APPENDIX 1

Levels and effects of persistent organic pollutants (POPs) in seabirds

Retinoids and α-tocopherol – potential biomarkers of POPs in birds?

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CONTENTS

List of papers

1. **Murvoll, K.M.**, Jenssen, B.M. and Skaare, J.U. 2005. Effects of pentabrominated diphenyl ether (PBDE-99) on vitamin status in domestic duck (*Anas platyrhynchos*) hatchlings. *J Toxicol Environ Health A* 68: 515-533.

2. **Murvoll, K.M.**, Skaare, J.U., Anderssen, E. and Jenssen, B.M. 2006. Exposure and effects of persistent organic pollutants in European shag (*Phalacrocorax aristotelis*) hatchlings from the coast of Norway. *Environ Toxicol Chem* (Volume 25). In press.

3. **Murvoll, K.M.**, Skaare, J.U., Moe, B., Anderssen, E. and Jenssen, B.M. (Accepted). Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North-Atlantic kittiwakes (*Rissa tridactyla)*. *Environ Toxicol Chem.*

4. **Murvoll, K.M.**, Skaare, J.U., Jensen, H. and Jenssen, B.M. (Submitted). Associations between persistent organic pollutants and vitamin status in Brünnich's guillemot and common eider hatchlings.

Summary

In the present thesis, levels of polychlorinated biphenyls (PCBs), some chosen organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) were analyzed by gas chromatography in the yolk sac of newly hatched chicks of European shag (*Phalacrocorax aristotelis*), kittiwake (*Rissa tridactyla*), Brünnich's guillemot (*Uria lomvia*) and common eider (*Somateria mollissima*) from the Norwegian coast and Svalbard. Levels of vitamin A (retinol), retinyl palmitate and vitamin E (α-tocopherol) were measured in plasma and liver of the hatchlings using high-performance liquid chromatography (HPLC). Using statistics, possible significant relationships between levels of the persistent organic pollutants (POPs) and vitamin levels were examined. Hence, the study aimed to elucidate retinoids and tocopherol as potential biomarkers of POP exposure. An exposure study on domestic duck (*Anas platyrhynchos*) eggs was also conducted to assess the effects of 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99) on vitamin levels under controlled laboratory conditions.

There were significant differences in POP levels between the bird species included in the present study. In general, kittiwake hatchlings had higher levels of POPs than the other species, followed by shag, Brünnich's guillemot and common eider hatchlings. Levels of organochlorine compounds in the hatchlings seemed to be higher than reported in sea bird eggs from the Canadian Arctic but lower than reported in eggs of other seabirds from the Netherlands, the Baltic, the Great Lakes and Japan. In contrast to this, the levels of PBDEs and HBCD seemed to be high in some of the species (kittiwakes, shags) relative to a European scale.

Negative relationships were revealed between POPs and morphology in Brünnich's guillemot hatchlings, indicating that this species may be more responsive with respect to effects of POPs on morphological variables than the other species included in the present study. The importance of considering possible confounding impacts of lipid content when studying effects of POPs on morphological variables was emphasized in shag hatchlings.

The study revealed negative correlations between POPs and liver tocopherol levels in domestic duck and shag hatchlings. In Brünnich's guillemot hatchlings, liver tocopherol levels also were negatively associated with POPs, but the relationships were less strong when

the effect of body mass on tocopherol levels was accounted for. In kittiwake and common eider hatchlings, however, there seemed to be a positive influence by POPs on tocopherol levels. Thus, the results should encourage further research on the effects of POPs on tocopherol levels (including oxidized forms of the vitamin).

In shag hatchlings, negative relationships between POPs and plasma retinol levels were observed, in line with several previous studies on birds. Since retinol was not influenced in any other species included in the study, tocopherol levels might be more responsive than retinol levels to POP exposure. Additional studies should, however, be conducted before certain conclusions are drawn.

Concerning the work needed for further development of vitamins as biomarkers of POP, effort should be done to characterize confounding factors, such as diet and condition of the avian mothers. Although there was no obvious link between the observed responses of vitamins to POP exposure and effects at higher biological levels (i.e. reproduction disturbances, population decline), the relevance of vitamins as potential biomarkers of POP exposure should not be repelled.

1. INTRODUCTION

At the beginning of World War II, several pesticides and other chemical agents were under experimental investigation (Ecobichon 2001). The continuing demand for many new materials in modern civilization and the concomitant development of the chemical industry resulted in production of man-made chemicals in large numbers and quantities, greatly contributing to our convenient and pleasant lifestyle (Tanabe et al 1994). However, many persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), also had undesirable outcomes. The chemicals were found to bioaccumulate in the food-chain (Reijnders 1980, Tanabe et al 1983) because of their resistance to biodegradation (recalcitrance) and high lipophilicity (Niimi 1987, Mackay et al 1992a; 1992b, Augistijn-Beckers et al 1994). In addition, negative effects on wildlife were revealed (Ratcliffe 1967, Delong et al 1973, Helander et al 1982, Bignert et al 1995). The reports on widespread environmental contamination of organochlorines (OCs) and the documentation on their toxic effects lead to restrictions on production and use of the compounds in industrialized countries (Peterle 1991a, AMAP 1998). In Norway, restrictions on use of PCBs and OCPs were given in the early 1970s (Ingebrigtsen et al 1984). Today there seems to be a decreasing temporal trend of PCBs and OCPs in biota (Bignert et al 1998, Braune et al 2001), but the compounds still exert a potential risk to human and wildlife health because of leakage from sediments and products (Tanabe 1988, AMAP 1998). Recently, other POPs such as brominated flame retardants (BFRs) have received much attention due to a reported temporal increase in human milk and Arctic biota (Meironyté et al 1999, Ikonomou et al 2002). Because of high production volumes of BFRs and structural resemblance of some of these compounds to well-known environmental contaminants, such as PCBs, concern has arisen for human and wildlife health (Darnerud 2003). Hence, due to continued discharges and leakage of POPs, monitoring of the environment with regard to these pollutants and their potential risk to wildlife is of great importance.

1.1 Organochlorines (OCs)

OCs are organic chemicals with one or more hydrogen atoms on the carbon skeleton substituted by chlorine atoms (Walker et al 2001). Although they have diverse chemical structures, the common characteristics for most of them are low water solubilities, high lipophilicity and resistance to biodegradation (Niimi 1987, Mackay et al 1992a; 1992b, Augistijn-Beckers et al 1994). These combined characteristics lead to bioaccumulation in fatty tissues of organisms (Reijnders 1980, Tanabe et al 1983, Barrett et al 1996, Champoux et al 2002). OCs are grouped in three major classes; industrial chemicals (e.g. PCBs), pesticides (e.g. dichlorodiphenyltrichloroethane; DDT) and by-products of combustion and industrial processes (e.g. polychlorinated dibenzo-*p*-dioxins; PCDDs). The present thesis deals with PCBs and some chosen pesticides (in addition to BFRs, see 1.2).

1.1.1 Polychlorinated biphenyls (PCBs)

Theoretically there are 209 PCB congeners (i.e. many variants or configurations of a common chemical structure), and approximately 120 of these are present in commercial products such as Aroclor 1254, Aroclor 1260 and Chlopen A60 (Walker et al 2001). Ballschmiter and Zell (1980) proposed a simple numbering system of the PCB congeners, giving each congener a number from one to 209. This system was later adapted by IUPAC (International Union of Pure and Applied Chemistry) (www.iupac.org).

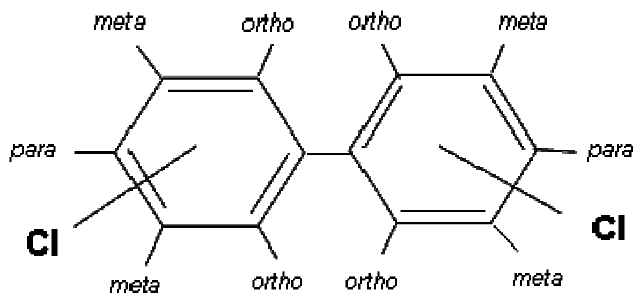


Figure 1: General molecular structure and Cl-substitution positions of PCBs.

