to seasonality for feather contamination were found. This may be seen in relation to seabirds', like the kittiwake, continuous exposure to seawater and may indicate that seabird feathers are a less good monitor for POP exposure than are feathers from terrestrial species.

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# Abbreviations

| | |
|---|---|
| ANOVA | analysis of variance |
| BFR | brominated flame retardants |
| DCA | detrended correspondence analysis |
| DCM | dichloromethane |
| DDE | dichlordiphenyldichloroethylene |
| DDT | dichlorodiphenyltrichloroethane |
| df | degrees of freedom |
| ELSD | evaporative light scattering detector |
| GC-MS | gas chromatography with mass spectrometry |
| GPC | gel-permeation chromatography |
| HCB | hexachlorobenzene |
| HCH | hexachlorocyclohexane |
| HPLC | high performance liquid chromatography |
| IUPAC | international union of pure and applied chemistry |
| $Na_2SO_4$ | sodium sulphate |
| Nd | not detected |
| NILU | Norwegian Institute for Air Research |
| NP | Norwegian Polar Institute |
| OCN | octachloronaphthalene |
| *p* (as in *p,p'*-DDE) | "para" explain the position of the chlorine atoms on the phenyls. |
| PCA | principal component analysis |
| PCB | polychlorinated biphenyls |
| POP | persistent organic pollutants |
| RDA | redundancy analysis |
| SD | standard deviation |
| SRM | standard reference material |
| TPE | thermoplastic elastomeres |
| UiB | University of Bergen |
| UN ECE | the United Nations Economic Commision for Europe |

Abbreviations and IUPAC names for the individual POPs, as well as names for the lipid classes included in this study, are listed in Appendix I and II.

# 1 Introduction

The Arctic may appear as a pristine and untouched piece of Earth, but even here the presence of a distant human civilisation can be experienced. As a footprint from human activity, organisms in the Arctic, so far from any sources of considerable size, are influenced by persistent organic pollutants (POPs) (Burkow and Kallenborn, 2000).

## 1.1 Persistent organic pollutants (POPs)

POPs are introduced to the environment mainly as industrial chemicals (e.g. polychlorinated biphenyls (PCBs)), industrial by-products (e.g. hexachlorobenzene (HCB)) and pesticides (e.g. DDT, HCB, HCH, chlordanes) (de March et al., 1998). The contaminants are a heterogeneous group with respect of chemical structures, but still they share several characteristics, such as being halogenated compounds (in this study with chlorine), they are semi-volatile by nature, they have low water solubility, but are highly lipophilic and are rather resistant to biodegradation. Together these characteristics lead to bioaccumulation of POPs in organisms' lipid rich tissue e.g. adipose and liver tissue (de March et al., 1998, de Wit et al., 2004).

### *Effects of POPs*

Several effects on wild life organisms have been related to POP contamination; e.g. PCBs, which were first introduced in the 1930s (de March et al., 1998), are associated with negative effects on immune functions, the endocrine- and nervous systems, reproduction and the ability to compete for food and habitat (de Wit et al., 2004, Gabrielsen, 2007, Gabrielsen and Sydnes, 2009). Among the pesticides e.g. DDT, which has been widely used since the 1940s on agricultural crops and as an agent against the malarial mosquito, is known to cause eggshell thinning in certain bird species (Timbrell, 2007, Gabrielsen and Sydnes, 2009).

### *Long range transport*

Within the Arctic areas there are no POP sources of considerable size, and the pollutants are mainly introduced by long-range transport from industrialised areas (Barrie et al., 1992, Macdonald and Bewers, 1996, Burkow and Kallenborn, 2000, de Wit et al., 2004). Because of the semi-volatile nature of most POPs, the fastest and most extensive transport towards the

Arctic is through the atmosphere. When the contaminants reach the cold Arctic area, the volatility of the contaminants decreases due to the lower temperatures, they are deposited into the oceans, and can enter the food webs (Macdonald and Bewers, 1996). Other pathways into the Arctic are ocean currents, large arctic rivers and the ice pack (Barrie et al., 1992, Macdonald and Bewers, 1996, Burkow and Kallenborn, 2000, de Wit et al., 2004). Also migrating animals like seabirds are known to be important transport vectors for POPs into some arctic food webs (Evenset et al., 2007).

Presence of POPs in areas distant from sources was first reported in the 1970s (de March et al., 1998, Gabrielsen, 2007). Since then it has become a global accepted issue, and the Arctic became an important indicator region for research regarding persistence and bioaccumulation (de Wit et al., 2004). Parallel to the increase in knowledge considering POPs toxic effects on wildlife species, and the awareness of long range transport, regulation and restrictions on production and use of certain POPs have been implemented. Evidence from research has been important in these regulations. The *UN ECE Convention on Long-Range Transboundary Air Pollution* (1979) (http://www.unece.org/env/lrtap/) and the *Stockholm Convention on persistent organic pollutants* (2001) (http://chm.pops.int/) have resulted in a global ban of several POPs. As a result the general presence of legacy POPs in living organisms recently has tended to decline (de Wit et al., 2004). Nevertheless the persistent POPs will remain in food webs, oceans and atmosphere for several decades. In addition new contaminants like brominated flame retardants (BFRs) are introduced to the ecosystems (Gabrielsen, 2007).

***Accumulation and biomagnification***

POPs in the ocean are entering the marine food web through planktons, the lowest trophic level, where contaminants diffuse into the organisms. Also fish experience passive contamination by diffusion over the gills, in addition to an input via dietary sources (Macdonald and Bewers, 1996, de Wit et al., 2004). Seabirds and mammals mainly obtain POPs from their diet. Because of slow metabolism, the contaminants bioaccumulate in the organisms, and are biomagnified up the food chain, with high levels in top predators like polar bear (*Ursus maritimus*) and glaucous gull (*Larus hyperboreus*) (Skaare et al., 2000, Fisk et al., 2001a, Gabrielsen, 2007). The accumulation of toxic substances is not uniform among species but varies due to variations in diet and ability to biotransformation and elimination (Borgå et al., 2007, Fisk et al., 2001b).

***Vulnerable arctic ecosystems***

Several characteristics of the arctic ecosystems make food webs here particularly vulnerable to bioaccumulative chemicals (Barrie et al., 1992, de Wit et al., 2004). In the cold arctic environment the persistence of POPs is even higher than in more temperate regions (de Wit et al., 2004). Throughout the year, arctic ecosystems experience great fluctuations in productivity (de Wit et al., 2004), and in periods when resources are scarce, the living organisms must rely on energy reserves stored as lipids. Parallel to the decrease in fat reserves there will be a release of lipid soluble contaminants to the circulation. During these periods with poor body conditions the POPs may be more of a risk for organisms, than the mean body burden of contaminants indicates (Macdonald and Bewers, 1996, de Wit et al., 2004). In addition to abiotic factors like temperature and seasonality, the presence of a sustainable population of higher level carnivores, like polar bear and glaucous gull, influences the arctic food webs vulnerability to toxic chemicals. Since POPs accumulate up the food chain the organisms on the top experience severe bioaccumulation which again could influence the whole food web through food web interactions (Barrie et al., 1992, de Wit et al., 2004).

## 1.2 High energy costs during reproduction in Arctic seabirds

Seabirds in arctic areas are breeding at low temperatures, both air and sea water, and the foraging and thermoregulatory costs are very high (Fyhn et al., 2001, Gabrielsen, 2009). The reproductive period may be very demanding in birds (Welcker et al., 2010) and under these hard conditions it is suggested that the birds work on their limit of physiological capacity (Drent and Daan, 1980, Fyhn et al., 2001, Bech et al., 2002). The activity required from adults is increasing, and the demands shift from self-maintenance and survival to egg-formation, incubation and growth of the chicks. In most avian species a decrease in body mass is observed during the breeding period (Moreno, 1989, Bech et al., 2002, Moe et al., 2002). In black-legged kittiwakes (*Rissa tridactyla*) breeding on Svalbard, a stable or even increasing body mass is observed during the incubation period. This period is followed by a decrease in body mass immediately after hatching. The decrease continues through the first part of the chick-rearing period, until the body mass is eventually stabilised (Bech et al., 2002, Moe et al., 2002, Henriksen et al., 1996). Due to their lipophilic nature, the concentration, and distribution of contaminants will depend on these changes in body mass and lipid composition during the breeding period. A decrease in body mass, and thereby lipid content, leads to a redistribution of POPs into lipid rich organs such as the liver (Henriksen et al., 1996).

## 1.3 Age-related accumulation of POPs

The major routes for excretion of POPs are through faeces and urine (de Wit et al., 2004). The contaminants may diffuse through the gut wall or metabolites are excreted together with bile (de Wit et al., 2004). Metabolism of POPs mainly occurs in the liver, where enzymes like cytochrome P450 is catalysing the process (Guengerich, 1991, de Wit et al., 2004).

According to the slow metabolism and thereby accumulating nature of POPs one would expect elevating concentrations with increasing age in arctic marine organisms (de Wit et al., 2004). Polar bears have shown to exhibit a rather high capacity to metabolise certain POPs when compared to other species compounds (Skaare et al., 2000, Kucklick et al., 2002, Verreault et al., 2005a). Nevertheless, POPs have been found to accumulate with age in polar bears, the accumulation appear to be especially striking in male bears (Bernhoft et al., 1997, Skaare et al., 2000). Also male harbour porpoise (*Phocoena phocoena*) show a pronounced effect of accumulation with increasing age (Kleivane et al., 1995). Only a lower degree of age accumulation of POPs are observes in female polar bears when compared to males. This is seen in relation to the female's reproductive role. Female mammals transfer POPs to their offspring through placenta and milk and can thereby lower the total body burden (Bernhoft et al., 1997, Skaare et al., 2000). The polar bear milk is very rich in lipids. Together with the long lactating period (up to 2.5 years) this is important for excretion of POPs in female polar bears (Bernhoft et al., 1997). The age accumulation of PCBs in polar bears seems to increase with increasing chlorine numbers. Higher chlorinated PCBs are most difficult to metabolise, and thereby most persistent (Bernhoft et al., 1997).

Because of the polar bear's high ability to metabolise certain POPs, its profile of contaminants is found to consist mainly of a few dominating compounds. In contrast, the profile in seabirds like glaucous gull is found to be far more complex. This indicates a lower capacity to metabolise such compounds (Verreault et al., 2005a). Despite the tendency of restricted metabolic capacity, POPs are not found to accumulate considerably with age in seabirds (Bustnes et al., 2003). Similar to female mammals, also female seabirds have an opportunity to transfer POPs to the offspring, but only through egg-yolk (Skaare et al., 2000). A study from Borgå et al. (2001) of black-legged kittiwakes (hereafter kittiwakes) and Brünnisch's guillemots (*Uria lomvia*) found that age accumulation was restricted to occur only between

chicks or first-year juveniles and adult birds. Similar Henriksen (1995) found stable PCB concentrations in kittiwakes from two years of age. Despite the low metabolic capacity (Skaare et al., 2000, Verreault et al., 2005a), an age-related equilibrium with a balance between exposure and elimination of PCBs, is seen both for male and female adult kittiwakes (Henriksen, 1995). This fact indicates that a significant mode of POP excretion, other than via the liver, is active in seabirds.

## 1.4 The preen gland

The preen gland (uropygial gland) is present in most birds and is largest in aquatic birds. It is a sebaceous gland appearing as a prominent swelling, dorsally at the base of the tail feathers (Stevens, 1996, Yamashita et al., 2007, Kent and Carr, 2001). An oily holocrine secretion is excreted from the gland and transferred to the bird`s feathers by preening, resulting in a waterproof plumage (Stevens, 1996, Kent and Carr, 2001). The preen oil has shown to be species specific when it comes to lipid composition (Jacob and Zeman, 1973) and also in quantity; for example Jacob (1976) found a daily excretion of 600 mg in Laridae. Earlier studies have indicated that this secretion may contain high concentrations of organic pollutants (Ingebrigtsen et al., 1981, Frank et al., 1983, Van Den Brink, 1997, Yamashita et al., 2007, Jaspers et al., 2008), and thereby could possibly make a significant contribution in excretion of POPs from the bird`s body (Ingebrigtsen et al., 1981, Frank et al., 1983, Ingebrigtsen et al., 1984, Petersen and Ólafsdóttir, unpublished data).

Recent research has pointed on bird feathers as a promising tool in non-destructive biomonitoring of POPs (Dauwe et al., 2005, Jaspers et al., 2006, Van den Steen et al., 2007). A feather is only growing in a limited period of time. Only in this restricted time, the feather is connected to blood vessels and the circulating POPs in the blood. Later the vessels atrophy and the feather become physiologically isolated from the bird. The measured contamination profile would reflect the time when blood vessels atrophy even if several months have passed, and possible changes in diet and body conditions have occurred (Jaspers et al., 2007b, Van den Steen et al., 2007). It is possible that the birds are reducing the body burden of POPs through loss of feathers in the moulting period (Van den Steen et al., 2007). External contamination of pollutants on feathers via air is significant when it comes to heavy metals, but the same has not been reported with POPs (Jaspers et al., 2007a). A study on common magpie (*Pica pica*) has indicated that oil from the preen gland might be the main source of external contamination by POPs onto feathers (Jaspers et al., 2008). This oil originate from

endogenous sources and the POP profile may therefore fit better with the profile in inner organs at the time of sampling than does the profile in the feather tissue alone. This would make feathers a more appropriate tool in order to monitor POPs. Several advantages would arise if feathers are found to be applicable for this purpose. Sampling of feathers would be non-destructive, as no birds will have to be sacrificed. This would be of great advantage in relation to endangered species, and generally to increase the sample sizes. In contrast to other non-destructive sampling strategies (e.g. blood samples), sampling of feathers do not require any special skills. In contrast to eggs, feathers are independent of sex, age and season.

## 1.5 Aims of the study

Several research projects have focused on metabolic degradation of persistent contaminants in the liver (Verreault et al., 2010). However, the knowledge regarding alternative excretion pathways for contaminants in birds is restricted. Transfer to eggs is an exception (Gabrielsen et al., 1995, Sandven, 2006, Verreault et al., 2006, Helgason et al., 2008).

The main aim for this study is to provide information on kittiwakes' ability to excrete POPs through the preen gland and thereby increase the knowledge regarding why POPs are not accumulating with age in seabirds, as seen in mammals. A second aim is to give further information on feathers applicability as a non-destructive tool for monitoring POPs. In order to answer these aims, samples from kittiwake feathers, liver and preen gland were analysed for POPs. Contaminant profiles for feathers, liver and preen gland were identified and compared, and differences related to sex and season were evaluated. In addition liver and preen gland samples were analysed for lipid class composition.

# 2 Materials and methods

## 2.1 Study area

Sampling for this project was conducted during the breeding season 2009. Kittiwakes were sampled from a small colony on the island Observasjonsholmen, situated in Kongsfjorden, close to Ny-Ålesund (78°55'N 11°55'E), Svalbard (Fig. 1). Kittiwakes constitute the majority of breeding birds in this colony. A low abundance of breeding black guillemots (*Cepphus grylle*) were also observed in the colony.


**Figure 1:** The study area Kongsfjorden in the Svalbard archipelago. The sampling locality was the island Observasjonsholmen as indicated on the map. Map: Oddveig Øien Ørvoll (Norwegian Polar Institute)

## 2.2 Study species

The black-legged kittiwake (Fig. 2) is the most numerous gull species in the world (del Hoyo et al., 1996). This medium sized gull has a circumpolar distribution where the breeding areas include arctic and boreal zones of the northern hemisphere (Strøm, 2006). The kittiwake is mainly a pelagic bird searching for food on or just beneath the sea surface and can be observed foraging together in flying or swimming flocks (Strøm, 2006). Polar cod (*Boreogadus saida*) and amphipods constitute the main diet for kittiwakes breeding on Svalbard (Mehlum and Gabrielsen, 1993, Strøm, 2006).

**Figure 2:** The study species black-legged kittiwake

The kittiwake is a numerous bird at Svalbard during the breeding season (Gabrielsen, 2009). Although the population on Svalbard has been reported as growing during the 20$^{th}$ century, counts since 1995 have revealed a recent decline in Svalbard's kittiwake population (Strøm, 2006). The kittiwake is a colony breeding species, where the colonies may consist exclusively of kittiwakes, or they can be shared between species. A common combination is breeding ledges shared between kittiwakes and Brünnich's guillemots (Strøm, 2006). Kittiwake colonies are generally found on high, steep cliffs on islands or mainland, near the sea. The birds are building their nests from plant material and faeces, on ledges and projections on the rock face (Strøm, 2006). Egg lying at Svalbard normally takes place in the middle of June and the usual clutch size is two eggs. The incubation time is 25-32 days and eggs and chicks are cared for by both parents. After hatching the chicks are fed regurgitated food until they are fledging five to six weeks old (Strøm, 2006). During their first years, and later outside the breeding season, kittiwakes disperse widely over the North-Atlantic (Coulson, 1966, Strøm,

2006). Three to five years old the kittiwakes reach sexual maturity (Strøm, 2006), and most of them seem to return to their natal area to breed (Coulson, 1966).