PCBs were made commercially available in about 1930 (Peterle 1991a). PCBs were used as hydraulic fluids, coolant-insulation fluids in transformers and plasticizers in paints due to their physical properties of chemical stability and low volatility (Walker et al 2001). In 1966, the Swedish scientist Sören Jensen discovered the presence of high PCB levels in environmental samples when analysing for the insecticide DDT (Jensen 1966). The further discovery of widespread environmental contamination (Risebrough et al 1976, Reijnders 1980, Tanabe et al 1983) and the subsequently reported negative impact on wildlife by PCBs (Delong et al 1973, Helander et al 1982) lead to restrictions on the production and use of the compounds (Peterle 1991a, AMAP 1998). In Norway, restrictions on the use of PCBs were imposed in

1971, and a ban was introduced in 1980 (Ingebrigtsen et al 1984). Today PCBs are globally banned in accordance with the Stockholm Convention of 17 May 2004 (www.pops.int). Some of the documented effects of exposure to PCBs on human health and wildlife are impaired immunity (DeSwart et al 1996), neurotoxicity (Stewart et al 2003), carcinogenicity (Cajaraville et al 2003), and hormonal and reproductive effects (Helander et al 1982, van den Berg et al 1994, Bustnes et al 2001).

1.1.2 Organochlorine pesticides (OCPs)

The OCPs are a relatively large group of chemicals. The chemicals have been used for the control of agricultural pests and diseases (Walker et al 2001).

p,p'-dichlorodiphenyldichloroethylene (*p,p'*-DDE) is the most stable metabolite of DDT (Peterle 1991a). The insecticidal properties of DDT were discovered by Paul Müller of the firm Ciba-Geigy in 1939. DDT was used for vector control during the Second World War, and thereafter it was widely used for control of agricultural pests, diseases (e.g. malaria mosquitoes) and insects (Walker et al 2001). The use of DDT has been restricted in several industrial countries for decades (Goldberg 1991), but has frequently been used in pest control programs in developing countries (Forget 1991). In Norway, the use of DDT was banned in 1988 (AMAP 1998). According to the Stockholm Convention, the global production and use of DDT is now limited to controlling disease vectors such as malaria mosquitoes (www.pops.int). Thus, some new use of DDT will also in future years lead to environmental releases. The metabolites *p,p'*-DDE and *o,p'*-dichlorodiphenyldichloroethane (*o,p'*-DDD) are primarily found at higher trophic levels. DDT and its metabolites are reported to affect reproduction, especially in birds due to egg-shell thinning (Ratcliffe 1967, Longcore and Stendell 1977, King et al 2003), to impair immunity (Wong et al 1992, Misumi et al 2005) and to influence hormonal systems (WHO 1989, Mayne et al 2004).

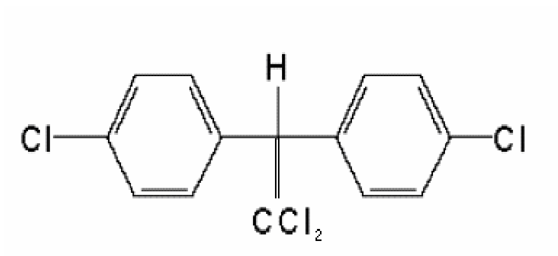


Figure 2: *p,p'*-DDE.

Hexachlorobenzene (HCB) is a by-product in the production of several chlorinated compounds. It had a limited use in the 1960s as a fungicide (AMAP 1998). The chemical bioaccumulates due to high lipophilicity and long half-life in biota (Niimi 1987, Augistijn-Beckers et al 1994). The worldwide production and use of the compound is now limited to narrowly prescribed purposes in accordance with the Stockholm Convention (www.pops.int). Several effects of exposure to HCB are reported, such as reproductive and developmental effects (Boersma et al 1986, Helberg et al 2005), interruption of the immune system (Bleavins et al 1983, Bustnes et al 2004) and tumour promotion (Stewart et al 1989, Randi et al 2003).

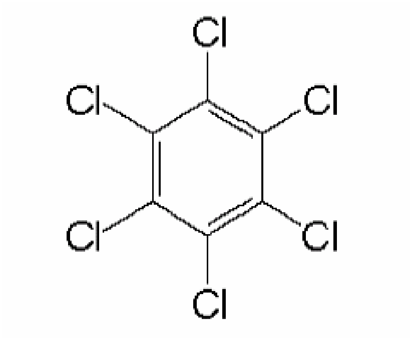


Figure 3: Hexachlorobenzene (HCB).

Oxychlordane is a toxic metabolite of the chlorinated pesticide chlordane, a mixture of at least 120 compounds. The most important components are *cis*-chlordane, *trans*-chlordane and *trans*-nonachlor (Dearth and Hites 1991). Chlordane is very persistent (Augistijn-Beckers et al 1994), and reproductive impacts (Lundholm 1988, Bustnes et al 2005), immunosuppression (Spyker-Cranmer et al 1982, Bustnes et al 2004) and cancer (WHO 1984) are among the documented toxic effects. According to the Stockholm Convention, the production and use of chlordane compounds are now limited to prescribed purposes (www.pops.int).

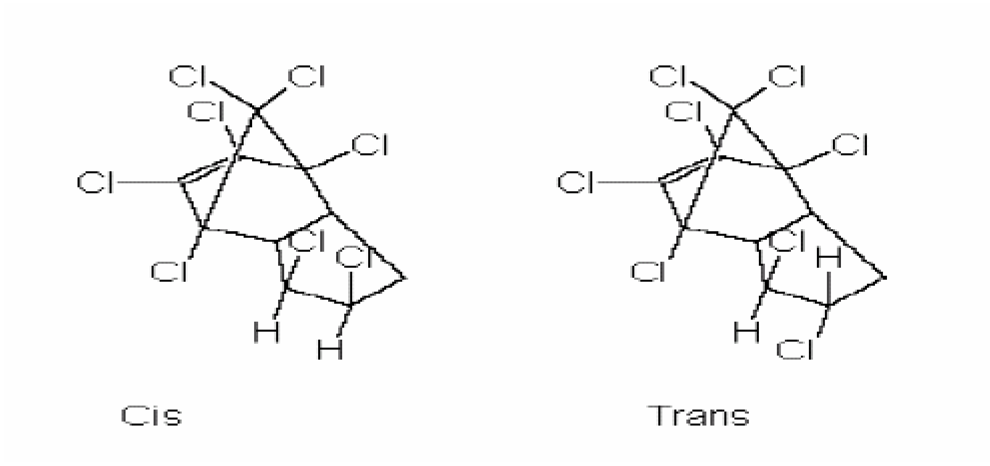


Figure 4: Two important components of chlordane, *cis*-chordane and *trans*-chlordane. Oxychlordane is a toxic metabolite of the chlordane mixture.

Mirex is an insecticide and fire retardant, used mainly in the USA and Canada. It has never been used in Norway (AMAP 1998). Mirex is extremely persistent in soils and sediment in addition to being lipophilic (Augistijn-Beckers et al 1994). Its present in the Arctic at low levels is consistent with its volatility and persistence, and today the production and use of mirex is limited to narrowly prescribed purposes in accordance to the Stockholm Convention (www.pops.int). Documented effects on vertebrates include reproductive impact (Naber and Ware 1965, Dai et al 2001) and reduced immune function (Wong et al 1992).

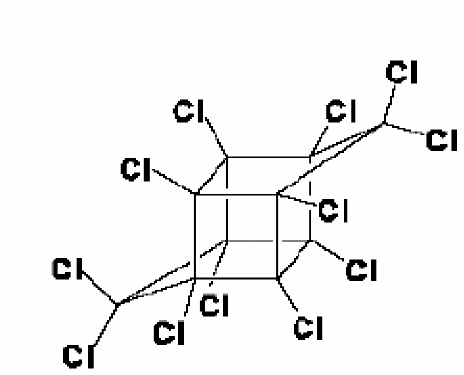


Figure 5: Mirex.

β-Hexachlorohexane (β-HCH) is an isomer of technical HCH, of which γ-HCH (lindane) is the most biologically active insecticidal isomer. β-HCH has been banned for use in the USA and most other circumpolar countries since the late 1970s (AMAP 1998). HCH is much less bioaccumulative than other OCs because of its relatively low lipophilicity and short half-life in biota (Niimi 1987). β-HCH is documented to be estrogenic (Van Velsen 1986, Steinmetz et al 1996) and to affect the immune system (Cornacoff et al 1988).

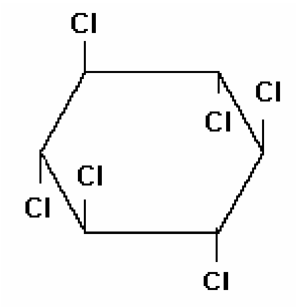


Figure 6: β-HCH.