## 2.3 Sampling procedures

Kittiwakes chosen for this study were all breeding. Due to differences in body conditions during the breeding season, the sampling was divided in two periods. Twelve birds were collected in the incubation period, assumed to be in rather good body conditions. Another 12 birds were sampled in the early chick rearing period with chicks varying in age between seven and 12 days. These birds' body conditions were expected to be poorer than that of the birds sampled in the incubation period, due to reproductive stress (Moe et al., 2002). The sampled birds consisted of an even number females and males. Not to destroy more nests than necessary, effort was made to collect pairs of kittiwakes. This was achieved for all but two birds.

Kittiwakes were caught on their nests using a long fishing-rod with a nylon noose. Between the two sampling periods a number of nests were inspected every two or three days until hatching, to estimate the age of the first hatched chick. In nests were chicks hatched before the nest inspection started, the age of the first hatched chick (assumed to be the larger one) was estimated from skull length (C. Bech unpublished data). In order to get pairs of kittiwakes the birds were caught when one parent came back to the nest from the sea. When caught, the birds were weighed and wing length and the skull length (head+bill) were measured, the latter being used in sex determination. When the skull length exceeds 92.1 mm the bird is considered to be male. This measurement is expected to give the correct sex in 87% of the kittiwakes (Barrett et al., 1985). Males were always assumed to be the bigger pair member. Sex was finally decided during dissection. Immediately after weighing and measuring, the kittiwakes were humanly killed by an instant head trauma. Eggs and chicks were collected from the nests and used in other projects, if the nest was within reach.

### 2.3.1 Dissection

The liver and the preen gland were dissected free. Liver samples were wrapped in aluminium foil and frozen. The preen gland was frozen in a glass container. Feathers collected from the back of the birds were frozen in plastic bags. Sex was finally decided by the presence or absence of ovaries. All samples were kept frozen at -20$^{\circ}$C until further sample preparation.

## 2.4 Chemical analyses

Sample preparation for POP analyses was conducted at the Norwegian Institute for Air Research (NILU) Tromsø, 14.09-28.10.2009 and 11.01-19.01.2010 under supervision of qualified chemists. Analysis, identification and quantification of POP compounds were done by a certified chemist from NILU. Analyses for lipid class composition were performed by UNILAB analyse As, Tromsø.

### 2.4.1 POPs analysed

Feathers, liver and preen gland were analysed for PCBs (PCB-99, -101, -105, -118, -123, -128, -138, -141, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189, and -194), *p,p'*-DDE, HCHs (α-HCH, β-HCH and γ-HCH), chlordanes (*trans*-chlordane, *cis*-chlordane, *oxy*-chlordane, *trans*-nonachlor and *cis*-nonachlor), mirex, hexachlorobenzene (HCB), heptachlor and heptachlor epoxide.

### 2.4.2 Extraction of POPs from liver and preen gland

This procedure is based on the published method by Herzke et al. (2003) with some refinements. Approximately 2.0 g liver tissue and 1.0 g tissue from the preen gland were homogenised with 40 g and 15 g sodium sulphate ($Na_2SO_4$, burnt at 600ºC for 8 hours) respectively, using a food blender ("Magic Bullet", Household Housewares, Los Angeles, USA) and left in the freezer over night. Homogenates were transferred to columns and spiked with 20 µl intern standard (POP I, NILU, Kjeller/Norway). Cold-column extraction was applied where non-polar solvents are separating non-polar lipids from the sample matrix. The extraction was performed in three elution steps with 50 ml cyclohexane:acetone (3:1) each. Columns and Turbovap® glasses (Caliper Life Sciences, Hopkinton, USA) were covered with aluminium foil to avoid evaporating of solvent and analytes. Isooctane was added as a keeper, to avoid entirely evaporation, before concentrating to 0.5 ml using a Turbovap® 500 Evaporation System (Caliper Life Sciences, Hopkinton, USA) at 35°C. Thereafter the extracts were transferred to 4 ml vials. Turbovap glasses were rinsed with *n*-hexane and dichloromethane (DCM). Extracts were kept in the fridge until the next sample preparation step.

### 2.4.3 Extraction of POPs from feathers

The feathers were washed with ultrapure MilliQ-water (MilliQ Advantage A10 Ultrapure Water Purification System) to remove dust and particles, covered with aluminium foil, and then left to dry at room temperature, in the fume hood, for at least two days. About 500 mg feathers were transferred to a 100 ml centrifugation glass. The largest feathers had to be cut in smaller pieces. 50 ml cyclohexane:acetone (3:1) was added before the sample was spiked with 20 µl intern standard. The centrifugation glass was put in an ultrasonic bath (Branson 5510) for 15 min before the solvent was transferred into a Turbovap glass. To avoid particles in the extract, it was filtered through a pipette filled with a piece of pre-washed cotton. The extraction procedure was repeated two more times. Isooctane was added and the extract was concentrated to 0.5 ml using Turbovap at 35°C water bath. Extracts were transferred to 4 ml vials with *n*-hexane and DCM as rinsing agents, and kept in the fridge until further sample preparation.

### 2.4.4. Lipid determination

Lipid content in the samples was gravimetrically determined. An aliquot from each extract was transferred to a 1.5 ml vial, weight and left to dry over two-three days, and then weight again. The percentage of extracted lipids was thereafter calculated.

### 2.4.5. Sample clean up

Lipids and other matrix substances may disturb the analysis on the analytical instruments and should therefore be removed. The first step in purifying the extracts included gel-permeation chromatography (GPC). To separate the analytes of interest from lipids, a Waters GPC system was used. The system consisted of dual packed Envirogel™ GPC Columns (Waters Corporation, Milford Massachusetts, USA) powered by a HPLC (high performance liquid chromatography) Pump (Waters Corporation, Milford Massachusetts, USA). DCM consisted the mobile phase. An aliquot of the extract was injected and separated on the column. Large molecules like lipids were separated from the smaller molecules; our analytes of interest, by the mean of different elution times through the column, large compounds cannot enter the pores of the stationary phase and pass the column fast. Smaller molecules are slower because the transport velocity is slower through the pores. Isooctane was added as a keeper and the samples were evaporated to about 0.5 ml with a RapidVap® Vacuum Evaporation System

(Labconco Corporation, Kansas City, USA). Glasses were rinsed with *n*-hexane and samples transferred to reagens-tubes.

Final clean up of remaining matrix was done by solid phase extraction using a Zymark Rapid Trace® solid phase extraction workstation (Caliper Life Sciences, Hopkinton, USA). Each sample was run through an individual column packed with 1.0 g florisil (burnt at 450°C for 8 hours, Merck Chemicals KGaA, Darmstadt, Germany) between two glass fiber frits. After clean up isooctane was added as a keeper and the samples were evaporated to 0.5 ml in the RapidVap® Vacuum Evaporation System before transferred to 1 ml vials and evaporated further under $N_2$. 20 µl recovery standard, Octachloronaphthalene (OCN, 200pg/µl dissolved in isooctane, Dr. Ehrenstorfer GmBH, Augsburg,Germany), was added and 70 µl sample was transferred to GC vials.

### 2.4.6. Quality assurance

For every batch (usually 7-10 samples) one blank was included, which imply an empty beaker treated the same way as a sample. Each batch also included a standard reference material (SRM 1588b: Organics in cod liver oil, National Institute of Standards and Technology, Gaithersburg, USA, or whale blubber SRM 1945: Organics in whale blubber, National Institute of Standards and Technology, Gaithersburg, USA). Prior to extraction an internal standard mixture was added to all samples. By this one can evaluate the recovery of the analytes in every individual sample; estimates to which degree our compounds of interest are extracted from the tissues.

### 2.4.7. Unfortunate contamination

Between steps in the process of sample clean up, samples were stored in tubes with caps and septa. These septa contained a TPE-polymer (thermoplastic elastomeres) which caused contamination of all samples. This type of septa and screw caps has been in use for many years in several different applications at NILU and has earlier been tested successfully in method development steps. Unfortunately a product change has been done on the septa, where the distributor missed to inform the customers about the replacement of the material. During storing, solvents in the samples dissolved a contaminating polymer from the septa. In order to remove this substance all samples had to be run a second time on the GPC.

### 2.4.8. Identification and quantification

Analysis, identification and quantification of the analytes were done by Dr. Sandra Huber (NILU).

Organohalogenated pesticides, DDE and PCBs were analysed by gas chromatography with mass-spectrometry (GC-MS) in selected ion monitoring (SIM) -mode. An Agilent 7890A GC with split/splitless injector coupled to an 5975 C MSD (Agilent, Böblingen, Germany) was used with helium as carrier gas and methane as reagent gas in negative chemical ionisation (NCI) mode. The injector was run in spitless-mode with a constant temperature at 250°C. Separation was achieved on a DB-5-ms column (30 m x 0.25 mm x 0.25 µm, Agilent, Böblingen, Germany) with temperature program as follows: 70°C (held 2 min), ramped at 15°C/min to 180°C, ramped at 5°C/min to 280°C, ramped at 30°C/min to 320°C (held 5 min). The carrier gas was set at constant pressure of 18 psi. Transferline temperature was set to 300°C, ion source temperature to 160°C and quadrupole temperature to 150°C. Quantification was performed by the internal standard method together with an one-point calibration (Bustnes et al., 2008, Huber, pers.comm.).

## 2.5 Lipid class determination

For lipid class determination, 24 samples were chosen by random selection. Premise for the selection were 12 samples from the preen gland and 12 from the liver. Also there should be an equal number from the incubation period and from the chick rearing period, last there should be an equal number female and male samples. Due to time restrictions these analyses were done by Unilab analyse AS (Tromsø). Neutral and polar lipid classes were separated and identified on a monolithic silica column using HPLC with an evaporative light scattering detector (ELSD). The method is described in (Graeve and Janssen, 2009).

### 2.5.1 Lipid classes analysed

The lipid classes analysed in kittiwake liver and preen gland samples were cholesteryl ester, wax ester, triacylglycerol, fatty alcohol, cholesterol, diacylglycerol, free fatty acid, monoacylglycerol, galactocerebroside, cardiolipin, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, phosphatidylserine, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine.

## 2.6 Statistics

Microsoft Excel® (version 2007) for Windows and the free statistical software R, version 2.10.1 (R Development Core Team, 2009) were both used in data investigation. The concentration mean for a compound was calculated only if more than 60% of the samples had a concentration above the LOD (level of detection) (Verreault et al., 2005b). Only these compounds were taken into further statistical analyses. For the compounds included in statistical analyses, values below the LOD were given a value LOD/2 to avoid missing values in the statistical analyses (Bernhoft et al., 1997). The significance level was set to $<0.05$ throughout this project.

### 2.6.1 Univariate statistics

The experimental design of this project creates a lack of independency among samples. From each individual kittiwake there are taken samples from three organs. The samples taken from the same individual will not be independent. In addition to the predictor variables tissue type, sampling period and sex, which are so called fixed effects, there will also be an unknown effect from the individual kittiwake the samples are collected from. This effect is categorised as a random effect. To correct for this random effect a linear mixed effect (lme) model is applied in analyses where tissue type is included as a variable.

Two-way ANOVA (analysis of variance) was applied to reveal differences in kittiwakes' body mass, according to sex and sampling period. Lipid content is percentage data; these were arcsine-square root transformed to meet the assumptions of constant variation and normal distribution. Initial to analysis a gross outlier (one sample) was removed from the dataset (more than 1.5 times the length of a box away from the box when inspecting a box plot). Differences in lipid content between liver and preen gland samples, between sexes and sampling periods were investigated using lme models with contrast analysis (comparing every possible combinations with each others). A significant difference was found between lipid content in liver and preen gland samples, something which influences the concentrations of POPs in the samples. In order to compare those tissues more properly, it was chosen to use POP concentrations based on lipid weights (([compound]/lipid%)*100) (lipid wt). For feathers, there are no lipid wt concentrations available due to the low lipid content in feathers and the assumption that most of the lipid content on the kittiwakes' feathers are derived from

preen gland oil. Because of the very low POP concentrations on feathers compared to liver and preen gland tissue, this is not assumed to lead to severe misinterpretations in the results.

According to Shapiro-wilks test the majority of the compounds' concentrations were not normally distributed ($p<0.05$). Prior to further analyses, all POP concentrations were natural log (ln) transformed, in order to reduce variance heterogeneity and obtain approximately normal distribution.

Due to the random effect from the experimental design lme model were fitted and followed by contrast analyses to reveal differences in the total POP concentrations (every compound's concentrations were summed up) on feather, in liver and preen gland, and also between sexes and sampling period. Further on two-way ANOVA followed by Tukey's multiple comparisons test for unplanned comparisons (Tukey HSD test) were applied to find differences between sexes and sampling periods in each compound within each tissue type. Pearson's correlation coefficients were calculated to reveal correlations between the concentrations in different tissues.

Only 23 (one lost during analysis) samples were available for comparisons of lipid class composition. To increase the sample size and statistical power, the female and male samples were pooled together. The percentage values were arcsine-square root transformed prior to analyses. Only three lipid classes were detected in both liver and preen gland samples and for these lme models were fitted followed by contrast analyses to detect possible differences between tissues, and sampling periods.

### 2.6.2 Multivariate statistics

A detrended correspondence analysis (DCA) was carried out on arcsine-square root transformed POP percentage of total POP data, and as well with arcsine square root transformed lipid class data included. In both analyses the gradient was shorter than 2 standard deviations (SD). This indicate that the variance will be optimally explained by linear ordination techniques, like Principal component analysis (PCA) and Redundancy analysis (RDA) (Wijngaarden et al., 1995). PCA was carried out on both sets of data to visualise the POP profiles in feather, liver and preen gland, as well as the pattern of POPs in relation to lipid classes. RDA was performed and further on tested with an ANOVA to reveal significant impact of the predictor variable, tissue type, on the distribution of POPs and lipid classes.

With ordination one investigates variability in samples. The samples are distributed along axes based on their composition of the species (here; POPs). In this study, no environmental variables are considered. The PCA diagram gives an overview of the variation in samples based on differences in POP profiles. Every arrow in a PCA diagram has the same length, but different orientation along the axis give the impression of variable lengths. The arrows in the PCA diagrams points in the direction of increasing contribution of each POP to the total POP concentration, or the increasing contribution of a lipid class to total lipid content. The relative distance between samples reflects the dissimilarity of the samples. Samples close together share a more common POP profile or lipid class arrangement, than samples further away from each others.

# 3 Results

## 3.1 Body mass

The body mass of the 24 sampled kittiwakes ranged from 313 g to 465 g. The mean body mass for female and male birds were 347.25 g and 411.75 g respectively. In the incubation period the mean body mass of kittiwakes (n=12) was 398.08 g and in early chick rearing period (n=12) 360.92 g (9% lower). For kittiwake females the mean body masses for incubation and chick rearing period were 366.5 g and 328.0 g respectively, and for males 429.67 g and 393.83 g respectively (Fig. 3a). The effects of sex and sampling period are both highly significant on kittiwakes' body mass (Two-way ANOVA: sex; F= 148.0, df = 69, p <0.001, period; F = 49.8, df = 69, p<0.001).

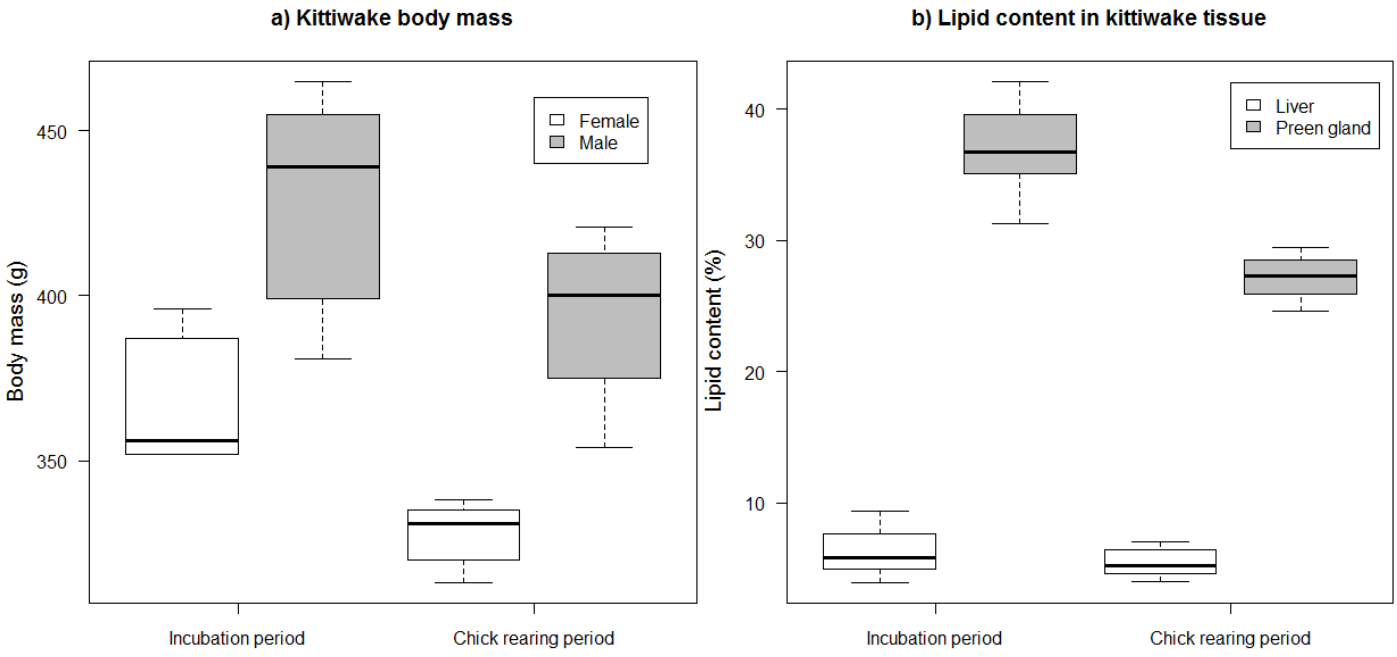


**Figure 3:** The body mass and lipid content in kittiwakes measured in the incubation- and chick rearing period. a) The body mass of male and female kittiwakes and b) the lipid content in kittiwake liver- and preen gland tissue. In this box-and-whisker plot, the median and first and third quartiles create the box, while the whiskers are max and min values, except when outliers are present. An outlier is defined as 1.5 times the length of the box away from the box.

## 3.2 Lipid content (%)

The mean ± SD of lipid content for all kittiwake samples were 33.83 ± 9.28 % and 5.78 ± 1.42 % for preen gland and liver samples respectively. When dividing the samples into incubation- and chick rearing period, the lipid content is 37.05 ± 3.06% and 30.61 ± 12.13% for preen gland in incubation- and chick rearing period respectively. For liver samples the

lipid content is 6.17 ± 1.68% and 5.39 ± 1.03% in first and second sampling period (figure 3b). Tissue type and sampling period were highly significant in explaining the lipid content (tissue; $F=2255.20$, $df=20$, $p<0.001$, period; $F=79.89$, $df=21$, $p<0.001$), in addition the interaction between these were found to be significant ($F=37.03$, $df=20$, $p<0.001$) Even if sex was not found to be significant ($p=0.28$) it occurred in a significant interaction with tissue type ($F=7.65$, $df=20$, $p=0.01$)

Because of the significant interactions, summary from the lme (Table 1) have to be further studied to reveal all results of interest. It is found that the higher lipid content in preen gland samples compared to liver samples is significant and independent of sampling period. In preen gland the lipid content decrease significantly from incubation to chick rearing period. In contrast such difference between sampling periods is not found in liver samples. Even if sex was not a significant predictor the interaction with tissue type made it important. A significant higher lipid content is found in male than female liver samples. In preen gland there is no difference between sexes.

**Table 1:** Summary from linear mixed effect model investigating lipid content in liver and preen glands of kittiwakes from Kongsfjorden, Svalbard. Interpretation: in every contrast analysis the first combination of predictor levels (first line) is basis for the interpretation of the three next lines. E.g. in contrast analysis 1, the first combination is female liver and sampling period 1; going to the second line the period is changed to period two, while tissue and sex remain unchanged. These two combinations are compared and a decreasing "value" and p=0.17, indicating similar lipid content in female liver samples from incubation- and chick rearing period. In contrary the first interaction (line 5) is compared with line 2 and the last line is compared to line 4. SE (standard error), Df (degrees of freedom).

| | *Value* | *SE* | *Df* | *t-value* | *p-value* |
|---|---|---|---|---|---|
| ***Contrast analysis 1:*** | | | | | |
| Female liver, period 1 | 0.23 | 0.01 | 21 | 25.49 | <0.001 |
| Period 2 | -0.02 | 0.01 | 21 | -1.41 | 0.17 |
| Preen gland | 0.43 | 0.01 | 20 | 32.67 | <0.001 |
| Male | 0.03 | 0.01 | 21 | 2.83 | <0.05 |
| Period 2:Preen gland | -0.09 | 0.02 | 20 | -6.02 | <0.001 |
| Preen gland: Male | -0.04 | 0.02 | 20 | -2.77 | <0.05 |
| ***Contrast analysis 2:*** | | | | | |
| Female preen gland, period 1 | 0.66 | 0.01 | 21 | 71.31 | <0.001 |
| Period 2 | -0.11 | 0.01 | 21 | -9.80 | <0.001 |
| Liver | -0.43 | 0.01 | 20 | -32.67 | <0.001 |
| Male | -0.01 | 0.01 | 21 | -1.10 | 0.28 |
| Period 2:Liver | 0.09 | 0.02 | 20 | 6.02 | <0.001 |
| Liver: Male | 0.04 | 0.02 | 20 | 2.77 | <0.05 |
| ***Contrast analysis 3:*** | | | | | |
| Female liver, period 2 | 0.22 | 0.01 | 21 | 23.86 | <0.001 |
| Period 1 | 0.02 | 0.01 | 21 | 1.41 | 0.17 |
| Preen gland | 0.34 | 0.01 | 20 | 24.89 | <0.001 |
| Male | 0.03 | 0.01 | 21 | 2.83 | <0.05 |
| Period 1:Preen gland | 0.09 | 0.02 | 20 | 6.02 | <0.001 |
| Preen gland:Male | -0.04 | 0.02 | 20 | -2.77 | <0.05 |
| ***Contrast analysis 4:*** | | | | | |
| Female preen gland, period 2 | 0.55 | 0.01 | 21 | 56.40 | <0.001 |
| Period 1 | 0.11 | 0.01 | 21 | 9.80 | <0.001 |
| Liver | -0.34 | 0.01 | 20 | -24.89 | <0.001 |
| Male | -0.01 | 0.01 | 21 | -1.10 | 0.28 |
| Period 1:Liver | -0.09 | 0.02 | 20 | -6.02 | <0.001 |
| Liver:Male | 0.04 | 0.02 | 20 | 2.77 | <0.05 |
| ***Contrast analysis 5:*** | | | | | |
| Male liver, period 1 | 0.26 | 0.01 | 21 | 28.76 | <0.001 |
| Period 2 | -0.02 | 0.01 | 21 | -1.41 | 0.17 |
| Preen gland | 0.38 | 0.01 | 20 | 29.45 | <0.001 |
| Female | -0.03 | 0.01 | 21 | -2.83 | <0.05 |
| Period 2:Preen gland | -0.09 | 0.02 | 20 | -6.02 | <0.001 |
| Preen gland:Female | 0.04 | 0.02 | 20 | 2.77 | <0.05 |
| ***Contrast analysis 6:*** | | | | | |
| Male preen gland, period 1 | 0.65 | 0.01 | 21 | 70.02 | <0.001 |
| Period 2 | -0.11 | 0.01 | 21 | -9.80 | <0.001 |
| Liver | -0.38 | 0.01 | 20 | -29.45 | <0.001 |
| Female | 0.01 | 0.01 | 21 | 1.10 | 0.28 |
| Period 2:Liver | 0.09 | 0.02 | 20 | 6.02 | <0.001 |
| Liver:Female | -0.04 | 0.02 | 20 | -2.77 | <0.05 |

## 3.3 POP compounds

The following compounds were detected above the LOD in over 60% of the samples: β-HCH, HCB, heptachlor, *cis*-chlordane, *oxy*-chlordane, *trans*-nonachlor, *cis*-nonachlor, mirex, *p,p*-DDE, PCB 99, PCB 105, PCB 118, PCB 128, PCB 138, PCB 141, PCB 149, PCB, 153, PCB 156, PCB 157, PCB 167, PCB 170, PCB 180, PCB 183, PCB 187, PCB 189 and PCB 194. In liver and preen gland samples all of the listed compounds were detected. In feather all except PCB 149 were found. ∑PCBs contribute 78, 80 and 81 % to ∑POPs in liver, feathers and preen gland respectively.

## 3.4 Level of POPs

The mean of ∑POPs in feather-, liver- and preen gland samples were 79.32 ± 47.86 (28.94-225.06) ng/g wet wt, 709.02 ± 458.33 (116.63-1488.99) ng/g wet wt and 2739.22 ± 1260.28 (1070.19-6624.96) ng/g wet wt respectively (Table 2). For liver and preen gland the concentrations are also given per lipid wt; 11976.50 ± 8141.90 (1777.20-26781.91) ng/g lipid wt and 8551.91 ± 4705.77 (2634.58-22462.51) ng/g lipid wt respectively (Table 2). Detailed tables of POP concentrations found in this study are given in appendix III (wet wt) and appendix IV (lipid wt).

**Table 2:** The mean, ± SD, median and data range of POP concentrations (ng/g wet wt) in feathers, liver and preen gland of adult kittiwakes from Ny-Ålesund, Svalbard.

| | | Feathers (ng/g wet wt) | | | | Liver (ng/g wet wt) | | | | Preen gland (ng/g wet wt) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | nd | nd | nd | nd | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **∑HCH**[a] | 19/24 | 1.20 ± 0.64 | 1.00 | 0.54-2.93 | 19/24 | 2.46 ± 1.43 | 2.25 | 0.45-5.67 | 24/24 | 29.21 ± 16.16 | 28.23 | 0.89-60.92 |
| **HCB** | 24/24 | 0.97 ± 0.95 | 0.73 | 0.29-4.83 | 24/24 | 17.02 ± 5.67 | 17.73 | 7.09-26.58 | 24/24 | 57.02 ± 16.76 | 55.04 | 35.65-116.75 |
| **Heptachlor** | 3/24 | nd | nd | nd | 24/24 | 8.06 ± 3.86 | 7.34 | 2.33-17.45 | 24/24 | 7.42 ± 3.87 | 6.33 | 2.98-20.24 |
| **∑chlordanes**[b] | 24/24 | 4.09 ± 2.76 | 2.74 | 1.49-10.71 | 24/24 | 26.41 ± 16.76 | 25.53 | 5.77-61.74 | 24/24 | 171.66 ± 69.06 | 154.78 | 56.74-319.60 |
| ***p,p'*-DDE** | 24/24 | 11.03 ± 12.86 | 6.61 | 0.88-62.43 | 24/24 | 50.69 ± 45.69 | 34.03 | 6.32-185.31 | 24/24 | 273.18 ± 200.31 | 228.85 | 48.33-746.05 |
| **Mirex** | 2/24 | nd | nd | nd | 24/24 | 13.90 ± 10.00 | 13.84 | 0.97-32.51 | 24/24 | 32.38 ± 12.05 | 31.75 | 8.55-57.59 |
| **∑PCBs**[c] | 24/24 | 62.09 ± 34.87 | 53.78 | 22.03-146.69 | 24/24 | 590.99 ± 401.66 | 557.40 | 86.85-1285.06 | 24/24 | 2168.35 ± 1149.77 | 1923.13 | 805.63-6083.33 |
| **∑POPs**[d] | 24/24 | 79.32 ± 47.86 | 69.40 | 28.94-225.06 | 24/24 | 709.02 ± 458.33 | 667.96 | 116.63-1488.99 | 24/24 | 2739.22 ± 1260.28 | 2383.50 | 1070.19-6624.96 |

[a] ∑HCH = sum of α-HCH, β-HCH and γ-HCH.

[b]∑chlordanes = sum of trans-chlordane, cis-chlordane, oxy-chlordane, trans-nonachlor and cis-nonachlor.

[c]∑PCBs = sum of PCB-99, -101, -105, -118, -123, -128, -138, -141, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189 and -194.

[d]∑POPs = sum of ∑HCH, HCB, Heptachlor, ∑chlordanes, p,p'-DDE, Mirex and ∑PCBs.

**Table 2:** The mean, ± SD, median and data range of POP concentrations (ng/g lipid wt) in liver and preen gland of adult kittiwakes from Ny-Ålesund, Svalbard.

| | | Liver (ng/g lipid wt) | | | | Preen gland (ng/g lipid wt) | | |
|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **∑HCH**[a] | 19/24 | 44.76 ± 20.80 | 43.60 | 11.49-91.88 | 24/24 | 95.42 ± 59.81 | 89.51 | 2.11-206.94 |
| **HCB** | 24/24 | 309.12 ± 100.32 | 313.97 | 134.71-500.00 | 0/24 | nd | nd | nd |
| **Heptachlor** | 24/24 | 153.40 ± 83.71 | 141.84 | 33.96-346.73 | 24/24 | 22.75 ± 10.65 | 21.33 | 5.67-48.05 |
| **∑chlordanes**[b] | 24/24 | 477.22 ± 278.68 | 401.40 | 112.98-1001.14 | 24/24 | 553.96 ± 285.77 | 467.67 | 144.91-1092.60 |
| ***p,p'*-DDE** | 24/24 | 795.01 ± 645.00 | 630.07 | 46.88-2479.50 | 24/24 | 806.60 ± 550.95 | 620.41 | 186.67-2095.81 |
| **Mirex** | 24/24 | 246.51 ± 158.67 | 245.53 | 24.98-490.36 | 24/24 | 102.66 ± 46.46 | 101.14 | 22.76-206.13 |
| **∑PCBs**[c] | 24/24 | 9959.79 ± 7259.70 | 7781.16 | 732.59-24569.59 | 24/24 | 6970.53 ± 4272.88 | 6302.77 | 2057.98-20802.98 |
| **∑POPs**[d] | 24/24 | 11976.50 ± 8141.90 | 9896.42 | 1777.20-26781.91 | 24/24 | 8730.87 ± 4734.24 | 8218.22 | 2733.39-22655.10 |

[a] ∑HCH = sum of α-HCH, β-HCH and γ-HCH.

[b]∑chlordanes = sum of trans-chlordane, cis-chlordane, oxy-chlordane, trans-nonachlor and cis-nonachlor.

[c]∑PCBs = sum of PCB-99, -101, -105, -118, -123, -128, -138, -141, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189 and -194.

[d]∑POPs = sum of ∑HCH, HCB, Heptachlor, ∑chlordanes, p,p'-DDE, Mirex and ∑PCBs

## 3.5 POP concentrations in kittiwakes related to differences in sex, type of tissue and sampling period

### 3.5.1 Overall

The sampling period and type of tissue are both found to be significant in explaining the ∑POPs level in kittiwakes (period; F=11.22, df=22, p<0.01, tissue; F=1733.22, df=44, p<0.001). The interaction between these is also significant (F=44.59, df=44, p<0.001). Overall no significant effect from sex is found (p=0.18) on the total POP concentration.

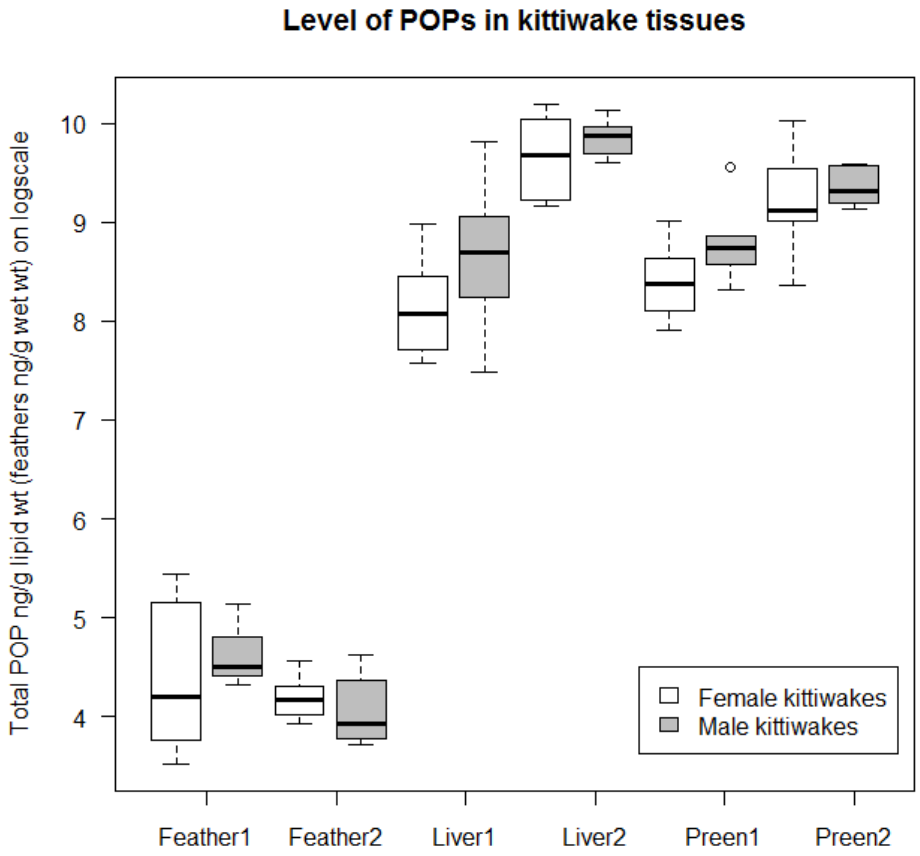


**Figure 4:** Level of POPs in female and male kittiwake feather (ng/g wet wt), and liver and preen gland (ng/g lipid wt) from the incubation period (1) and the chick rearing period (2).