1.2 Brominated flame retardants (BFRs)

Flame retardants are materials added or applied to a material to increase the fire resistance of that product. In order to meet fire safety regulations, flame retardants are applied to combustible materials, such as plastics, wood paper and textiles (de Wit 2002, Alaee et al 2003). Halogens are very effective in capturing free radicals that are produced during combustion processes and hence removing the capability of the flame to propagate. Due to different stability among the halogens, chlorinated and brominated compounds, and not fluorinated and iodinated compounds, are used as flame retardants. However, with higher trapping efficiency and lower decomposing temperature, brominated compounds have become more popular than their organochlorine counterparts as flame retardants (Alaee et al 2003). The BFRs are divided into three subgroups from the incorporation of brominated compounds into the polymers; monomers, reactive and additive. Brominated monomers are incorporated into polymers containing both brominated and nonbrominated monomers. Reactive flame retardants, such as tetrabromobisphenol A (TBBPA), are chemically bonded to plastics. Additive flame retardants, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), are blended with the polymers and are more likely to leach out of the products (Alaee et al 2003). Most used BFRs are polybrominated biphenyls (PBBs), TBBPA and its derivatives, PBDEs and HBCD (Darnerud 2003). The present thesis deals with PBDEs and HBCD (in addition to OCs, see 1.1).

1.2.1 Polybrominated diphenyl ethers (PBDEs)

There are theoretically 209 PBDE congeners. Diphenyl ether molecules contain ten hydrogen atoms, which can be substituted by bromine. The structure of PBDEs is corresponding to that of PCBs, and the individual PBDE congeners are numbered according to the IUPAC system, based on the position of the halogen atoms on the carbon skeleton (de Wit 2002). The commercial PBDE products predominately consist of so-called penta-, octa- and decabromodiphenyl ether products (Darnerud 2003). The Penta-BDE (Bromkal 70) consists of mainly tetra- and pentabrominated congeners (i.e. PBDE-47 and PBDE-99, respectively), whereas the Octa-BDE contains mainly hepta-, octa- and nonabrominated diphenyl ethers (e.g. PBDE-183 [hepta] and PBDE-203 [octa]). Commercially produced Deca-BDE contains mainly the decabrominated diphenyl ether PBDE-209 (Alaee et al 2003).

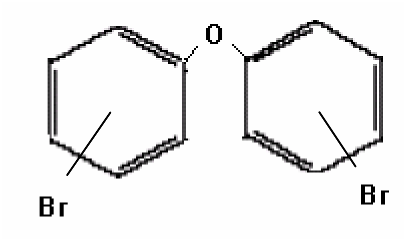


Figure 7: General molecular structure of PBDEs.

The PBDEs have been used in plastic components in electronic equipment, in textiles, in cars and in building materials (Darnerud 2003). In the environment, PBDEs were first discovered in Sweden in fish samples taken downstream from several textile industries (de Wit 2002). During recent years, the compounds have been detected in sediments, birds, seals and human blood serum (Allchin et al 1999, Sjödin et al 1999, Ikonomou et al 2002, Herzke et al 2003, Vorkamp et al 2004). Although the knowledge about the effects of PBDEs in wildlife and man is scarce (Darnerud 2003), several critical effects of the compounds have been reported in rodents, such as effects on neurobehavioral development (Eriksson et al 2001), as well as effects on thyroid hormone homeostasis (Darnerud and Sinjari 1996) and on vitamin A status (Hallgren et al 2001). The use of the commercial mixtures Penta-BDE and Octa-BDE was prohibited in all applications for the EU Market from 15 August 2004 (www.bsef-site.com/regulation), and the Penta-BDE is also voluntarily withdrawn from the Japanese market (Alaee et al 2003). In addition, California, Maine and Michigan of the USA have prohibited the use of Penta-BDE from 2006-2008 (www.bsef-site.com/regulation). However, due to the recalcitrance of the compounds, they will persist in the environment for decades. In addition, no global ban on the production and use of the compounds is yet adopted.

1.2.2 Hexabromocyclododecane (HBCD)

HBCD has been used for about 20 years in foams and expanded polystyrene (de Wit 2002), which is largely used in insulation panels and blocks for building constructions (Darnerud 2003). The few available studies indicate that HBCD has low water solubility and a high bioaccumulation potential (de Wit 2002). The compound has been detected in various environmental compartments and biota (de Wit 2002), but there is a lack of studies of high quality with regard to toxic effects (Darnerud 2003). However, behavioural effects may be a sensitive endpoint for HBCD (Eriksson et al 2002), although also other physiological effects are possible.

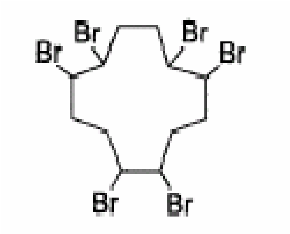


Figure 8: HBCD.

1.3 Toxic mechanisms of persistent organic pollutants (POPs)

The mechanisms of toxic action of the POPs are diverse and all of them still not fully solved (Darnerud et al 2001). However, the toxicity of PCBs and OCPs has been extensively studied (Parkinson 2001). For the PCBs, a considerable amount of evidence supports a certain mechanism of toxic action of the non-*ortho* (e.g. PCB-77, -126, -169) and mono-*ortho* (e.g. PCB-105, -118, -156, -157) congeners (Brouwer 1991). These compounds have a planar configuration and elicit toxicity through the nuclear aryl hydrocarbon receptor (AhR), of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has the highest affinity (dioxin-like toxicity) (Safe 1994). Binding to the Ah-receptor mediates the induction of cytochrome P450 (CYP) 1A enzymes in the liver, which are constituents of the most important enzyme system involved in xenobiotic biotransformation (Safe 1994). Di-*ortho* PCB congeners (e.g. PCB-128, -137, -138, -153) do not as easily induce CYP 1A enzymes as the more planar congeners, but are able to induce CYP 2B enzymes via another nuclear receptor (Boon et al 1992, Parkinson 2001). Also OCPs induce CYP 2B enzymes (Parkinson 2001). Inducers of the CYP enzymes will affect the metabolism and toxicokinetics of other contaminants and may lead to increased formation of reactive intermediates, giving increased toxicity (additive or synergistic effects), or increased detoxification (antagonistic effects). The CYP inducers will also be substrates for the enzymes and thus be metabolized to degradation products (Boon et al 1992). Hydroxy- and methylsulfonyl PCB metabolites formed by actions of detoxifying enzymes have been shown to cause a variety of toxic effects, including reduced vitamin A and thyroid hormone levels (Brouwer et al 1998). Metabolites of DDT are reported to elicit various toxic effects, such as impaired reproduction (Ratcliffe 1967, Longcore and Stendell 1977, King et al 2003), suppressed immunity (Wong et al 1992**,** Misumi et al 2005) and endocrine disruption (WHO 1989, Mayne et al 2004).

The knowledge about the toxicology of PBDEs and HBCD is scarce (Darnerud 2003). However, many of the adverse effects of these brominated compounds resemble those of PCBs, which may indicate similar toxic mechanisms. Actually, tetra-, penta- and hexabrominated diphenyl ethers have been found to induce the CYP enzymes (von Meyerinck et al 1990, Pettersson et al 2001). In cultured chick embryo livers, the most potent CYP 1A inducer of the tested congeners was PBDE-99 (Pettersson et al 2001).

1.4 Persistent organic pollutants in seabirds

Highly lipophilic and persistent organic compounds such as POPs are released into the sea, where they bioaccumulate in marine organisms (Macdonald and Bewers 1996). The POP bioaccumulation depends on the uptake and elimination ability of the organism, and the compounds' physico-chemical properties (Walker et al 2001). Although diet and trophic position is the dominant factor influencing concentrations of hydrophobic and recalcitrant compounds in seabirds (Borgå et al 2004), several other factors will also be of importance when considering accumulation of POPs, e.g. the organisms' age (Donaldson et al 1997), condition and reproductive status (Henriksen et al 1996) and migration pattern (Buckman et al 2004).

Uptake of organic compounds in seabirds takes place through the food chain (biomagnification) (Walker et al 2001). The xenobiotic metabolism activity is related to the organism's metabolic rate, which generally increases from marine invertebrates to vertebrates (Livingstone et al 1992) and further from fish (poikilotherms) to seabirds and mammals (homeotherms). However, many seabirds have shown low ability to metabolize contaminants via CYP enzymes (Walker 1992). The reason why fish-eating birds are deficient in CYP enzymes could be that there has been no requirement for sophisticated enzymic detoxication. In contrast, many herbivore terrestrial birds have evolved systems to detoxify lipophilic xenobiotics in their food, which contains a wide range of lipophilic compounds that are normal constituents of plants, but are not produced by animals (Walker 1992). Thus, when considering the problem of bioaccumulation in marine ecosystems, particular attention should be paid to fish-eating birds because they are top-predators and because they seem to have low metabolic capacity towards lipophilic contaminants. Several studies on fish-eating birds have also shown negative relationships between high levels of OC pollution and reproductive and developmental parameters, such as malformations of chicks, reduced eggshell thickness and reduced hatching and breeding success (Fox et al 1991, Bignert et al 1995, Dirksen et al 1995).