A summary from the lme model is given in Table 4. ∑POPs concentration is significant higher in liver and preen gland tissue than in feathers independent of sampling period. In the incubation period there is no significant difference in contamination between liver and preen gland tissue. In contrast there is a significant higher concentration in liver tissue compared to preen gland tissue in the chick rearing period. There is a significant increase in contamination

in both liver and preen gland tissue from the incubation to the chick rearing period. In contrast there is a trend towards decreasing contamination in feathers in the chick rearing period (p=0.07) (Fig. 4).

**Table 4:** Summary from the linear mixed effect model for ∑POPs in kittiwakes from Kongsfjorden, Svalbard. Interpretation: in every contrast analysis the first combination of predictor levels (first line) is basis for the interpretation of the three next lines. E.g. in contrast analysis 1, the first combination is tissue feather and sampling period 1; going to the second line the period is changed to period two, while tissue still is feather. These two combinations are compared and a decreasing "value" and p=0.07, indicates a trend (not significant) towards decreasing ∑POPs concentrations in kittiwake feathers from first to second sampling period. In contrary the interactions (two last lines) are compared to the second line.

| | *Value* | *SE* | *Df* | *t-value* | *p-value* |
|---|---|---|---|---|---|
| ***Contrast analysis 1:*** | | | | | |
| Feather, period 1 | 4.49 | 0.14 | 44 | 32.33 | <0.001 |
| Period 2 | - 0.37 | 0.20 | 22 | - 1.89 | 0.07 |
| Liver | 3.91 | 0.13 | 44 | 29.97 | <0.001 |
| Preen gland | 4.11 | 0.13 | 44 | 31.48 | <0.001 |
| Period2: Liver | 1.73 | 0.19 | 44 | 9.38 | <0.001 |
| Period2: Preen gland | 1.05 | 0.19 | 44 | 5.67 | <0.001 |
| ***Contrast analysis 2:*** | | | | | |
| Preen gland, period 1 | 8.60 | 0.14 | 44 | 61.89 | <0.001 |
| Period 2 | 0.67 | 0.20 | 22 | 3.43 | <0.01 |
| Liver | - 0.20 | 0.13 | 44 | - 1.51 | 0.14 |
| Feather | - 4.11 | 0.13 | 44 | - 31.48 | <0.001 |
| Period 2:Liver | 0.68 | 0.19 | 44 | 3.71 | <0.001 |
| Period 2:Feather | - 1.05 | 0.19 | 44 | -5.67 | <0.001 |
| ***Contrast analysis 3:*** | | | | | |
| Liver, period 1 | 8.40 | 0.14 | 44 | 60.47 | <0.001 |
| Period 2 | 1.36 | 0.20 | 22 | 6.91 | <0.001 |
| Preen gland | 0.20 | 0.13 | 44 | 1.51 | 0.14 |
| Feather | - 3.91 | 0.13 | 44 | - 29.97 | <0.001 |
| Period 2:Preen gland | - 0.68 | 0.19 | 44 | - 3.71 | <0.001 |
| Period 2:Feather | - 1.73 | 0.19 | 44 | - 9.38 | <0.001 |
| ***Contrast analysis 4:*** | | | | | |
| Feather, period 2 | 4.12 | 0.14 | 44 | 29.65 | <0.001 |
| Period 1 | 0.37 | 0.20 | 22 | 1.89 | 0.07 |
| Liver | 5.64 | 0.13 | 44 | 43.23 | <0.001 |
| Preen gland | 5.16 | 0.13 | 44 | 39.50 | <0.001 |
| Period 1:Liver | - 1.73 | 0.19 | 44 | - 9.38 | <0.001 |
| Period 1:Preen gland | - 1.05 | 0.19 | 44 | - 5.67 | <0.001 |
| ***Contrast analysis 5:*** | | | | | |
| Preen gland, period 2 | 9.28 | 0.20 | 44 | 66.74 | <0.001 |
| Period 1 | - 0.67 | 0.20 | 22 | - 3.43 | <0.01 |
| Liver | 0.49 | 0.13 | 44 | 3.73 | <0.001 |
| Feather | - 5.16 | 0.13 | 44 | - 39.50 | <0.001 |
| Period 1:Liver | - 0.68 | 0.19 | 44 | - 3.71 | <0.001 |
| Period 1:Feather | 1.05 | 0.19 | 44 | 5.67 | <0.001 |

### 3.5.2 Liver

The mean liver concentration of β-HCH, HCB, heptachlor, *oxy*-chlordane, *cis*-nonachlor, mirex, PCB 99, PCB 105, PCB 118, PCB 138, PCB 149, PCB 153, PCB 156, PCB 157, PCB 167, PCB 180, PCB 183, PCB 187, PCB 189, and PCB 194 increased from the incubation- to the chick rearing period. Even if not significant, the other compounds also showed increasing means from the incubation period to the chick rearing period. Significant difference between sexes was revealed only in *oxy*-chlordane and mirex. Even not significant, near every compounds have higher concentration mean in male than female kittiwakes (Table 5).

***Table 5:*** Two-way ANOVA and Tukey HSD test parameters, for contaminant concentrations in kittiwake liver in respect of sampling period and sex. Only ANOVA parameters for significant results are given. Tukey HSD are given also for non significant result, to be able to track patterns. 1=incubation period, 2=chick rearing period, m=male, f=female.

| | ANOVA | | | | Tukey HSD | |
|---|---|---|---|---|---|---|
| **Compound** | **Significant predictors** | **F** | **df** | **p** | **Incubation vs. Chick rearing period** | **Male vs. female** |
| **β-HCH** | Period | 18.56 | 21 | <0.001 | 2>1 | m>f |
| **HCB** | Period | 62.58 | 21 | <0.001 | 2>1 | m>f |
| **Heptachlor** | Period | 20.78 | 21 | <0.001 | 2>1 | m<f |
| ***cis*-chlordane** | | | | | 2>1 | m>f |
| ***oxy*-chlordane** | Sex<br>Period | 7.55<br>71.69 | 21<br>21 | <0.05<br><0.001 | <br>2>1 | m>f |
| ***trans*-nonachlor** | | | | | 2>1 | m>f |
| ***cis*-nonachlor** | Period | 5.60 | 21 | <0.05 | 2>1 | m>f |
| **Mirex** | Sex<br>Period<br>Sex:period | 9.71<br>55.31<br>4.43 | 20<br>20<br>20 | <0.01<br><0.001<br>0.05 | <br>2>1 | m>f |
| ***p,p*-DDE** | | | | | 2>1 | m>f |
| **PCB 99** | Period | 10.58 | 21 | <0.01 | 2>1 | m>f |
| **PCB 105** | Period | 25.80 | 21 | <0.001 | 2>1 | m>f |
| **PCB 118** | Period | 26.47 | 21 | <0.001 | 2>1 | m>f |
| **PCB 128** | | | | | 2>1 | m<f |
| **PCB 138** | Period | 31.62 | 21 | <0.001 | 2>1 | m>f |
| **PCB 141** | | | | | 2>1 | m>f |
| **PCB 149** | Period | 36.62 | 21 | <0.001 | 2>1 | m>f |
| **PCB 153** | Period | 34.16 | 21 | <0.001 | 2>1 | m>f |
| **PCB 156** | Period | 33.28 | 21 | <0.001 | 2>1 | m>f |
| **PCB 157** | Period | 16.60 | 21 | <0.001 | 2>1 | m>f |
| **PCB 167** | Period | 35.36 | 21 | <0.001 | 2>1 | m>f |
| **PCB 170** | | | | | 2>1 | m>f |
| **PCB 180** | Period | 39.63 | 21 | <0.001 | 2>1 | m>f |
| **PCB 183** | Period | 39.85 | 21 | <0.001 | 2>1 | m>f |
| **PCB 187** | Period | 33.21 | 21 | <0.001 | 2>1 | m>f |
| **PCB 189** | Period | 29.29 | 21 | <0.001 | 2>1 | m>f |
| **PCB 194** | Period | 32.81 | 21 | <0.001 | 2>1 | m>f |
| **∑POPs** | Period | 39.96 | 21 | <0.001 | 2>1 | m>f |

### 3.5.3 Preen gland

The preen gland samples show a pattern, similar to that of the liver, with increase from incubation to chick rearing period in the following compounds' mean concentrations; β-HCH, HCB, *oxy*-chlordane, mirex, PCB 99, PCB 105, PCB 118, PCB 128, PCB 138, PCB 149, PCB153, PCB156, PCB167, PCB170, PCB 183, PCB187, PCB 189 and PCB 194. Except for *trans*-nonachlor and *cis*-chlordane (which are decreasing) the rest of the compounds did also increase even if not significant. Only mirex and PCB 149 had higher concentrations in male than female kittiwakes. Also here higher mean values for males are found in almost every compound, although not significant (Table 6).

**Table 6:** Two-way ANOVA and Tukey HSD test parameters, for contaminant concentrations in kittiwake preen gland in respect of sampling period and sex. Only ANOVA parameters for significant results are given. Tukey HSD comparisons are given also for non significant result, to be able to track patterns. 1=incubation period, 2=chick rearing period, m=male, f=female.

| | ANOVA | | | | Tukey HSD | |
|---|---|---|---|---|---|---|
| **Compound** | **Significant predictors** | **F** | **df** | **p** | **Incubation vs. Chick rearing period** | **Male vs. female** |
| **β-HCH** | Period | 10.22 | 21 | <0.01 | 2>1 | m<f |
| **HCB** | Period | 15.73 | 21 | <0.001 | 2>1 | m<f |
| **Heptachlor** | | | | | 2>1 | m>f |
| ***cis*-chlordane** | | | | | 2<1 | m<f |
| ***oxy*-chlordane** | Period | 21.54 | 21 | <0.001 | 2>1 | m>f |
| ***trans*-nonachlor** | | | | | 2<1 | m>f |
| ***cis*-nonachlor** | | | | | 2>1 | m>f |
| **Mirex** | Sex<br>Period | 4.37<br>6.98 | 21<br>21 | <0.05<br><0.05 | <br>2>1 | m>f |
| ***p,p*-DDE** | | | | | 2>1 | m>f |
| **PCB 99** | Period | 21.53 | 21 | <0.001 | 2>1 | m>f |
| **PCB 105** | Period | 9.62 | 21 | <0.01 | 2>1 | m>f |
| **PCB 118** | Period | 23.05 | 21 | <0.001 | 2>1 | m>f |
| **PCB 128** | Period | 20.51 | 21 | <0.001 | 2>1 | m>f |
| **PCB 138** | Period | 14.98 | 21 | <0.001 | 2>1 | m>f |
| **PCB 141** | | | | | 2>1 | m>f |
| **PCB 149** | Sex<br>Period | 4.92<br>19.49 | 21<br>21 | <0.05<br><0.001 | <br>2>1 | m>f |
| **PCB 153** | Period | 16.37 | 21 | <0.001 | 2>1 | m>f |
| **PCB 156** | Period | 14.97 | 21 | <0.001 | 2>1 | m>f |
| **PCB 157** | | | | | 2>1 | m>f |
| **PCB 167** | Period | 7.72 | 21 | <0.05 | 2>1 | m>f |
| **PCB 170** | Period | 11.51 | 21 | <0.01 | 2>1 | m>f |
| **PCB 180** | | | | | 2>1 | m>f |
| **PCB 183** | Period | 10.91 | 21 | <0.01 | 2>1 | m>f |
| **PCB 187** | Period | 10.99 | 21 | <0.01 | 2>1 | m>f |
| **PCB 189** | Period | 8.62 | 21 | <0.01 | 2>1 | m>f |
| **PCB 194** | Period | 9.81 | 21 | <0.01 | 2>1 | m>f |
| **∑POPs** | Period | 16.37 | 21 | <0.001 | 2>1 | m>f |

### 3.5.4 Feathers

Samples from kittiwake feathers show a rather different pattern (Fig. 4) regarding sampling periods. The mean concentrations of HCB, *trans*-nonachlor, *cis*-nonachlor and, *p,p'*-DDE are significant lower in the chick rearing period, than in the incubation period. Even if the difference is not significant, there is observed a possible trend towards lower mean concentrations in all other compounds except PCB 153 and PCB 194. No compounds detected in feathers had significant higher levels in male than in female kittiwakes, but also here it higher concentrations were measured for males in most compounds (Table 7).

**Table 7:** Two-way ANOVA and Tukey HSD test parameters, for contaminant concentrations in kittiwake feathers in respect of sampling period and sex. Only ANOVA parameters for significant results are given. Tukey HSD comparison are given also for non significant result, to be able to track patterns. 1=incubation period, 2=chick rearing period, m=male, f=female. nd = not detected.

| | ANOVA | | | | Tukey HSD | |
|---|---|---|---|---|---|---|
| **Compound** | **Significant predictors** | **F** | **df** | **p** | **Incubation vs. Chick rearing period** | **Male vs. female** |
| **β-HCH** | | | | | 2<1 | m<f |
| **HCB** | Period | 12.11 | 21 | <0.01 | 2<1 | m>f |
| **Heptachlor** | | | | | 2<1 | m<f |
| ***cis*-chlordane** | | | | | 2<1 | m>f |
| ***oxy*-chlordane** | | | | | 2<1 | m>f |
| ***trans*-nonachlor** | Period | 27.51 | 21 | <0.001 | 2<1 | m<f |
| ***cis*-nonachlor** | Period | 31.79 | 21 | <0.001 | 2<1 | m<f |
| **Mirex** | | | | | 2<1 | m<f |
| ***p,p*-DDE** | period | 5.85 | 21 | <0.05 | 2<1 | m<f |
| **PCB 99** | | | | | 2<1 | m>f |
| **PCB 105** | | | | | 2<1 | m>f |
| **PCB 118** | | | | | 2<1 | m>f |
| **PCB 128** | | | | | 2<1 | m<f |
| **PCB 138** | | | | | 2<1 | m>f |
| **PCB 141** | | | | | 2<1 | m<f |
| **PCB 149** | | | | | nd | nd |
| **PCB 153** | | | | | 2>1 | m>f |
| **PCB 156** | | | | | 2<1 | m<f |
| **PCB 157** | | | | | 2<1 | m>f |
| **PCB 167** | | | | | 2<1 | m>f |
| **PCB 170** | | | | | 2<1 | m>f |
| **PCB 180** | | | | | 2<1 | m>f |
| **PCB 183** | | | | | 2<1 | m>f |
| **PCB 187** | | | | | 2<1 | m>f |
| **PCB 189** | | | | | 2<1 | m<f |
| **PCB 194** | | | | | 2>1 | m>f |
| **∑POPs** | | | | | 2<1 (p=0.07) | m>f |

## 3.6 Correlations between POP concentrations in the different kittiwake tissues

The correlation coefficient between POP concentrations shows a variable pattern according to which tissues are examined (Table 8). Several significant positive correlations are found between concentrations in preen gland and liver tissue. Also a trend for increasing correlation is seen from incubation to chick rearing period. *p,p'*-DDE concentrations are also correlated between preen gland and feather, as well as between liver and feather. No significant negative correlations are found.

**Table 8:** Pearson's correlation coefficients (r) calculated between log concentrations of contaminants on kittiwake feathers (ng/g wet wt), in liver (ng/g lipid wt), and preen gland (ng/g lipid wt) in incubation period (1) and chick rearing period (2). When less than 60% of samples had concentrations above LOD, the result is reported as not detected (Nd). (*p<0.05; **p<0.01:***p<0.001).

| | **Preen gland vs. liver** | | **Preen gland vs. feather** | | **Liver vs. feather** | |
|---|---|---|---|---|---|---|
| ***Period*** | *1* | *2* | *1* | *2* | *1* | *2* |
| *β*-HCH | 0.51 | 0.73** | 0.17 | 0.07 | 0.65* | 0.06 |
| HCB | 0.45 | -0.03 | 0.54 | 0.17 | -0.12 | 0.12 |
| Heptachlor | 0.48 | 0.38 | -0.09 | Nd | 0.55 | Nd |
| Chlordanes | 0.63* | 0.84*** | 0.35 | 0.35 | 0.04 | 0.45 |
| Mirex | 0.72** | 0.59* | 0.02 | Nd | 0.39 | Nd |
| *p,p'*-DDE | 0.77** | 0.97*** | 0.76** | 0.82** | 0.81** | 0.86*** |
| ∑PCBs | 0.64* | 0.88*** | 0.42 | 0.64* | 0.38 | 0.51 |
| ∑POPS | 0.74** | 0.86*** | 0.53 | 0.52 | 0.47 | 0.43 |

## 3.7 POP profiles

POP profiles or pattern show the percentage contribution from the different compounds to the total POP concentration.

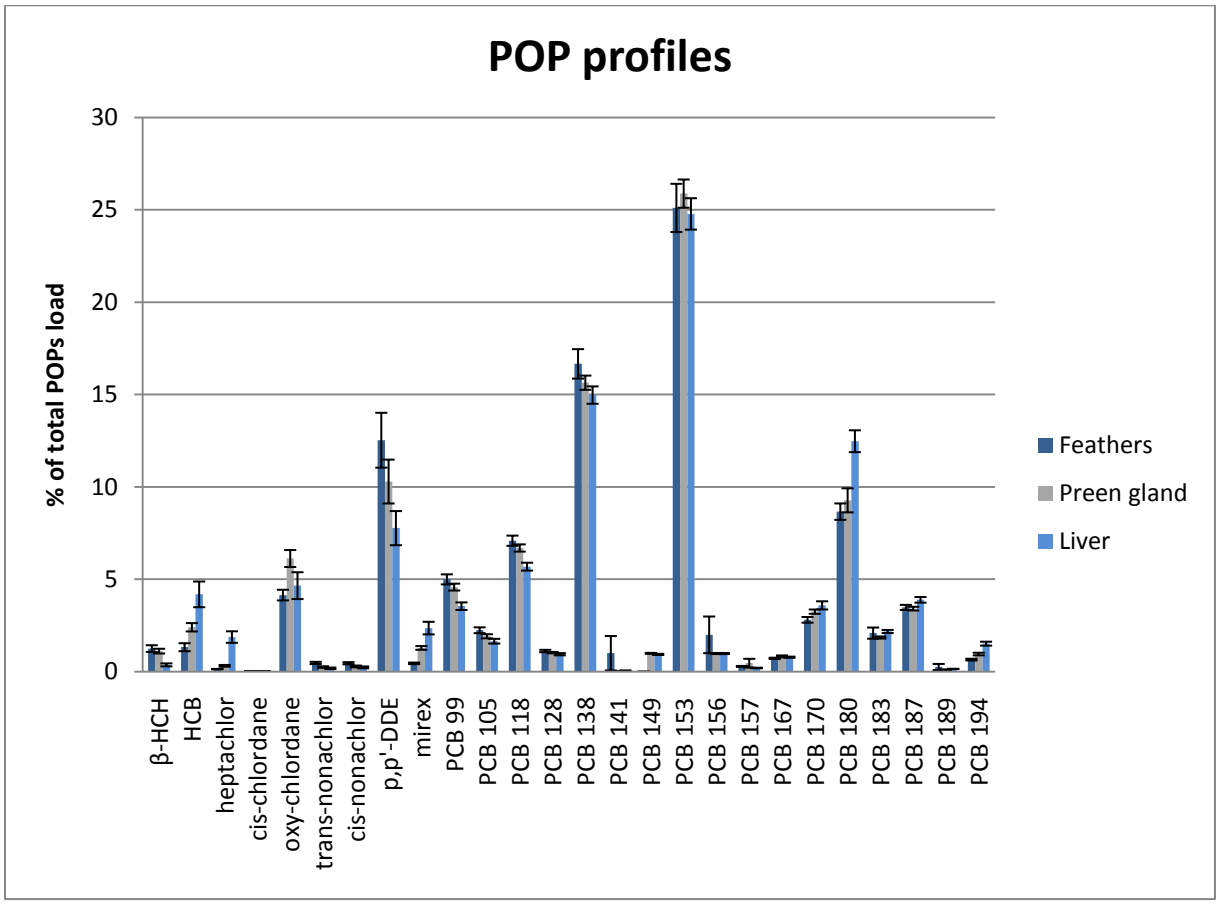


**Figure 5:** The POP profiles for feathers, preen gland and liver in kittiwakes, 95% confidence intervals are indicated with error bars.

For feather, liver and preen gland PCB 153 (25.1%, 24.8% and 25.9% in feather, liver and preen gland respectively), PCB 138 (16.7%,15.0% and 15.6%), *p,p*-DDE (12.5%, 7.8% and 10.3%) and PCB 180 (8.7%, 12.5% and 9.3%) are the main contaminant contributors to the total concentration of contaminants in kittiwakes (Fig. 5).

In several compounds the 95% confidence intervals (Fig. 5), indicated with error bars, are not overlapping between tissues. This indicates a significant difference in the compound's mean contribution to the total POP load. Within most of the compounds the preen gland seem to be situated between the liver and feathers, meaning the feather POP profile are more similar to the preen gland than the liver. E.g. β-HCH (1.3% and 0.4% in feather and liver respectively), *p,p'*-DDE (12.5% and 7.8%), and PCB 99 (5.0% and 3.6%) seem to contribute significant more in feather- than in liver POP profile. The error bars indicate significant difference

between feather and liver in at least 12 of the compounds, while only in approximately four compounds a difference is seen between feathers and preen gland. Even though differences are spotted in several compounds, are most of them really small.

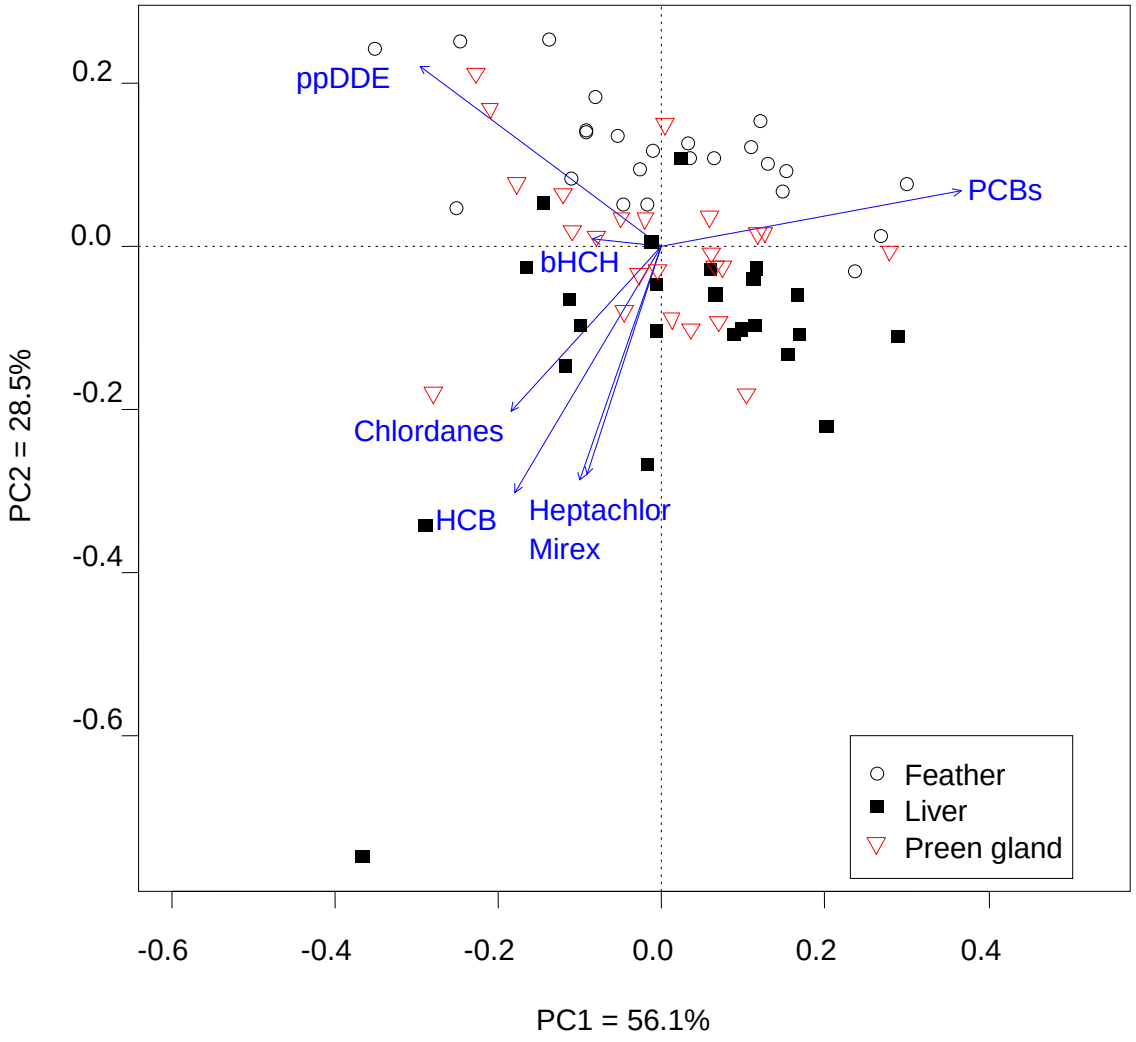


**Figure 6:** Indirect ordination analysis from a principal component analysis (PCA) presenting the POPs (β-HCH, HCB, heptachlor, ∑chlordanes, *p,p'*-DDE and ∑PCBs) profile in kittiwake feather, liver and preen gland. The arrows (POPs) point in the direction of increasing mean contribution to the total POP load. Percent variability explained by the two first principal components (PC1 and PC2) is given. The figure is slightly modified to make readable.

Inspection of the PCA plot (Fig. 6) reveals a tendency for feather, liver and preen gland samples to separate along the second axis. Also here feather samples are slightly more similar to preen gland than liver profile. This is in accordance with Figure 5. Liver and preen gland samples are more related to higher contribution of chlordanes, HCB, heptachlor and mirex to the POP profile, than are feather samples. The RDA analysis showed significant contribution from the predictor variable tissue type ($F = 7.12$, $df = 69$, $p = 0.005$) on the POPs profile.

However, the variation in POP pattern explained by this predictor is very small (less than 5%).This is in accordance with the interpretation of the barplot where several significant differences were spotted, but most of them were very small. Cumulatively, the two principal components (PC1 and PC2) accounted for 84.6% of the total variation in the POP pattern.

### 3.8 Lipid class composition in liver and preen gland samples

The lipid classes wax esters (WE), triacylglycerols (TAG), cholesterols (C), diacylglycerols (DAG), galactocerebrosides (GAL), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), phosphatidylinositols (PI), phosphatidylserines (PS), phosphatidylcholines (PC), sphingomyelins (SPM) and lysophosphatidylcholines (LPC) are detected in one or more of the analysed samples.

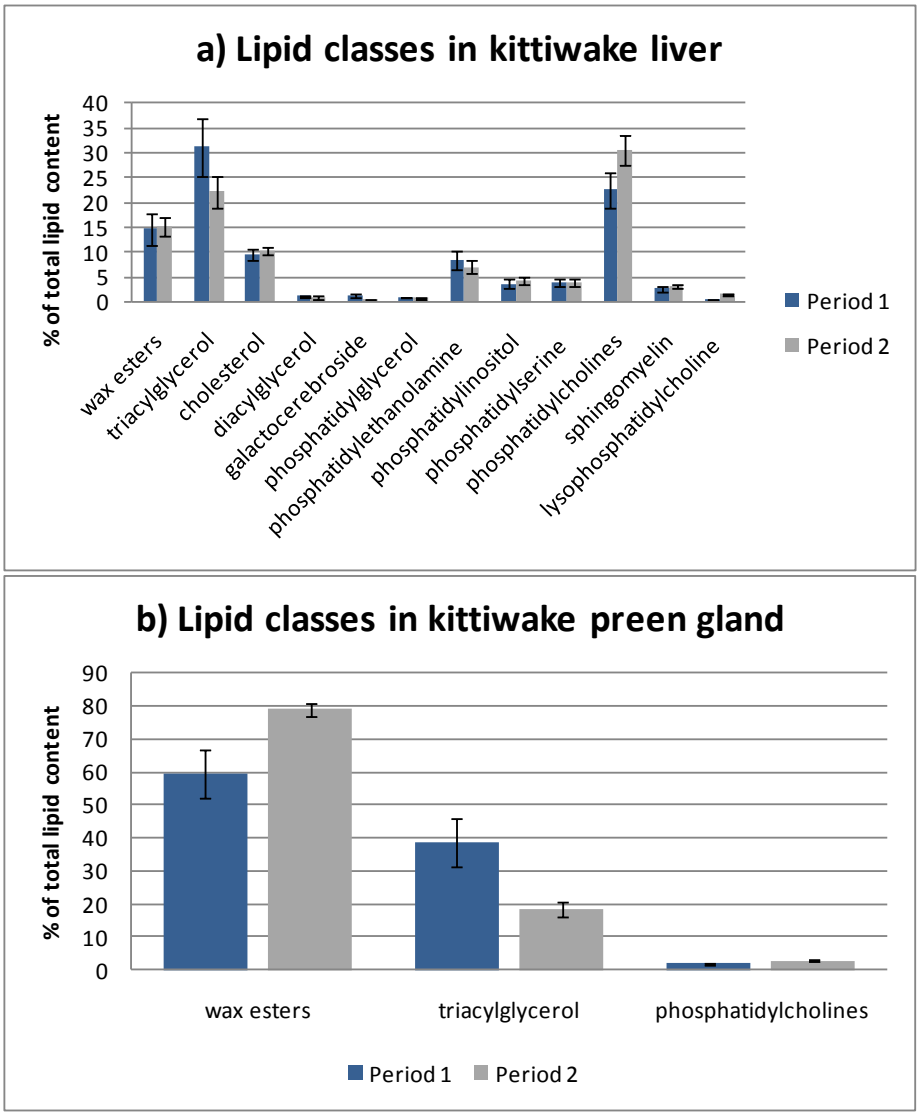


**Figure 7:** The mean contribution from each lipid class in the kittiwakes' liver (a), and preen gland (b) as a percent of total lipid content, in the incubation (1) and chick rearing period (2). 95% confidence interval is indicated with error bars.

Only a few lipid classes contribute most of the lipids to liver and preen gland (Fig. 7). Those most important are wax esters (14.9 % and 70.1 % in liver and preen gland respectively), triacylglycerols (26.7 % and 27.5 %), cholesterols (9.9 % and 0%) and phosphatidylcholines (26.6 % and 2.3 %). Detailed lipid compositions are listed in Appendix V.

Wax esters, triacylglycerols and phosphatidylcholines were the only lipid classes detected in both liver and preen gland tissue. Overall the contribution of wax esters is higher in preen gland- than liver tissue (70 % and 15 %). Wax esters have a similar contribution to total lipid content in liver in both sampling periods, while in preen gland the contribution is higher in chick rearing period than incubation period. Triacylglycerols show a similar contribution in liver- and preen gland tissue and a declining contribution from incubation- to chick rearing period. There are less phosphatidylcholines in preen gland- than liver tissue (27 % and 2 %). The contribution of phosphatidylcholines in both liver and preen gland is increasing from incubation to chick rearing period. Test parameters are listed in Table 9.