Although fish-eating birds in general show relatively low ability to metabolize contaminants, between-species differences in metabolic ability exist among seabirds (Borgå et al 2001; 2005, Fisk et al 2001a; 2001b). In addition, the classifications of birds with reference to development patterns could possibly influence metabolism of contaminants in embryos and hatchlings. The traditional classifications of development patterns of birds recognize several categories arranged along a gradient (altricial-precocial) according to a combination of morphological, physiological and behavioural characteristics of the neonates (Starck and Rickleffs 1998). The precocial extreme of the spectrum includes species whose young are totally independent of their parents, and in some species the chicks can fly from the first day of postnatal life. Semi-precocial species have chicks with relatively less developed locomotor activity, stronger nest attendance, and complete dependence of the parents for food. Species whose chicks remain in nest for much or all of their development are altricial. Fully altricial hatchlings hatch with closed eyes en exhibit little motor activity other than begging and no visible feathers (Starck and Rickleffs 1998). Precocial hatchlings are covered with down and are able to respond effectively to heat and cold in contrast to altricial hatchlings, which have little ability to regulate body temperature at ambient temperatures below 35ºC or above 40ºC (Dawson and Whittow 2000). Hence, when studying levels and effects of POPs in embryos or hatchlings of seabirds, it could be of relevance to focus at different species according to the altricial-precocial spectrum. Their different metabolic ability could influence the levels and pattern of, and responses to, the compounds.

Furthermore, when studying levels and effects of POPs it could be of interest to include populations of the same species from different geographical locations. Populations of seabirds situated close to sources of production and/or widespread use of POPs are expected to show higher contaminant levels than remote populations far from the sources. Thus, when studying different populations of birds, this could give opportunities of providing information on spatial trends of POPs and on possible differences within the same species in responses to POP exposure.

1.5 Environmental monitoring and biomarkers

Traditionally, monitoring of environmental pollution has focused to a large extent on chemical measurements of well-known contaminants in sediments, water or living organisms. However, chemical data alone is insufficient to reliably assess the potential biological responses of the complex mixture of contaminants in the natural environment (Peakall and McBee 2001). There are cases where chemicals may interact in a way that results in an increase (synergism) or decrease (antagonism) in their overall effect compared with the sum of the effects of the individual components (Peterle 1991b). Unknown substances could also lead to a discrepancy between the actual and predicted risk of the contaminants. In addition, a great variety of organisms and different environmental and biological processes have to be considered (Fent 2001). Thus, to provide more detailed information on the possible effects of contaminants, *biomarkers* have been introduced as helpful tools in environmental monitoring (Huggett et al 1992, Walker et al 2001). Some inconsistency, however, applies to the definition of biomarker (Walker et al 2001). The most common use of the biomarker term refers to biochemical, physiological or histological changes as well as aberrations in organisms that can be used to estimate either exposure to chemicals or resultant effects (Huggett et al 1992). Peakall (1994) defines a biomarker as “a biological response to a chemical or chemicals that gives a measure of exposure and sometimes, also, of toxic effects” whereas Walker et al (2001) defines biomarker as “any biological response to an environmental chemical at the individual level or below demonstrating a departure from the normal status”. Changes at organizational levels above that of the individual, i.e. population, community and ecosystem, are termed bioindicators (Walker et al 2001).

A number of classifications of biomarkers have been proposed. The most widely used is division into "biomarkers of exposure" and "biomarkers of effect". Biomarkers of exposure are those that indicate exposure of the organism to chemicals, but give no information on the degree of adverse effects that this changes causes. Biomarkers of effect are those which demonstrate an adverse effect of the organism (Walker et al 2001). However, Peakall and McBee (2001) argue that this classification is misleading. All biomarkers indicate exposure and demonstrate an effect of some kind.

The idea of measuring a biological parameter as an indicator of the well-being of an organism is very old. In ancient China, physicians were able to assess the health of their patients by tasting urine, by smelling faeces, etc. Modern medicine has refined this approach, and measurements of heart rate, body temperature, blood counts, enzyme levels etc. are now commonly used to monitor the patient's health (Depledge 1994). Due to the complexity of ecosystems, the development of biomarkers in ecotoxicology has to deal with many challenges that medical toxicology more easily solves. Nevertheless, the parallel to medical toxicology should only encourage the further work on biomarkers for environmental monitoring.

The specificity of biomarkers to chemical contaminants varies greatly. Both specific and non-specific (general) biomarkers have their place in environmental assessment (Peakall and McBee 2001). Further, some biomarkers can be applied throughout the animal kingdom, for example the inhibition of acetylcholinesterase, whereas the induction of vitellogenin is confined to those vertebrates that lay eggs. Some biomarkers respond instantaneously while others need years to develop. The response may also be transient or irreversible (Huggett et al 1992). Hence, a number of aspects need to be considered when evaluating biomarkers. In addition to the factors already mentioned, it will also be of importance that the biomarker is sensitive compared to other endpoints, such as mortality or reproductive impairment, and the responses should be distinguished from natural stressors. A biomarker will also be more useful if there is a clear linkage to effects at higher levels of organization (Peakall and McBee 2001).

Seabirds have been used in several environmental monitoring studies. Fish-eating birds may be well suited for the assessment of effects of POPs due to their wide distribution (Fox 1993).

They also bioaccumulate relatively high levels of POPs due to their higher trophic levels and due to their limited abilities to metabolize anthropogenic compounds (Walker 1992). In birds induction of CYP 1A enzymes (Sanderson et al 1994, Henriksen et al 2000), retinol homeostasis (Spear and Moon 1986, Boily et al 1994, Kuzyk et al 2003), thyroid function (van den Berg et al 1994, Verreault et al 2004) and various malformations (Fox et al 1991) have been studied as responses to POP exposure and thus as potential biomarkers of POPs. However, still there is a need of more studies to establish the application of biomarkers in environmental monitoring programmes.

1.6 Vitamins

Vitamin A (retinol) and vitamin E (tocopherol) are water-insoluble and lipophilic vitamins (Combs 1992, Traber et al 1993), which are found in plants as carotinoids (pro-vitamin A), tocopherols and tocotrienols (Combs 1992, Sheppard et al 1993). The most biologically active form of vitamin E is α-tocopherol (Sheppard et al 1993).

The liver stores 50-80 % of retinol in vertebrates as retinyl esters, of which retinyl palmitate is the most important (Blomhoff 1994, Karadas et al 2005). Mobilization of retinol from the liver occurs by hydrolysis of retinyl esters. However, the transport of retinol in plasma is dependent on specific transport molecules, retinol-binding proteins (RBPs), which have been shown to be partly evolutionary conserved across the avian and mammalian species (Soprano and Blaner 1994). In plasma, retinol-RBP binds to transthyretin (TTR), the transport protein of thyroid hormones. The RBP-TTR complex is vital to prevent filtration of retinol-RBP through the kidneys (Combs 1992). In contrast, there is no evidence of a specific vitamin E plasma carrier protein. Tocopherol is transported in the blood within plasma lipoproteins and erythrocytes, which has two important consequences: One is the protection by tocopherol from free-radical attack on fatty acids and lipids. The other is that tocopherol concentrations do not entirely depend on dietary intake (Traber et al 1993). Following absorption of tocopherol from the intestine, tocopherol is incorporated in chylomicrons (mammals) or portomicrons (birds) (Traber et al 1993, Surai 1999). Avian portomicrons (large lipoproteins) are transported directly via the portal system to the liver prior to plasma entry (Surai 1999). In contrast, mammalian chylomicrons are catabolized to chylomicron remnants and then taken up by the liver. Once in the liver, α-tocopherol is secreted into the bloodstream within lipoproteins (Traber et al 1993).

Retinol is essential for normal vision, reproduction, cellular immune function and the maintenance of differentiated epithelia and mucous secretions in higher animals (Fox 1993). Deficiency in retinol also influences growth and development of tissues and organs of embryos and young animals (Spear et al 1986). In birds, nutritional excess or deficiency is associated with changes in several reproductive parameters, such as secondary sexual characteristics, spermatogenesis, egg-laying, egg size, embryo survival and hatching success (Boily et al 1994). In the avian embryo, antioxidant defence based on natural antioxidants such as vitamin A and vitamin E is responsible for the restriction and prevention of oxidative chain formation and propagation (Surai 1999).