**Table 9:** Summaries from linear mixed effect models for contribution of wax esters, triacylglycerols and phosphatidylcholines in kittiwake liver and preen gland tissues. Interpretation: in every contrast analysis the first combination of predictor levels (first line) is basis for the interpretation of the three next lines. E.g. in contrast analysis 1, the first combination is tissue liver and sampling period 1; going to the second line the period is changed to period two, while tissue still is liver. These two combinations are compared and a increasing "value" and p=0.80, indicates similar contribution from wax esters to total lipid content in incubation- and chick rearing period. In contrary the interaction (last line) are compared to the second line.

| | *Value* | *SE* | *Df* | *t-value* | *p-value* |
|---|---|---|---|---|---|
| ***Wax esters*** | | | | | |
| ***Contrast analysis 1:*** | | | | | |
| Liver, period 1 | 0.38 | 0.05 | 10 | 8.57 | <0.001 |
| Period 2 | 0.02 | 0.06 | 10 | 0.26 | 0.80 |
| Preen gland | 0.51 | 0.05 | 9 | 9.48 | <0.001 |
| Period 2:Preen gland | 0.19 | 0.07 | 9 | 2.56 | <0.05 |
| ***Contrast analysis 2:*** | | | | | |
| Liver, period 2 | 0.40 | 0.05 | 10 | 8.94 | <0.001 |
| Period 1 | -0.02 | 0.06 | 10 | -0.26 | 0.80 |
| Preen gland | 0.70 | 0.05 | 9 | 13.85 | <0.001 |
| Period 1:Preen gland | -0.19 | 0.07 | 9 | -2.56 | <0.05 |
| ***Contrast analysis 3:*** | | | | | |
| Preen gland, period 1 | 0.89 | 0.05 | 10 | 18.45 | <0.001 |
| Period 2 | 0.21 | 0.07 | 10 | 3.12 | <0.05 |
| Liver | -0.51 | 0.05 | 9 | -9.48 | <0.001 |
| Period 2:Liver | -0.19 | 0.07 | 9 | -2.56 | <0.05 |
| ***Triacylglycerol*** | | | | | |
| ***Contrast analysis 1:*** | | | | | |
| Period 1 | 0.62 | 0.05 | 11 | 13.26 | <0.001 |
| Period 2 | -0.15 | 0.07 | 10 | -2.32 | <0.05 |
| ***Phosphatidylcholine*** | | | | | |
| ***Contrast analysis 1:*** | | | | | |
| Liver, period 1 | 0.49 | 0.03 | 10 | 16.58 | <0.001 |
| Period 2 | 0.10 | 0.04 | 10 | 2.90 | <0.05 |
| Preen gland | -0.40 | 0.04 | 10 | -11.54 | <0.001 |
| ***Contrast analysis 2:*** | | | | | |
| Liver, period 2 | 0.59 | 0.03 | 10 | 19.98 | <0.001 |
| Period 1 | -0.10 | 0.04 | 10 | -2.90 | <0.05 |
| Preen gland | -0.40 | 0.04 | 10 | -11.54 | <0.001 |
| ***Contrast analysis 3:*** | | | | | |
| Preen gland, period 1 | 0.09 | 0.03 | 10 | 2.88 | <0.05 |
| Period 2 | 0.10 | 0.04 | 10 | 2.90 | <0.05 |
| Liver | 0.40 | 0.04 | 10 | 11.54 | <0.001 |

The PCA plot (Fig. 8) are clearly separating the liver and preen gland samples along the first axis on the basis of the different POPs' contribution to total POP concentration, and different lipid classes to the total lipid content in the samples. Wax esters' contribution is increasing towards the preen gland samples. This is in accordance to the univariate analysis. *β*-HCH seems to be the POP most related to wax esters and thereby preen gland tissue. The RDA analysis showed significant contribution from the predictor variable tissue type (F = 56.9, df =21, p = 0.005), but only 22% of the variation in POP profile and lipid class contribution is explained by this predictor.

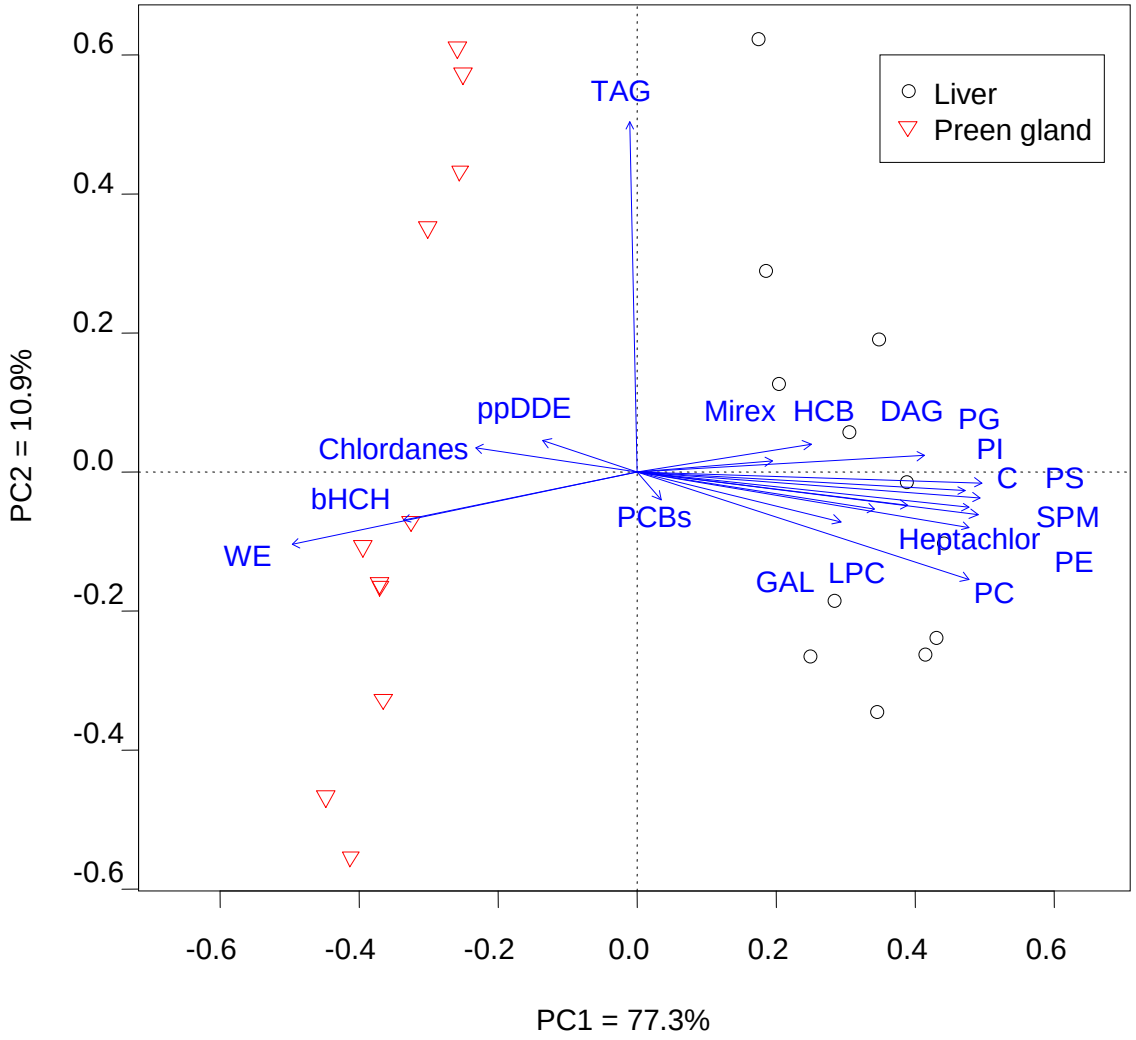


**Figure 8:** Indirect ordination analysis from a principal component analysis (PCA) presenting the POP profile and lipid class contribution in kittiwake liver and preen gland. The arrows (POPs and lipid classes) point in the direction of increasing contribution to the total POP load and total lipid content. Percent variability explained by the two first principal components (PC1 and PC2) is given. The plot is slightly modified to make readable.

The PCA plot are clearly separating the liver and preen gland samples along the first axis on the basis of the different POPs' contribution to total POP concentration, and different lipid classes to the total lipid content in the samples. Wax esters' contribution is increasing towards the preen gland samples. This is in accordance to the univariate analysis. $\beta$-HCH seems to be the POP most related to wax esters and thereby preen gland tissue. The RDA analysis showed significant contribution from the predictor variable tissue type ($F = 56.9$, $df = 21$, $p = 0.005$), but only 22% of the variation in POP profile and lipid class contribution is explained by this predictor.

# 4 Discussion

This study provides further evidence for excretion of POPs through the preen gland in birds. Kittiwake feathers were found to be contaminated by POPs and the POP profiles in feather and preen gland were found to be more similar than the feather and liver profiles. This indicates a transfer of POPs from the preen gland to the feathers. The wet wt concentrations of POPs measured in the preen gland were significant higher than the liver concentrations. The high level of POPs in preen gland tissues creates a basis for the kittiwake to excrete POPs through the preen gland. Similar to liver the POP concentrations in the preen gland increased from the incubation- to the chick rearing period. This could give the kittiwake an even greater opportunity to excrete POPs in energy demanding periods where the contaminants are most concentrated.

Every single POP compound (except PCB 149) detected in liver and preen gland samples was also detected in the feathers. This is an advantage for the use of feathers in monitoring POPs. However, there was a lack of correlation between feather- and the inner organ concentration, and a deviating response to seasonality for feather contamination. This may be seen in relation to seabirds', like the kittiwake, continuous exposure to seawater and may indicate that seabird feathers are a less good monitor for POP exposure than are feathers from terrestrial species.

## 4.1 Level of POPs compared with earlier contaminant studies

The lipid wt levels of contaminants other than ∑PCBs and DDT correspond to previous reported levels for kittiwake liver from the Barents Sea. ∑PCBs and DDT concentrations from the present study are only half of the earlier reported levels (Borgå et al., 2001). However, when evaluating wet wt data, which is more important to the toxicology of the compounds (Henriksen et al., 1996), the present study shows higher contaminant levels in kittiwake liver than do the study by Savinova et al. (1995) also from Kongsfjorden. ∑PCBs wet wt levels were also higher than reported from Hornøya in 1992 (Henriksen et al., 1996). No other studies are available in order to make comparison of contaminant concentrations in the preen gland and feathers in kittiwake. Jaspers et al. (2007b) report 220 ng/g wet wt ∑PCBs from tail feathers of herring gull (*Larus argentatus*) from Belgium, versus 62 ng/g wet wt in the present study's kittiwakes. These studies are not directly comparable, because of a non consistent sampling procedure, different analytical approaches, and the Belgium birds'

vicinity to contamination sources. Even if the present study's unfortunate contamination of samples was successfully eliminated, it was impossible to analyse for the lowest chlorinated PCBs as well as some DDT derivatives. The POP burden measured in different studies may be influenced by a different number of compounds analysed. Analytical differences between studies may also influence the comparisons. The low sample size in the present study, and the relatively high variation found, may bias the results. Therefore conclusions related to trends and significant differences should be drawn with caution.

## 4.2 Excretion of POPs through the preen gland in kittiwakes

***POPs are detected in kittiwake feathers***

As the bird is preening the plumage with oil from the preen gland, a main evidence to indicate excretion of POPs through the preen gland would be detection of POPs in the feathers. The present study's detection of POPs in kittiwake feathers is in accordance with earlier studies and can be seen as a first step to indicate excretion of POPs through the preen gland (Frank et al., 1983, Jaspers et al., 2008, Petersen and Ólafsdóttir, unpublished data). However, the POPs in feathers may have three possible sources of origin. First, the pollution may be remains from the feathers growing period. When feathers stop growing, the blood vessels atrophy and blood containing circulating POPs will be enclosed in the feather tissue (Jaspers et al., 2007b, Van den Steen et al., 2007). The cyclohexane/acetone treatment used for extraction of POPs in feathers, may have extracted POPs from inside the feather tissue. Second the POPs may be deposited onto the plumage by airborne pollution as has been reported for certain heavy metals (e.g. Dauwe et al., 2003). The study by Jaspers et al. (2007a) rejected this possibility in the common buzzards' (*Buteo buteo*) feathers. Third, the POPs may origin from the preen oil excreted by the preen gland as suggested by earlier studies on common loons (*Gavia immer*) and common magpies (Frank et al., 1983, Jaspers et al., 2008). The present study found significant but small differences in POP profiles between feathers, liver and preen gland. Despite the differences' small magnitude this study found that the POP profile on feathers was more similar with the POP profile in the preen gland that the profile from the liver. This support the suggestion that POPs on the kittiwakes feathers, at least partly origin from the preen gland.

***High POP concentrations are detected in preen gland of kittiwakes***

The mean wet wt concentration of POPs in preen gland samples were found to be significantly higher than concentrations measured in the liver. Due to the high lipid content in preen gland tissue, this was expected. When correcting for the differences in lipid content the POP concentrations in liver and preen gland were very similar (Fig. 4). The high concentrations in preen gland tissue is in accordance with earlier studies (Ingebrigtsen et al., 1981, Frank et al., 1983, Ingebrigtsen et al., 1984) and is a premise for significant excretion of POPs through the preen gland. The high levels of contaminants in the preen gland along with the finding of contamination on kittiwakes' feather, and a POP profile in feathers being most similar to that of the preen gland, indicate that the preen gland can be seen as an organ for excretion of POPs in kittiwakes (Frank et al., 1983).

***Lack of sex differences***

Female seabirds transfer contaminants to their offspring through egg-yolk (e.g. Gabrielsen et al., 1995, Sandven, 2006, Verreault et al., 2006, Helgason et al., 2008), and may thereby reduce their body burden of POPs compared to male seabirds (Skaare et al., 2000). In every tissues investigated, the present study shows only a trend (not significant) for lower contamination in female than male kittiwakes. The lack of a significant sex difference in contaminant level falls in line with a non consistent pattern regarding sex differences in contamination concentrations found in other seabird studies. Some studies find differences between the sexes (Gabrielsen et al., 1995) while others do not (Sagerup et al., 2009b). A low sample size (n=24), and low contaminant concentrations, may have biased the results because of a high within group variation compared with the between group variations. In this study the lipid content in the preen gland was found to be similar between female and male kittiwakes, which may indicate that both sexes would benefit equally from excretion of POPs through the preen gland. Knowledge regarding a possible sex difference in the preen gland's size is needed to better exclude such differences.

***POPs in the incubation- and chick rearing period***

The higher body mass reported for male compared to female kittiwakes was expected according to the species characteristics (Barrett et al., 1985, Moe et al., 2002). The significant decrease in body mass (9%) from the incubation- to the chick rearing period can be seen in relation to the increasing energy demand, which arise when the eggs hatches and the chicks must be fed. This is in accordance with mass loss generally found in breeding birds (Moreno,

1989) including kittiwakes (Henriksen et al., 1996, Moe et al., 2002). As expected the wax producing preen gland tissue was richer in lipid than the liver tissue (approximately 5 times higher). As the energy demand increases from the incubation- to the chick rearing period, kittiwakes are losing weight, which is mainly subcutaneous body fat (Moe, 1998, Gabrielsen, 2009). In the preen gland tissue this results in a decrease of the lipid content. A similar reduction of lipid in liver samples is not expected as it serves life functions. The birds are first using lipid stored in subcutaneous fat, before a fat reduction occur in central organs such as the liver Even in a selection severely emaciated and self-dead glaucous gulls, the total lipid content in liver was comparable with healthy gulls (data from Sagerup et al., 2009a).

Both liver and preen gland lipid wt concentrations of POPs increase from the incubation- to the chick rearing period. This is in accordance with earlier findings in liver and blood of kittiwakes (Henriksen et al., 1996, Nordstad, 2009). In the breeding season the kittiwakes' energy demand increase and shifts from self maintenance to chick care. The body mass in birds is decreasing because body-lipids are invested in reproduction. The lipophilic contaminants are thereby redistributed and concentrated in the smaller amount body-lipids which is left (Henriksen et al., 1996). This variation in lipid content, and a possible difference in lipid composition, could also influence the tissues' affinity for the lipid soluble compounds (Henriksen et al., 1996). Even if the mean POP concentration does not exceed the threshold for negative effects when the bird maintain a good body condition, the effect threshold may be exceeded in energy demanding periods for birds where contaminants are redistributed (de Wit et al., 2004).

The seasonality shown for contaminant concentrations in feathers was rather different from that found for liver and preen gland samples. The total POP contamination in kittiwake feathers showed a trend towards a decrease in the concentration from the incubation- to the chick rearing period. The only earlier study found,- which compare feather contamination in birds with different body conditions is the study of common loon by Frank et al. (1983). In contrast to the present study they report higher contamination levels in feathers from emaciated common loons compared with healthier birds. These emaciated common loons are not directly comparable to the present study, as they were not sampled with respect to any specific period when birds are known to be in poor body conditions, like the chick rearing period. Further were these birds sampled as carcasses, some partially decomposed with an unknown cause of death.

Feathers are not connected to the birds' circulation system, and the only POPs that might change concentrations in relation to seasonal factors are those contributed from external sources. In the study of common magpie by Jaspers et al. (2008), POPs originating from the preen gland is suggested to be the main contributor to external contamination in bird's feathers. The trend for decreasing contamination levels in feathers in the chick rearing period may indicate that the energy reserves are too scarce for kittiwakes to sustain the same preen oil production in the chick rearing period compared to periods in which the birds are in good body condition, such as before breeding or in the autumn. As reported in the present study, the POPs may be more concentrated in the preen gland during the chick rearing period, but due to fat deficiency, the production of preen oil might be lower. Theoretically a gland excreting POPs should be most efficient in periods with high concentrations of contaminants, such as found for the chick rearing period. Nevertheless low contaminate levels are detected in kittiwakes' feathers in the chick rearing period, thought to be the result of a fat deficiency. This indicates that the kittiwake cannot utilise the extra advantage of an organ excreting POPs, which arise in periods where POPs are found in high concentrations. A second explanation for the lower or stable contaminate levels found on kittiwake feathers from the chick rearing period may be directly related to a changing activity pattern of the parent birds. During the chick rearing period the parent birds continuously have to provide food to the chicks (Bech et al., 2002), which involves increasing exposure to seawater and consequently causing the contamination to be washed away from the feathers.

From the present study it is impossible to predict the amount of POPs which could possibly be excreted by the preen gland, except tentatively. Frank et al. (1983) related the findings of high contaminant concentrations in the preen gland, as well as high levels in common loons' feathers to excretion of POPs through the preen gland. However, the study by Frank et al. (1983) minimises the possible effect of this excretion on the bird's total POP body burden, by assuming the amount of excreted POPs to be minor. Without knowledge of species specific preen oil production it is impossible to exactly predict the amount of POPs that could possibly be excreted through the preen gland. Jacob (1976), reports a daily excretion of 600 mg preen oil for birds in the Laridae family. Jacob (1976) does not specify species or body mass for the Laridae in question. Species included in the Laridae family range in body mass from 100 g to about 2000 g (del Hoyo et al., 1996). If one assume the kittiwake's size to be a third of the assumed "average" Laridae from the study by Jacob (1976), the expected daily preen oil

excretion is assumed to be 200 mg. Verreault et al. (2007) report total body burden of POPs per gram for glaucous gull to be approximately 40% of the level measured in liver. With this estimate an average kittiwake from this study (380g) will have a total POP body burden of 100 000 ng. The estimated yearly excretion from preen gland would then be approximately 20% of the estimated body burden. These calculations are not done with exact data and include a high degree of uncertainty. However, the calculation may indicate that the amount of POPs excreted by the preen gland should not be neglected.

***No restriction to which POP compounds may be excreted through the kittiwake's preen gland***

The liver was found to exhibit a greater diversity in lipid class composition than do the preen gland. This can be seen in relation to the livers diversity of functions e.g. production of bile, energy storing and break down of toxic substances (Nelson and Cox, 2008), while the preen gland's main function is to produce preen oil (Stevens, 1996, Kent and Carr, 2001). In accordance to the preen gland's function, the water repellent wax esters dominated the lipid composition in preen gland samples. Similar results have been found in studies on northern fulmar (*Fulmarus glacialis*), and alcids (Jacob and Zeman, 1971, Jacob and Zeman, 1973). From this difference in lipid composition one might expect specific POPs to be overrepresented in one of the tissues, due to higher affinity to certain lipid classes (Bernhoft et al., 1997). However the present study found all POP compounds present in both liver and preen gland. Significant differences were detected in the POP profiles, but the magnitude of those was small. This may indicate that in these organs, the specific POP compounds are mainly dissolved in the lipid pool as a whole, and are not strictly associated to certain lipid classes (Gabrielsen et al., 1995). From this one may assume that there are no specific restrictions to which POP compounds may possibly be excreted through the kittiwakes' preen gland.

## 4.3 Feather as a biomonitor for POPs in seabirds

***POPs were detected on feathers***

The concentration of POPs measured in kittiwake feathers were found to be low compared to inner organ concentrations. This is in accordance with feathers' low lipid content. However, out of totally 26 POP compounds, 25 were detected above the LOD in feather samples.

***POP profile on feathers***

Although the differences between profiles are small in magnitude, e.g. β-HCH, *p,p'*-DDE and some of the lower chlorinated PCBs seem to contribute relatively more to the POP profile in feathers than to the inner organ profiles. This trend might have been more pronounced without the unfortunate contamination, which precluded the analysing of the lowest chlorinated PCBs. These findings are consistent with results from the studies by Dauwe et al. (2005) and Jaspers et al. (2007b) with 8 bird species (both aquatic and terrestrial). These studies relate the result to POP profiles in blood from the feather formation period. Due to the extraction with cyclohexane and acetone, it is likely that POPs inside the feather tissue, together with surface POPs from the preen gland constitute the total POP concentration. Exogenous air contamination could be a plausible explanation, since e.g. lower chlorinated PCBs are found to contribute more to contamination in the air than do higher chlorinated congeners (Vorhees et al., 1997). However, as discussed above Jaspers et al. (2007a) rejects airborne pollution as source of feather contamination in common magpie feathers. Nevertheless, in order to use feather in POP monitoring, the possibility for several origins for POPs on feather must be taken into account.

***Lack of correlations between contaminant concentrations on feathers and in inner organs***

The present study shows a lack of correlation in contaminant level between feathers and the inner organs. The only correlation found between feather and liver concentrations are that of *p,p'*-DDE and *β*-HCH (incubation period). *p,p'*-DDE and total PCB (chick rearing period) are correlated between feather and preen gland. This deviate from a rather high correlation found in the studies by Jaspers et al. (2008) (preen gland) and Jaspers et al. (2007a) (liver). These studies included feather from the common magpie and the common buzzard (*Buteo buteo*), both terrestrial birds. In consistence with the present study, Jaspers et al. (2007b) found no correlation between the contaminant level in feather and liver in herring gull. This could be an indication that POPs excreted by the preen gland are not accumulated to the same degree on feathers from seabirds, as in feathers from strictly terrestrial birds. Seabirds are continuous exposed to seawater where preen oil and POPs are washed away. Another possibility could be that individual differences may have biased the results. The kittiwakes from the present study were all breeding. Some were caught on their way in from the sea, the other after several hours on the nest. This may have created individual differences in the POP concentrations in

feathers found in the present study, resulting in noise in the statistical analyses and few significant correlations.

As discussed above the contamination on feathers showed a trend to decrease from the incubation- to the chick rearing period. Similar to the lack of correlation between feather and inner organ concentrations, this deviating seasonality response may be seen in relation to the seabirds' exposure to seawater. In the chick rearing period the parent birds have to increase their activity in order to provide food to the chicks. This means an even more frequently exposure to seawater, and thereby lower contamination on feathers collected in the chick rearing period. Generally the measured contaminant levels in kittiwake feathers were very low, which itself could bias the results. "Noise" would constitute a larger proportion of the variation, which could make trends more difficult to track.

From this study's result and the results presented by Jaspers et al. (2007b) it may be argued that POPs detected on seabird feathers may be more related to the blood profile from the moment when the blood vessels atrophy, than is seen for terrestrial birds due to seawater exposure. Since this time both diet and environment may have changed (Jaspers et al., 2007b, Van den Steen et al., 2007). The lack of correlation between feather and liver concentrations may be a result of this. The liver is a highly metabolically active organ with rather high turnover of POPs, and assumed to represent recent contaminant exposure (Jaspers et al., 2006). There is a possibility that the POP concentrations would correlate better with e.g. muscle tissue, which is less metabolically active than the liver (Jaspers et al., 2006). This study indicate less applicability for feathers in monitoring POPs for seabirds, than is earlier found for terrestrial birds. Further analytical analysis is needed, to investigate e.g. correlation between feather concentration and tissues other than liver and preen gland.

## 4.4 Concluding remarks

The main aim for this study was to increase the knowledge on seabirds' excretory pathways for POPs, other than liver and eggs. This was done in order to explain the stable contaminant levels found in adult seabirds, which is in contrast to the age-accumulation observed in mammals. The preen gland was hypothesised to play an important role in reducing seabirds' body burden of contaminants, by excreting POPs onto the feathers. The second aim of the

study was to investigate if feathers could be used as a non-destructive method for monitoring POPs in seabirds.

From the present study a further step is taken in explaining alternative excretory ways for POPs in seabirds. Detectable amounts of contaminants in the kittiwakes' feathers, rather similar POP profile in feathers and preen gland, and a considerable high concentration of POPs in preen gland tissues, can be seen as indications that the preen gland may function as an organ for excretion of POPs in kittiwakes. However, this study is not enough to explain the stable contaminate levels found in seabirds compared to mammals. This study provide no exact estimation for the amount of POPs that can be excreted by the preen gland. Further studies should focus on species specific preen oil production, in order to calculate the exact excretion of POPs through the preen gland. By a species specific estimation of the yearly amount POPs excreted through the preen gland, one would further increase the knowledge of this organs importance for the minor accumulation by age, found in several seabirds. Also by studying age-accumulation of POPs in birds without preen gland, e.g. emus, parrots and pigeons one would likely increase the knowledge of this gland's importance in POP excretion.

In several studies feathers have shown to be a promising tool for non destructive monitoring of POPs in birds. This study supports the applicability in relation to detectable amounts of POPs on the feathers. However, the present study also indicates that feathers for monitoring POPs may be less applicable for seabirds than strictly terrestrial birds. In order to investigate this, POPs should be measured in birds with different body conditions, to support or reject the trend for lower feather concentrations in energy demanding periods. Also effort should be done to search for individual differences in feathers from bird coming directly from the sea, compared with those parents staying at the nest for a long time. Consistency in which feathers are sampled would improve the comparisons between studies. Feathers are a common feature to all birds, and advantages will arise in POP monitoring if feathers are found to be a satisfying monitoring tool. In contrast to other non-destructive sampling procedures (e.g. blood samples), no special skills are required to collect feathers. In contrast to eggs, feathers can be sampled independent of season, age and sex, but most important, no birds need to be sacrificed. This is important, both for conservation of endangered species and to increase sample size in order to improve the statistical analyses.

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# 6 Appendices

## Appendix I

IUPAC abbreviation and names of the POPs analysed.

| Abbreviation | IUPAC-name |
|---|---|
| α-HCH | α-1,2,3,4,5,6-Hexachlorocyclohexane |
| β-HCH | β-1,2,3,4,5,6-Hexachlorocyclohexane |
| γ-HCH | γ-1,2,3,4,5,6-Hexachlorocyclohexane |
| HCB | Hexachlorobenzene |
| Heptachlor | 1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-Tetrahydro-4,7-Methano-1*H*-indene |
| Heptachlor epoxide | 2,3-epoxy-1,4,5,6,7,8,8,-heptachloro-2,3,3a,4,7,7a-hexahydro-4,7- Endomethanoindane |
| *oxy*-chlordane | 1-exo-2-endo-4,5,6,7,8,8a-Octochloro-2,3-exo-epoxy-2,3,3a,4,7,7a-Hexahydro-4,7-Methanoindane |
| *cis*-chlordane | cis-1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-Tetrahydro-4,7-Methanoindane |
| *trans*-chlordane | trans-1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-Tetrahydro-4,7-Methanoindane |
| *trans*-nonachlor | trans-1,2,3,4,5,6,7,8,8-Nonachloro-3a,4,7,7a-Tetrahydro-4,7-Methanoindane |
| *cis*-nonachlor | cis-1,2,3,4,5,6,7,8,8-Nonachloro-3a,4,7,7a-Tetrahydro-4,7-Methanoindane |
| *p,p'*-DDE | p,p'-Dichlorodiphenyltrichloroethane |
| Mirex | 1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-Metheno-2H-Cyclobuta[cd]pentalene |
| PCB-99 | 2,2',4,4',5-Pentachlorobiphenyl |
| PCB-101 | 2,2',4,5,5'-Pentachlorobiphenyl |
| PCB-105 | 2,3,3',4,4'-Pentachlorobiphenyl |
| PCB-118 | 2,3',4,4',5-Pentachlorobiphenyl |
| PCB-123 | 2',3,4,4',5-Pentachlorobiphenyl |
| PCB-128 | 2,2'3,3'4,4'-Hexachlorobiphenyl |
| PCB-138 | 2,2',3'4,4',5'-Hexachlorobiphenyl |
| PCB-141 | 2,2',3,4,5,5'-Hexachlorobiphenyl |
| PCB-149 | 2,2'3,4',5',6-Hexachlorobiphenyl |
| PCB-153 | 2,2',4,4',5,5'-Hexachlorobiphenyl |
| PCB-156 | 2,3,3',4,4',5-Hexachlorobiphenyl |
| PCB-157 | 2,3,3',4,4',5'-Hexachlorobiphenyl |
| PCB-167 | 2,3',4,4',5,5'-Hexachlorobiphenyl |
| PCB-170 | 2,2',3,3',4,4',5-Heptachlorobiphenyl |
| PCB-180 | 2,2',3,4,4',5,5'-Heptachlorobiphenyl |
| PCB-183 | 2,2',3,4,4',5',6-Heptachlorobiphenyl |
| PCB-187 | 2,2',3,4',5,5',6-Heptachlorobiphenyl |
| PCB-189 | 2,3,3',4,4',5,5'-Heptachlorobiphenyl |
| PCB-194 | 2,2',3,3',4,4',5,5'-Octachlorobiphenyl |

## Appendix II

Abbreviation and names of the lipid classes analysed.

| Abbreviation | Name |
|---|---|
| CE | Cholesteryl ester |
| WE | Wax ester |
| TAG | Triacylglycerol |
| FAOH | Fatty alcohol |
| C | Cholesterol |
| DAG | Diacylglycerol |
| FFA | Free fatty acid |
| MAG | Monoacylglycerol |
| GAL | Galactocerebroside |
| CL | Cardiolipin |
| PG | Phosphatidylglycerol |
| PE | Phosphatidylethanolamine |
| PI | Phosphatidylinositol |
| PA | Phosphatidic acid |
| PS | Phosphatidylserine |
| PC | Phosphatidylcholines |
| SPM | Sphingomyelin |
| LPC | Lysophosphatidylcholine |

## Appendix III

Concentrations of POPs (ng/g wet wt) and lipid content (%) in kittiwake feather, liver and preen gland. Sample size is indicated (n). Mean ± SD, median and range are reported when the given POP were detected in ≥60% of the samples. Compounds reported in ≤60% of the samples are reported not detected (nd).

| | Feathers (ng/g wet wt) | | | | Liver (ng/g wet wt) | | | | Preen gland (ng/g wet wt) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | nd | nd | nd | nd | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **α-HCH** | 2/24 | nd | nd | nd | 3/24 | nd | nd | nd | 11/24 | nd | nd | nd |
| **β-HCH** | 19/24 | 1.15 ± 0.60 | 0.93 | 0.54-2.83 | 19/24 | 2.45 ± 1.42 | 2.25 | 0.45-5.62 | 23/24 | 30.17 ± 15.38 | 28.74 | 6.07-60.54 |
| **γ-HCH** | 13/24 | 0.11 ± 0.04 | 0.10 | 0.04-0.17 | 0/24 | nd | nd | nd | 8/24 | nd | nd | nd |
| **∑HCH** | 19/24 | 1.20 ± 0.64 | 1.00 | 0.54-2.93 | 19/24 | 2.46 ± 1.43 | 2.25 | 0.45-5.67 | 24/24 | 29.21 ± 16.16 | 28.23 | 0.89-60.92 |
| **HCB** | 24/24 | 0.97 ± 0.95 | 0.73 | 0.29-4.83 | 24/24 | 17.02 ± 5.67 | 17.73 | 7.09-26.58 | 24/24 | 57.02 ± 16.76 | 55.04 | 35.65-116.75 |
| **Heptachlor** | 3/24 | nd | nd | nd | 24/24 | 8.06 ± 3.86 | 7.34 | 2.33-17.45 | 24/24 | 7.42 ± 3.87 | 6.33 | 2.98-20.24 |
| ***trans*-chlordane** | 13/24 | 0.05 ± 0.06 | 0.03 | 0.01-0.23 | 0/24 | nd | nd | nd | 0/24 | nd | nd | nd |
| ***cis*-chlordane** | 10/24 | nd | nd | nd | 19/24 | 0,06 ± 0.05 | 0.05 | 0.01-0.20 | 22/24 | 0.54 ± 0.24 | 0.46 | 0.20-1.06 |
| **oxy-chlordane** | 24/24 | 3.31 ± 2.25 | 2.30 | 1.12-8.42 | 24/24 | 24.58 ± 16.14 | 23.10 | 4.69-58.66 | 24/24 | 158.25 ± 67.43 | 140.65 | 43.65-303.55 |
| ***trans*-nonachlor** | 24/24 | 0.39 ± 0.41 | 0.26 | 0.07-2.01 | 24/24 | 0.83 ± 0.69 | 0.67 | 0.15-3.42 | 24/24 | 6.27 ± 4.45 | 5.23 | 1.66-19.30 |
| ***cis*-nonachlor** | 24/24 | 0.34 ± 0.27 | 0.27 | 0.08-1.35 | 24/24 | 0.95 ± 0.52 | 0.78 | 0.28-1.80 | 24/24 | 6.64 ± 3.96 | 6.08 | 2.87-19.54 |
| **∑chlordanes** | 24/24 | 4.09 ± 2.76 | 2.74 | 1.49-10.71 | 24/24 | 26.41 ± 16.76 | 25.53 | 5.77-61.74 | 24/24 | 171.66 ± 69.06 | 154.78 | 56.74-319.60 |
| ***p,p*-DDE** | 24/24 | 11.03 ± 12.86 | 6.61 | 0.88-62.43 | 24/24 | 50.69 ± 45.69 | 34.03 | 6.32-185.31 | 24/24 | 273.18 ± 200.31 | 228.85 | 48.33-746.05 |
| **Mirex** | 2/24 | nd | nd | nd | 24/24 | 13.90 ± 10.00 | 13.84 | 0.97-32.51 | 24/24 | 32.38 ± 12.05 | 31.75 | 8.55-57.59 |