Tocopherol is essential for normal neurological structure and function (Traber et al 1993). As a part of the antioxidant defences, tocopherol is also important in reducing the negative effects of cellular oxidative stress (Saito 1990). In avian embryos, vitamin E is considered to play a particular important role in the antioxidant defence being actively accumulated in embryonic tissues (Surai 1999).

1.7 Vitamins as biomarkers of POPs

Several studies report associations between retinoid status and OC compounds in rodents (Brouwer et al 1983), seals (Brouwer et al 1989, Jenssen et al 2003), polar bears (Skaare et al 2001) and birds (Spear and Moon 1986, van den Berg et al 1994, Murvoll et al 1999, Champoux et al 2002, Kuzyk et al 2003, Martinovic et al 2003). Thus, retinoid status has been proposed as a promising biomarker for exposure to such compounds (Simms and Ross 2000). Further, in rats and mice, exposure to PBDE mixtures have been shown to cause a reduction in hepatic retinol levels (Hallgren et al 2001). Studies also document that α-tocopherol is influenced by PCBs (Palace et al 1996, Twaroski et al 2001). It is therefore possible that alterations in tocopherol levels could be a useful biomarker of exposure to POP compounds.

Proposed mechanisms for the reducing effect of OCs on retinoid stores are reviewed by Chen et al (1992) and include (1) increased glomular filtration due to conformational changes by hydroxy-metabolites in transport molecules for retinol in plasma (TTR-RBP), (2) loss or transformation of stellate storage cells in the liver and (3) increased metabolism of several retinol forms by induced phase I and phase II enzymes. Alternatively, increased utilization of retinol as an antioxidant may also deplete retinol stores (Palace et al 1996).

The influence on α-tocopherol by PCBs is believed to be caused by the cellular oxidative stress initiated by the substances, which cellular antioxidant defences, such as tocopherol, try to counteract (Saito 1990). It is assumed that the oxidative stress induced by specific environmental contaminants is due to the interaction of these compounds with the aryl hydrocarbon (Ah) receptor and activation of the CYP 1A enzymes (Toborek et al 1995) which lead to formation of reactive oxygen species (ROS). Some PBDEs have also been shown to induce CYP 1A enzymes (von Meyerinck et al 1990, Pettersson et al 2001), and these compounds may therefore also initiate the formation of ROS and oxidative stress. Thus, tocopherol might be a potential biomarker of the oxidative stress exerted by POPs.

2. OBJECTIVES

The aim of the present thesis was to provide up-to-date information on levels of PCBs, some OCPs, PBDEs and HBCD in different seabirds along the coast of Norway and from Svalbard, and to contribute to the development of vitamins as potential biomarkers of POP exposure. Several knowledge gaps make the use of biomarkers in environmental monitoring limited. However, in the future, biomarkers would possibly make monitoring of species and ecosystems more comprehensive and nuanced.

To accomplish the aim, the following was done:

1. Concentrations of POPs

   The concentrations of 23 PCB congeners, 5 OCPs, 6 PBDEs and HBCD were analyzed in the yolk sac of newly hatched chicks of four seabird species; European shag (*Phalacrocorax aristotelis*), kittiwake (*Rissa tridactyla*), Brünnich's guillemot (*Uria lomvia*) and common eider (*Somateria mollissima*). Samples from shag hatchlings were taken at Sklinna (65°12' N, 11°00' E), an island at the coast of Norway, while samples from kittiwake hatchlings were taken at Runde (62°24' N, 5°36' E), another island on the coast of Norway, and from Kongsfjorden (78°55' N, 12°30' E) close to Ny-Ålesund at Svalbard. Samples of Brünnich's guillemot and common eider hatchlings were taken from Kongsfjorden (see Fig. 9).

2. Responses in vitamin levels

   An exposure study on domestic duck (*Anas platyrhynchos*) eggs was performed using injections of PBDE-99 to study possible effects of this congener on vitamin levels under controlled laboratory conditions. Vitamin levels (retinol, retinyl palmitate and α-tocopherol) were measured in liver and plasma of newly hatched chicks of domestic ducks, shags, kittiwakes, Brünnich's guillemot and common eider. Possible relationships between POP levels and vitamins were statistically examined. In both field and lab studies the age of the hatchlings were standardized (< 12 hrs) to reduce confounding factors. In kittiwakes, also sex of the hatchlings was determined using PCR techniques, allowing a correction for sex variation in the kittiwake data.

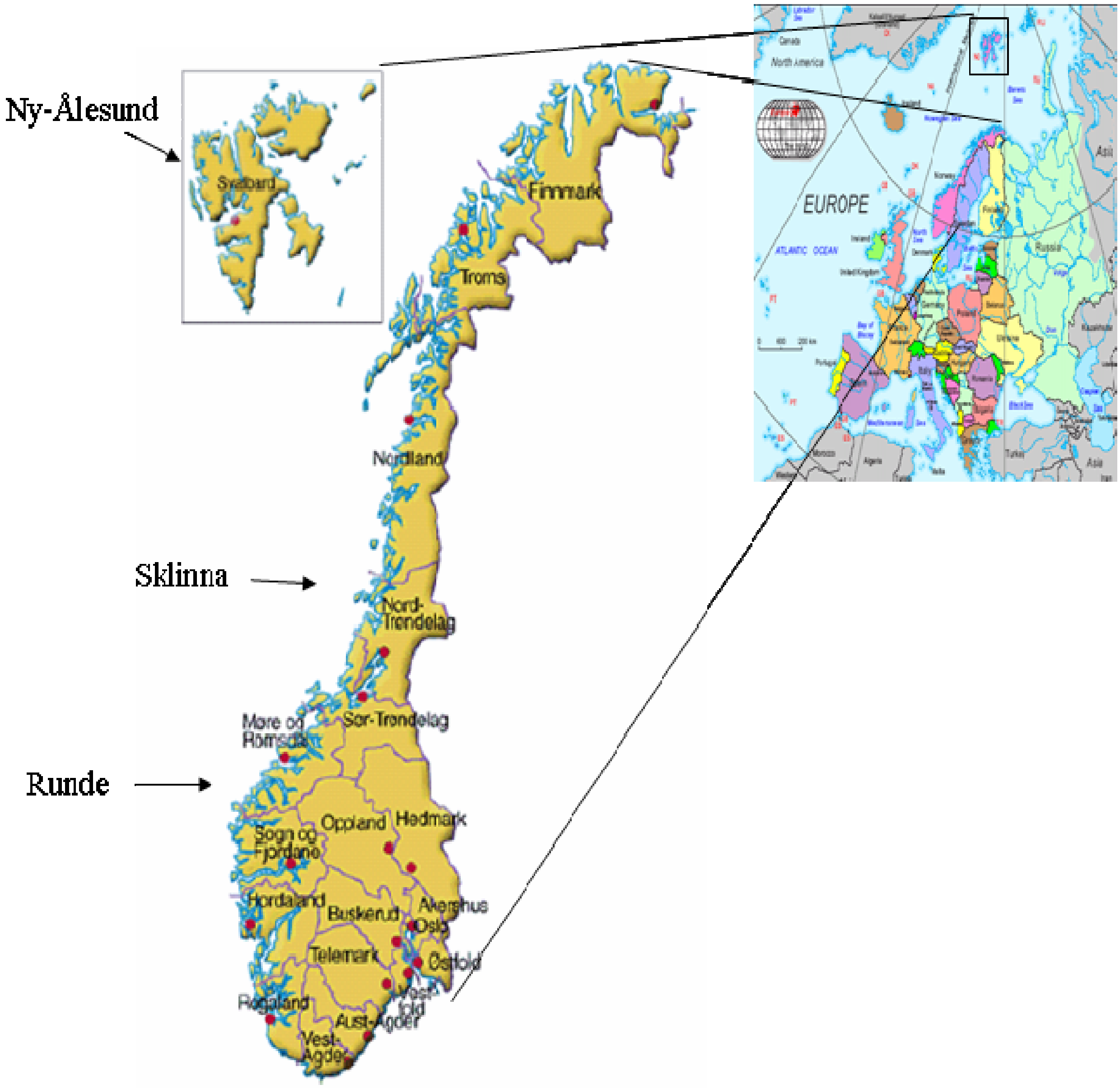


Figure 9: A map indicating where Runde, Sklinna and Ny-Ålesund are situated.