## Appendix III continued

| | | Feathers (ng/g wet wt) | | | | Liver (ng/g wet wt) | | | | Preen gland (ng/g wet wt) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | nd | nd | nd | nd | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **PCB 99** | 23/24 | 4.19 ± 2.59 | 3.07 | 1.59-10.64 | 23/24 | 27.03 ± 15.36 | 29.96 | 4.54-54.93 | 24/24 | 124.40 ± 554.83 | 118.20 | 45.40-251.95 |
| **PCB 101** | 5/24 | nd | nd | nd | 8/24 | nd | nd | nd | 0/24 | nd | nd | nd |
| **PCB 105** | 23/24 | 1.92 ± 1.27 | 1.35 | 0.69-4.81 | 24/24 | 11.04 ± 6.89 | 11.08 | 2.03-27.88 | 24/24 | 50.65 ± 21.12 | 51.26 | 19.51-105.46 |
| **PCB 118** | 24/24 | 5.75 ± 3.97 | 4.59 | 1.36-16.54 | 24/24 | 39.71 ± 24.88 | 38.21 | 7.53-91.62 | 24/24 | 179.47 ± 70.78 | 162.53 | 75.50-334.02 |
| **PCB123** | 1/24 | nd | nd | nd | 12/24 | 4.47 ± 3.65 | 3.54 | 0.76-10.43 | 4/24 | nd | nd | nd |
| **PCB 128** | 23/24 | 0.96 ± 0.72 | 0.77 | 0.18-3.01 | 23/24 | 6.75 ± 4.33 | 6.09 | 1.08-17.27 | 24/24 | 28.15 ± 14.04 | 27.01 | 12.17-75.03 |
| **PCB 138** | 23/24 | 14.03 ± 8.26 | 11.07 | 4.76-34.78 | 24/24 | 107.16 ± 65.86 | 109.83 | 16.84-217.33 | 24/24 | 426.81 ± 196.16 | 399.37 | 160.21-1029.20 |
| **PCB 141** | 7/24 | nd | nd | nd | 19/24 | 0.32 ± 1.19 | 0.05 | 0.01-5.23 | 19/24 | 0.26 ± 0.12 | 0.24 | 0.05-0.49 |
| **PCB 149** | 0/24 | nd | nd | nd | 24/24 | 6.65 ± 4.15 | 7.16 | 1.19-14.04 | 24/24 | 26.47 ± 9.81 | 25.82 | 10.53-47.09 |
| **PCB 153** | 23/24 | 20.91 ± 11.85 | 17.73 | 6.96-50.89 | 24/24 | 188.59 ± 134.13 | 176.06 | 27.51-472.70 | 24/24 | 728.76 ± 427.89 | 653.21 | 249.31-2285.49 |
| **PCB 156** | 24/24 | 1.15 ± 1.86 | 0.68 | 0.29-9.67 | 24/24 | 7.37 ± 4.98 | 7.21 | 1.09-15.60 | 24/24 | 27.58 ± 14.69 | 25.69 | 8.72-72.47 |
| **PCB 157** | 24/24 | 0.21 ± 0.11 | 0.19 | 0.07-0.46 | 22/24 | 1.54 ± 1.01 | 1.63 | 0.25-3.57 | 24/24 | 9.99 ± 19.02 | 5.33 | 2.01-97.76 |
| **PCB 167** | 24/24 | 0.56 ± 0.32 | 0.49 | 0.08-1.33 | 24/24 | 5.93 ± 4.10 | 5.09 | 0.83-14.57 | 24/24 | 24.44 ± 19.06 | 18.56 | 7.91-82.17 |
| **PCB 170** | 24/24 | 2.16 ± 1.18 | 1.97 | 0.23-4.94 | 23/24 | 30.34 ± 23.04 | 25.21 | 3.64-78.84 | 24/24 | 93.77 ± 66.27 | 75.54 | 31.75-343.34 |
| **PCB 180** | 24/24 | 6.55 ± 3.29 | 5.76 | 1.06-14.56 | 24/24 | 99.32 ± 75.02 | 80.78 | 11.59-239.16 | 22/24 | 293.97 ± 185.52 | 239.58 | 103.73-912.26 |
| **PCB 183** | 24/24 | 1.47 ± 0.78 | 1.15 | 0.49-3.46 | 24/24 | 16.90 ± 12.13 | 15.05 | 2.15-39.70 | 24/24 | 51.68 ± 29.35 | 43.28 | 18.71-147.57 |
| **PCB187** | 24/24 | 2.70 ± 1.58 | 2.26 | 0.76-6.83 | 24/24 | 28.75 ± 19.03 | 27.73 | 4.30-62.68 | 24/24 | 93.76 ± 50.02 | 81.65 | 43.47-268.88 |
| **PCB189** | 24/24 | 0.13 ± 0.31 | 0.06 | 0.03-1.58 | 24/24 | 1.22 ± 1.03 | 0.90 | 0.13-3.70 | 24/24 | 3.30 ± 3.32 | 2.29 | 0.94-17.58 |
| **PCB 194** | 23/24 | 0.51 ± 0.26 | 0.41 | 0.23-1.17 | 24/24 | 12.68 ± 11.26 | 8.97 | 1.03-43.64 | 24/24 | 28.46 ± 26.81 | 20.39 | 9.35-139.66 |
| **∑PCBs** | 24/24 | 62.09 ± 34.87 | 53.78 | 22.03-146.69 | 24/24 | 590.99 ± 401.66 | 557.40 | 86.85-1285.06 | 24/24 | 2168.35 ± 1149.77 | 1923.13 | 805.63-6083.33 |
| **∑POPs** | 24/24 | 79.32 ± 47.86 | 69.40 | 28.94-225.06 | 24/24 | 709.02 ± 458.33 | 667.96 | 116.63-1488.99 | 24/24 | 2739.22 ± 1260.28 | 2383.50 | 1070.19-6624.96 |

## Appendix IV

Concentrations of POPs (ng/g lipid wt) and lipid content (%) in kittiwake liver and preen gland. Sample size is indicated (n). Mean ± SD, median and range are reported when the given POP were detected in ≥60% of the samples. Compounds reported in ≤60% of the samples are reported as not detected (nd).

| | | Liver (ng/g lipid wt) | | | Preen gland (ng/g lipid wt) | | | |
|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **a-HCH** | 3/24 | nd | nd | nd | 11/24 | nd | nd | nd |
| **b-HCH** | 19/24 | 44.65 ± 20.65 | 43.60 | 11.49-91.5 | 23/24 | 98.65 ± 57.77 | 103.48 | 15.50-205.66 |
| **g-HCH** | 0/24 | nd | nd | nd | 8/24 | nd | nd | nd |
| **∑HCH** | 19/24 | 44.76 ± 20.80 | 43.60 | 11.49-91.88 | 24/24 | 95.42 ± 59.81 | 89.51 | 2.11-206.94 |
| **HCB** | 24/24 | 309.12 ± 100.32 | 313.97 | 134.71-500.00 | 0/24 | nd | nd | nd |
| **Heptachlor** | 24/24 | 153.40 ± 83.71 | 141.84 | 33.96-346.73 | 24/24 | 22.75 ± 10.65 | 21.33 | 5.67-48.05 |
| ***trans*-chlordane** | 0/24 | nd | nd | nd | 0/24 | nd | nd | nd |
| ***cis*-chlordane** | 15/24 | 3.15 ± 7.08 | 1.08 | 0.62-28.52 | 22/24 | 1.61 ± 0.70 | 58.20 | 0.51-3.03 |
| **oxy-chlordane** | 24/24 | 445.34 ± 270.82 | 352.09 | 89.11-951.18 | 24/24 | 513.69 ± 279.16 | 428.41 | 111.58-1071.63 |
| ***trans*-nonachlor** | 24/24 | 13.41 ± 9.12 | 11.42 | 3.25-42.10 | 23/24 | 19.30 ± 12.50 | 16.00 | 6.73-51.05 |
| ***cis*-nonachlor** | 24/24 | 16.62 ± 7.95 | 15.65 | 5.59-30.21 | 23/24 | 21.18 ± 11.05 | 19.21 | 8.79-50.62 |
| **∑chlordanes** | 24/24 | 477.22 ± 278.68 | 401.40 | 112.98-1001.14 | 24/24 | 553.96 ± 285.77 | 467.67 | 144.91-1092.60 |
| ***p,p*-DDE** | 24/24 | 795.01 ± 645.00 | 630.07 | 46.88-2479.50 | 24/24 | 806.60 ± 550.95 | 620.41 | 186.67-2095.81 |
| **Mirex** | 24/24 | 246.51 ± 158.67 | 245.53 | 24.98-490.36 | 24/24 | 102.66 ± 46.46 | 101.14 | 22.76-206.13 |

## Appendix IV continued

| | | Liver (ng/g lipid wt) | | | | Preen gland (ng/g lipid wt) | | |
|---|---|---|---|---|---|---|---|---|
| ***Analyte*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Median*** | ***Range*** |
| **Lipid content (%)** | 24/24 | 5.78 ± 1.42 | 5.56 | 3.90-9.32 | 24/24 | 33.83 ± 9.25 | 32.77 | 24.60-68.78 |
| **PCB 99** | 23/24 | 450.19 ± 277.85 | 329.92 | 40.13-887.85 | 24/24 | 403.29 ± 241.60 | 383.05 | 115.96-861.58 |
| **PCB 101** | 8/24 | nd | nd | nd | 0/24 | nd | nd | nd |
| **PCB 105** | 24/24 | 186.32 ± 132.57 | 178.03 | 13.44-606.83 | 24/24 | 160.63 ± 77.05 | 144.13 | 49.84-302.21 |
| **PCB 118** | 24/24 | 658.89 ± 425.77 | 573.09 | 53.69-1485.61 | 24/24 | 572.76 ± 279.41 | 556.04 | 188.92-1134.60 |
| **PCB123** | 12/24 | 81.21 ± 71.62 | 57.60 | 17.44-241.28 | 4/24 | nd | nd | nd |
| **PCB 128** | 23/24 | 110.75 ± 71.42 | 98.78 | 8.11-231.67 | 24/24 | 89.69 ± 53.03 | 78.60 | 31.08-256.57 |
| **PCB 138** | 24/24 | 1802.51 ± 1194.84 | 1498.54 | 143.03-3874.03 | 24/24 | 1371.54 ± 748.63 | 1315.18 | 409.20-3519.55 |
| **PCB 141** | 18/24 | 7.11 ± 26.62 | 0.79 | 0.25-113.75 | 19/24 | 0.77 ± 0.37 | 0.80 | 0.20-1.35 |
| **PCB 149** | 24/24 | 111.20 ± 74.25 | 92.02 | 9.54-227.58 | 24/24 | 84.81 ± 39.90 | 81.05 | 26.88-161.02 |
| **PCB 153** | 24/24 | 3190.52 ± 2458.40 | 2422.30 | 237.16-9037.82 | 24/24 | 2357.32 ± 1582.80 | 2071.08 | 636.78-7815.65 |
| **PCB 156** | 24/24 | 124.33 ± 90.45 | 101.27 | 9.59-291.69 | 24/24 | 88.94 ± 54.04 | 83.48 | 21.81-247.83 |
| **PCB 157** | 22/24 | 26.91 ± 20.12 | 21.49 | 2.14-68.24 | 24/24 | 29.28 ± 47.46 | 17.97 | 5.14-244.63 |
| **PCB 167** | 24/24 | 100.20 ± 75.70 | 78.20 | 7.39-278.60 | 24/24 | 78.34 ± 63.51 | 60.35 | 20.19-280.99 |
| **PCB 170** | 23/24 | 506.39 ± 417.30 | 351.28 | 32.50-1507.43 | 24/24 | 301.08 ± 235.28 | 248.27 | 81.09-1174.10 |
| **PCB 180** | 24/24 | 1679.97 ± 1347.66 | 1379.38 | 107.79-4572.56 | 22/24 | 941.59 ± 671.00 | 752.81 | 264.95-3119.63 |
| **PCB 183** | 24/24 | 285.07 ± 217.02 | 242.10 | 19.19-689.40 | 24/24 | 165.15 ± 106.05 | 138.65 | 47.78-504.64 |
| **PCB187** | 24/24 | 487.24 ± 351.13 | 476.10 | 35.39-1198.35 | 24/24 | 298.50 ± 182.77 | 264.72 | 111.04-919.48 |
| **PCB189** | 24/24 | 20.54 ± 17.63 | 15.58 | 1.13-70.76 | 24/24 | 10.70 ± 11.61 | 7.91 | 2.41-60.11 |
| **PCB 194** | 24/24 | 213.57 ± 198.42 | 167.87 | 12.37-834.35 | 24/24 | 92.19 ± 94.48 | 63.28 | 23.88-477.58 |
| **∑PCBs** | 24/24 | 9959.79 ± 7259.70 | 7781.16 | 732.59-24569.59 | 24/24 | 6970.53 ± 4272.88 | 6302.77 | 2057.98-20802.98 |
| **∑POPs** | 24/24 | 11976.50 ± 8141.90 | 9896.42 | 1777.20-26781.91 | 24/24 | 8730.87 ± 4734.24 | 8218.22 | 2733.39-22655.10 |

## Appendix V

The sample size (n), mean ± SD and the range for the contribution of lipid classes (%) to the total lipid content in Kittiwake liver and preen gland. Not detected is reported as nd.

| | Liver | | | Preen gland | | |
|---|---|---|---|---|---|---|
| ***Lipid class*** | ***n*** | ***Mean ± SD*** | ***Range*** | ***n*** | ***Mean ± SD*** | ***Range*** |
| Cholesteryl ester | 0/12 | nd | nd | 0/11 | nd | nd |
| Wax ester | 12/12 | 14.9 ± 6.3 | 6.5-24.0 | 11/11 | 70.1 ± 15.3 | 46.8-88.2 |
| Triacylglycerol | 12/12 | 26.7 ± 12.1 | 15.2-54.5 | 11/11 | 27.5 ± 15.8 | 9.0-51.5 |
| Fatty alcohol | 0/12 | nd | nd | 0/11 | nd | nd |
| Cholesterol | 12/12 | 9.9 ± 2.1 | 6.2-12.3 | 0/11 | nd | nd |
| Diacylglycerol | 10/12 | 1.3 ± 0.5 | 0.7-2.0 | 0/11 | nd | nd |
| Free fatty acid | 0/12 | nd | nd | 0/11 | nd | nd |
| Monoacylglycerol | 0/12 | nd | nd | 0/11 | nd | nd |
| Galactocerebroside | 5/12 | 1.7 ± 0.2 | 1.4-2.0 | 0/11 | nd | nd |
| Cardiolipin | 0/12 | nd | nd | 0/11 | nd | nd |
| Phosphatidylglycerol | 12/12 | 0.9 ± 0.4 | 0.4-1.5 | 0/11 | nd | nd |
| Phosphatidylethanolamine | 12/12 | 7.6 ± 3.9 | 1.7-14.2 | 0/11 | nd | nd |
| Phosphatidylinositol | 12/12 | 3.9 ± 1.9 | 1.4-7.4 | 0/11 | nd | nd |
| Phosphatidic acid | 0/12 | nd | nd | 0/11 | nd | nd |
| Phosphatidylserine | 12/12 | 3.9 ± 1.8 | 1.4-6.9 | 0/11 | nd | nd |
| Phosphatidylcholines | 12/12 | 26.6 ± 8.9 | 11.3-37.2 | 11/11 | 2.3 ± 0.7 | 1.6-3.6 |
| Sphingomyelin | 12/12 | 2.9 ± 0.9 | 1.4-4.2 | 0/11 | nd | nd |
| Lysophosphatidylcholine | 12/12 | 0.9 ± 0.7 | 0.2-2.3 | 0/11 | nd | nd |