3. METHODS

3.1 Sampling

At hatching, body mass, tarsus length and head size were recorded before blood samples (1-2 ml) of anaestisized chicks were taken by heart-puncture using hypodermic needles. A small fraction of the collected blood was used to measure hematocrit, and the remaining blood was centrifuged. The plasma was transferred to cryo vials and kept frozen at -40ºC. To prevent photodegradation of retinol and tocopherol, the plasma-filled vials were covered with aluminium foil. The yolk sac and liver were removed and weighed, before packed in aluminium foil and stored at -40ºC. All samples were taken within 12 hrs after hatching to reduce the risk of confounding due to normal temporal variations in vitamin levels during the first 24 hours after hatchling. The sampling was approved by Norwegian Animal Research Authority (Oslo, Norway).

The yolk sac of the hatchlings was the most appropriate matrix for the POP analysis (see 3.2) due to the available amounts. Plasma and liver samples from the hatchlings were of small quantities, but could be used for the analysis of vitamin levels (see 3.3). Hence, POP levels and vitamin levels were measured in different matrices. The statistical analysis of possible relationships between the POPs (predictor variables) and vitamins (response variables) could thus be confounded. Nevertheless, Henriksen et al (1996) found that lipid weight PCB concentrations were highly significantly correlated between tissues (liver vs. fat vs. brain) in adult kittiwakes, and from this it was assumed that the levels of POPs in yolk sacs of hatchlings probably correlated with levels of POPs in plasma and liver.

3.1.1 Exposure study

In the laboratory study, the yolk of eggs of domestic duck was injected by PBDE-99 (0.05. 0.5 and 5 µg/ml) (**Paper I**). After the injection, the eggs were sealed with paraffin and placed in an incubator. The eggs hatched after 27-28 days of incubation.

3.1.2 Field studies

In the field studies, eggs of kittiwakes, Brünnich's guillemot and common eider were taken from the nests and transferred to a field laboratory where the eggs were put in incubators

(**Paper III, IV**). The eggs hatched during 1-25 days. European shag hatchlings were collected from their nests within 12 hours after hatching (**Paper II**). The eggs/hatchlings were not artificially exposed to POPs, and hence the POP levels measured represented natural levels.

3.2 Analysis of POPs

The analyses of POPs were carried out at the Environmental Toxicology Laboratory at the Norwegian School of Veterinary Science as described in **Paper I-II**. In short, the organic compounds were extracted with acetone and cyclohexane, concentrated by evaporation and lipids removed from the extracts with sulphuric acid. The extracts were then injected to a gas-chromatograph (GC) with a MS- (brominated compounds) and EC- (chlorinated compounds) detector (**Paper I-II**).

The laboratory is accredited according the requirements of NS-EN ISO/IEC 17025:2000 for the relevant analytical methods. The laboratory's accredited analytical quality for the chlorinated and brominated compounds has been approved in several international intercalibration tests.

The 23 PCB congeners analyzed represented both lower- and higher chlorinated PCBs. Some PCBs previously shown to contribute very little to ∑PCBs in seabirds (i.e. PCB-31, -87, -136, -110, -151, -132, -141, -199, -206, -209) (Murvoll 1996) were not included. In addition, the choice of PCB congeners was based on the configuration of the compounds to include the most persistent ones (i.e. PCB-153, -138, -128, -180, -170) (Boon et al 1997, see 4.2.3.1) and inducers of the Ah receptor (e.g. PCB-105, -118, -156, -157) (Boon et al 1992, see 1.3). The chosen OCPs were included to represent the most abundant OCPs in marine biota (AMAP 1998). Furthermore, the 6 PBDE congeners analyzed represented both lower- and higher brominated PBDEs. The chosen PBDEs were also believed to be the most abundant ones based on information from available studies on seabirds in 2002 (Lundstedt-Enkel et al 2001).

3.3 Analysis of vitamins

The analyses of vitamins were carried out at Department of Biology, Norwegian University of Science and Technology (NTNU) as described in **Paper I**. In short, the vitamins were extracted with hexane, concentrated by evaporation under pure N_2 and dissolved in methanol (mobile phase). Before evaporation, liver samples also had to be sonicated to break the cells and to facilitate the extraction of vitamins. The extracts were then injected to a high-performance liquid chromatograph (HPLC) with a fluorescence detector (**Paper I**).

The vitamins were chosen due to the previously documented negative relationships between retinol and OCs in birds and between tocopherol and PCBs in vertebrates (see 1.7). Plasma and liver are most frequently used as matrices for analysis of these lipid-soluble vitamins. In addition to retinol, levels of retinyl palmitate were measured due to the possible mobilization of retinol from retinyl palmitate in the liver in response to POP exposure (Murk et al 1991, Murk et al 1994).

3.4 Statistical analysis

To investigate possible relationships between POP concentrations and vitamin levels, univariate (**Paper I, IV**) and multivariate (**Paper II, III**) regression tests were used. In addition to possible relationships between POP levels and vitamins, it was also statistically explored if there were any correlations between POPs and morphological variables.

4. DISCUSSION

4.1 The choice of seabird species

The seabird species (i.e. shag, kittiwake, Brünnich's guillemot, common eider) included in the present study were chosen since they represented different trophic levels and belonged to different developmental categories along the altricial-precocial spectrum for birds. Common eider, feeding on benthic organisms (Dahl et al 2003), occupies a lower trophic position compared to Brünnich's guillemot, primarily feeding on crustaceans and fish (Borgå et al 2001), and kittiwakes and shags, feeding predominately on fish (Barrett et al 1990, Borgå et al 2001). According to the altricial-precocial spectrum, shag represented the altricial species, kittiwake and Brünnich's guillemot represented the semi-precocial species and common eider (and domestic duck) represented the precocial species (Starck and Rickleffs 1998).

The species of the present thesis have also been used in previous studies on POP levels (Barrett et al 1996, Murvoll et al 1999, Franson et al 2004), which could be interesting in the light of temporal trends. Moreover, samples from kittiwake hatchlings from both the coast of Norway and from Svalbard were of special usefulness in the comparisons of POP levels between Norway and Svalbard (Arctic) (i.e. spatial differences) due to the reduction of confounding factors when using the same species in accordance to the same methodology and analytical procedures. Also possible differences in responses to POP exposure within a species could be revealed when studying kittiwakes from the two populations. Furthermore, ecological monitoring of some of the colonies from where the hatchling samples were taken provided opportunities of linking observed effects at lower biological levels to those observed at higher biological levels.

4.2 Levels of POPs in hatchlings of the present study

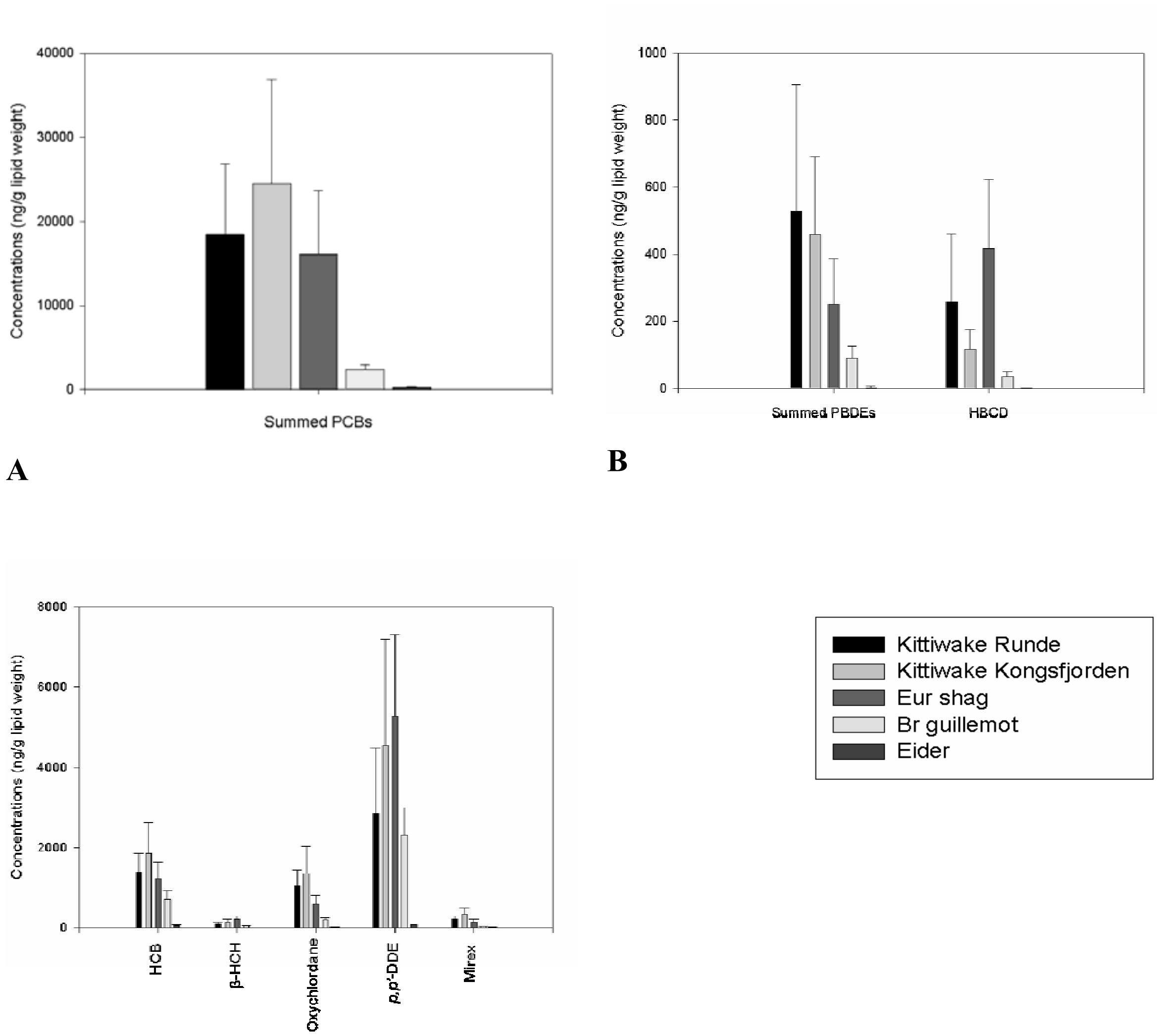
4.2.1 Levels of POPs

The concentrations of POPs in different seabirds vary due to several factors. Diet and trophic position is the dominant factor influencing concentrations of hydrophobic and recalcitrant compounds (Borgå et al 2004). However, differing metabolizing capacity between species is also of importance (Borlakoglu et al 1990, Fisk et al 2001a), in addition to age, (Donaldson et al 1997), condition and reproductive status (Henriksen et al 1996) and migration pattern (Buckman et al 2004). Newly hatched chicks reflect the avian mothers' contamination, although some elimination could happen as the metabolizing capacity increases during embryonic development (Lorenzen et al 1997, Hoffman et al 1998).

In general, kittiwake hatchlings had significant higher levels of POPs in comparison to European shag, Brünnich's guillemot and common eider hatchlings. Further, all POP levels were significantly higher in shag hatchlings compared to Brünnich's guillemot and common eider hatchlings ($p<0.00$ for all POPs). There were also significant differences in POP levels between Brünnich's guillemot and common eider hatchlings (**Paper IV**). Figure 10 A-C shows the POP levels of the seabird hatchlings of the present thesis.

As stated above, several factors influence differences in POP levels between different species and different locations. Kittiwakes were higher contaminated by most POPs compared to other species, despite that they occupy a fairly similar intermediate trophic position as European shags (Nils Røv pers comm.) and Brünnich's guillemots (AMAP 1998, Hop et al 2002). In addition to the influence of minor discrepancies in trophic positions, the higher POP concentrations in kittiwakes could be due to the fact that kittiwakes migrate widely and may thus ingest contaminants outside the study area (Barrett et al 1996, **Paper III**). Differences in metabolizing capacities between the species could also affect POP levels. However, kittiwakes are believed to have a higher metabolic activity and excretion efficiency than Brünnich's guillemots (Borgå et al 2001), which make it reasonable to assume that higher rate of food intake (Ellis and Gabrielsen 2002) and diet are more important than elimination ability in explaining high POP levels of kittiwakes. Higher levels of POPs in kittiwakes than in Brünnich's guillemots from the Arctic environment is in accordance to previous studies (Borgå et al 2001, Hop et al 2002).

Some exceptions from the general trend of kittiwakes having the highest POP levels were found: The kittiwakes from Runde and shags from Sklinna seemed to have ∑PCBs and HCB levels of same magnitude (∑PCBs: $p=0.39$, HCB: $p=0.47$), whereas the levels of *p,p'*-DDE and HBCD were higher in shag hatchlings than in kittiwake hatchlings from both Runde and Kongsfjorden ($p<0.04$). The PCBs have been prohibited in most industrial countries for decades, and local sources and input are at present minimized, although some leakage from



C

Figure 10:

A. Concentrations of ∑PCBs in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14). In shag hatchlings, 12 individuals had not detectable levels of PCB-52. In common eider hatchlings, no individuals had detectable levels of PCB-52, -47 and -114. One common eider hatchling had not detectable levels of PCB-157.

B. Concentrations of ∑PBDEs and HBCD in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14). In shag hatchlings, PBDE-28 was not detected in two individuals. In common eider hatchlings, PBDE-28 was not detected in any individuals, and PBDE-47, -99, -100, -153, -154 were detected in three to eleven hatchlings. HBCD was detected in only on hatchling of common eider.

C. Concentrations of OCPs in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14).

products and sediments still lead to environmental releases (AMAP 1998). Hence, migration of kittiwakes to potential higher contaminated area may not be of same importance as earlier with regard to accumulation of PCB levels. Thus, it is possible that the levels of PCBs in the hatchlings now to a greater extent reflect local contamination on the Norwegian coast. Since shags from Sklinna actually reside not far from Runde during winter time (Røv 1991), this could explain the fairly similar levels of PCBs to kittiwakes from Runde. The higher levels of *p,p'*-DDE in shag hatchlings compared to kittiwake hatchlings may also be explained by the assumption that OC levels in birds along the coast of Norway reflect the local contamination. Hence, differences in food choice, in addition to between-species differences in elimination properties, are probable causes of the observed higher levels of *p,p'*-DDE in shag hatchlings.

With respect to the high levels of HBCD in shag hatchlings, this could be due to local sources, although no survey with regard to BFRs is yet conducted nearby Sklinna where shags breed. However, a national survey of brominated compounds in sediments and mussels has revealed very high levels of both HBCD and PBDEs in Åsefjorden, approximately 40 km from Runde (Fjeld et al 2004, **Paper III**). The high levels are believed to be due to local discharges. Since shags from Sklinna reside not far from Runde during winter time (Røv 1991), it may be speculated that the local source in Åsefjorden contribute to the high HBCD levels observed in the shag hatchlings. In addition, species-dependent differences in uptake and/or metabolism of this congener and different food choice will influence the levels of HBCD.

It should be noted that shags are lean bird species, having a very low lipid content (6.84 %) compared to the other species (kittiwakes: 17.5-19.8 %, Brünnich's guillemots: 28.1 %, common eiders: 20.9 %). This will make lipid weight concentrations relatively high compared to wet weight concentrations in shags. Being an altricial species (Starck and Rickleffs 1998), shag hatchlings may also metabolize POPs slower than semi-precocial and precocial species. This could be of relevance in explaining the relatively high levels of POPs in shag hatchlings when taking into consideration that the adult shag mothers reside along the coast of Norway during the year (Røv 1991).

Kittiwake hatchlings from Kongsfjorden had significantly higher levels of ∑PCB and OCPs than those from Runde (**Paper III**). A possible explanation to this could be that the expected decrease of PCB and OCP concentrations in biota, following the regulatory restrictions on the use of these compounds, is slower in Arctic regions than in more temperate regions because the Arctic region serves as sinks for some POPs (Wania and Mackay 1993). This could probably also be of importance in explaining the similar levels of *p,p'*-DDE in Brünnich's guillemot hatchlings to kittiwake hatchlings from Runde (p=0.84), being significantly higher contaminated by POPs in general. Although not discussed in **Paper III** because of high degree of uncertainty, another cause for the differences observed between kittiwakes from Kongsfjorden and Runde could be related to energy expenditure of the adult female prior to egg-laying in the breeding season or during the winter. Values of field metabolic rates (FMR) of chick rearing kittiwakes in Kongsfjorden (Fyhn et al 2001) and on Runde (Gangås 1994) indicate higher energy expenditure in kittiwakes from Kongsfjorden. Thus a higher rate of food intake and bioaccumulation of PCBs and OCPs could be expected in Arctic kittiwakes. This contrasts, however, to the conclusion by Golet et al (2000) that energy expenditure in breeding kittiwakes is relatively invariant between populations from different environmental conditions. It is very difficult to assess possible differences in energy expenditure or food intake during winter between the two study populations of kittiwakes because specific wintering areas are not known. However, there is a tendency for birds breeding in northern colonies to migrate further north and east than those breeding in southern colonies (Anker-Nilssen et al 2000). If this applies to the populations in question, on one hand it could imply higher winter energy expenditure in the kittiwakes from Kongsfjorden (due to higher costs of thermoregulation), but on the other hand it could imply that kittiwakes from Runde feed on more contaminated food during winter. Hence, different POP accumulation because of possible disparities in energy expenditure and food intake between the two populations of kittiwakes can not yet be concluded.

In contrast to what was found for OC compounds, kittiwake hatchlings from Runde had higher levels of HBCD than those from Kongsfjorden (**Paper III**). It is possible that this could be explained by a local source on the coast (Åsefjorden) close to Runde, identified in a national survey of brominated compounds (Fjeld et al 2004, **Paper III**).

The shag hatchlings had higher levels of POPs than Brünnich's guillemot and common eider. None of these species migrate widely, but reside along the coast of Norway and in Nordic waters during the years (Røv 1991, Anker-Nilssen 2000, Borgå et al 2005). Only minor influence of migration on the POP burdens in these species should thus be expected due to their resident behaviour. From the species' breeding sites, it could probably also be expected that shags from the coast of Norway had higher levels of POPs than Brünnich's guillemots and common eiders from the remote Arctic. In addition, common eider feeds on benthic organisms (Dahl et al 2003, **Paper IV**) and occupies a lower trophic position compared to Brünnich's guillemot, primarily feeding on crustaceans and fish (Borgå et al 2001), and shag, feeding on predominately on fish (Barrett et al 1990). In addition, common eider hatchlings are precocial (Starck and Rickleffs 1998) and thus have a higher metabolic rate, indicating a potential of faster elimination of POPs compared to semi-precocial Brünnich's guillemot hatchlings and altricial shag hatchlings. POP level differences between shag and Brünnich's guillemot hatchlings could also be explained by different food choice and disparities with regard to metabolic activity. Being a semi-precocial species (Starck and Rickleffs 1998), Brünnich's guillemot hatchlings might metabolize POPs faster than altricial shag hatchlings do.

4.2.2 Levels of POPs in comparison to other studies

It should be noted that comparison of contamination levels between studies often is difficult due to differences in matrix analyzed and in the analytical procedures. Nevertheless, comparisons could give valuable information on spatial and temporal trends of POP levels. In comparison with previous studies on PCB and OCP levels, kittiwake and shag hatchlings herein had similar, or somewhat higher, levels than reported earlier along the Norwegian coast and at Svalbard (Barrett et al 1996, **Paper II-III**). Hatchlings of Brünnich's guillemots had lower and corresponding levels of PCB and OCPs, respectively, compared to earlier reports (Barrett et al 1996, **Paper IV**). Based on other studies of temporal trends of PCBs in seabirds (Bignert et al 1998) the PCB concentrations from 1990s until early 2000s should be expected to be decreasing. As discussed in **Paper III**, the lack of an obvious decline in OC levels from the 1990s in some of the species included in the present study, despite the regulatory restrictions on the use of the compounds, could be that the expected decline was most evident during the 1980s and early 1990s, and that PCB and OCP levels will fluctuate around these relatively low levels in years to come (Barrett et al 1996).

The levels of most OCs were higher in both kittiwake and Brünnich's guillemot hatchlings than reported in eggs of the same species from the Canadian Arctic (**Paper III-IV**). The same applied to common eider hatchlings and eggs (Franson et al 2004, **Paper IV**). The shag hatchlings seemed to have relatively low levels of OC compounds in comparison with

Phalacrocoracidae species from other European waters, the Great Lakes and Japan (**Paper II**, Kumar et al 2005).

As shown in Table 1, the PBDE levels of the species included in the present study were low compared to the reported levels in eggs of Great Lakes herring gull (*Larus argentatus*) (Norstrom et al 2002), in eggs of great blue herons (*Ardea herodias)* and double-crested cormorants (*Phalacrocorax auritus*) from British Colombia (Elliott et al 2005) and in eggs of common cormorant (*Phalacrocorax carbo*) from Japan (Watanabe et al 2004). However, the PBDE levels in kittiwake and shag hatchlings were high compared to levels of brominated compounds reported in eggs of guillemot (*Uria aalge*) from the Baltic (Sellström et al 2003), in eggs of black guillemot (*Cepphus grylle*) from the Greenland (Vorkamp et al 2004) and in eggs of fulmars (*Fulmarus glacialis*) from the Faroe Islands (Fängström et al 2005). Moreover, the HBCD levels were very high in shag hatchlings compared to all other available studies on HBCD levels in seabirds (**Paper II**). In a European scale, the relatively high levels of BFRs seem to be in contrast to the situation for the OC levels, as the reported PCB levels in guillemots from the Baltic are much higher than in birds from the Norwegian coast, e.g. shags (Bignert et al 1998). In addition to migration of kittiwakes to potential higher contaminated areas, the most obvious explanation of the high BFR levels is linked to local sources (i.e. Åsefjorden). However, as mentioned above, to our knowledge no survey is conducted nearby Sklinna where shags breed, and possible local sources remain to be substantiated. Since shags from Sklinna reside not far from Runde during winter time (Røv 1991), the local source in Åsefjorden (Fjeld et al 2004), close to Runde, may contribute to the high BFR levels observed also in the shag hatchlings. Another possible link between the high levels of BFRs in shags and the local source in Åsefjorden could be the Norwegian spring-spawning herring (*Clupea harengus*). This herring spawn at the coastal banks off Møre where Åsefjorden is situated, and the juveniles migrate northward to the Barents Sea to feed. A minor part of the juveniles also feed in the coastal areas from Trøndelag to Finnmark, while the adult stock mainly migrates westward to feed (Sissener and Bjørndal 2005). Hence, herring in the Møre area could accumulate BFRs and transfer the pollution to shags, breeding at the coast of Trøndelag (Sklinna), where the herring migrates to feed.

Table 1: Levels of polybrominated diphenyl ethers (PBDEs) in different species of seabirds. ww; wet weight concentrations, lw; lipid weight concentrations.

Species	Matrix	Year	Location	∑PBDEs ww	∑PBDEs lw	PBDE-47	PBDE-99	References
Common eider	Yolk sac	2002	Norw Arctic	0.4 ng/g[1]	2.1 ng/g[1]	0.74 ng/g lw	0.71 ng/g lw	Present thesis
Brünnich's guillemot	Yolk sac	2002	Norw Arctic	27 ng/g[1]	90 ng/g[1]	60 ng/g lw	11 ng/g lw	Present thesis
Kittiwake	Yolk sac	2002	Norw Arctic	79 ng/g[1]	461 ng/g[1]	285 ng/g lw	34 ng/g lw	Present thesis
Kittiwake	Yolk sac	2002	Norw coast	103 ng/g[1]	528 ng/g[1]	351 ng/g lw	44 ng/g lw	Present thesis
Eur shag	Yolk sac	2002	Norw coast	17 ng/g[1]	251 ng/g[1]	82 ng/g lw	23 ng/g lw	Present thesis
Glaucous gull	Egg	2002	Norw Arctic	53.6 ng/g[2]	556 ng/g[2]			SFT 2004
Black guillemot	Egg	2000	Greenland	2.5 ng/g[1]				Vorkamp et al 2004
Guillemot	Egg	2001	Baltic			89 ng/g lw	12 ng/g lw	Sellström et al 2003
Herring gull	Egg	2000	Great Lakes	188-1362 ng/g[3]				Norstrom et al 2002
Common cormorant	Egg		Japan		1400 ng/g[4]			Watanabe et al 2004
Great blue heron	Egg	2002	Br Colombia	455 ng/g[5]				Elliott et al 2005
Double-crested cormorant	Egg	2002	Br Colombia	62.5 ng/g[5]				Elliott et al 2005
Fulmar	Egg	2000-2001	Faroe Islands		21 ng/g[6]	4.3 ng/g lw	4.6 ng/g	Fängström et al 2005

Footnotes: [1]=$\sum PBDE_{6}$, [2]=$\sum PBDE_{9}$, [3]=$\sum PBDE_{7}$, [4]= $\sum PBDE_{20}$, [5]= $\sum PBDE_{18}$, [6]= $\sum PBDE_{5}$

4.2.3 Pattern of POPs

4.2.3.1 PCBs

In all species included in the study, PCB-153 was the most abundant congener, which could be expected from the persistency towards biodegradation of this congener (Boon et al 1997). The pattern of PCBs in shag (**Paper II**) seemed to be in accordance to earlier reports. Also the PCB pattern observed in both populations of kittiwakes (PCB-153 > PCB-138 > PCB-180 > PCB-118), Brünnich's guillemot (PCB-153 > PCB-138 > PCB-118 > PCB-99) and common eider (PCB-153 > PCB-138 > PCB-180 > PCB-118) hatchlings was in fairly good accordance with the pattern reported in previous studies from Norway and Svalbard (Savinova et al 1995, Henriksen et al 1996, Hop et al 2002, Borgå et al 2005) and from Great Britain (Malcolm et al 2003).

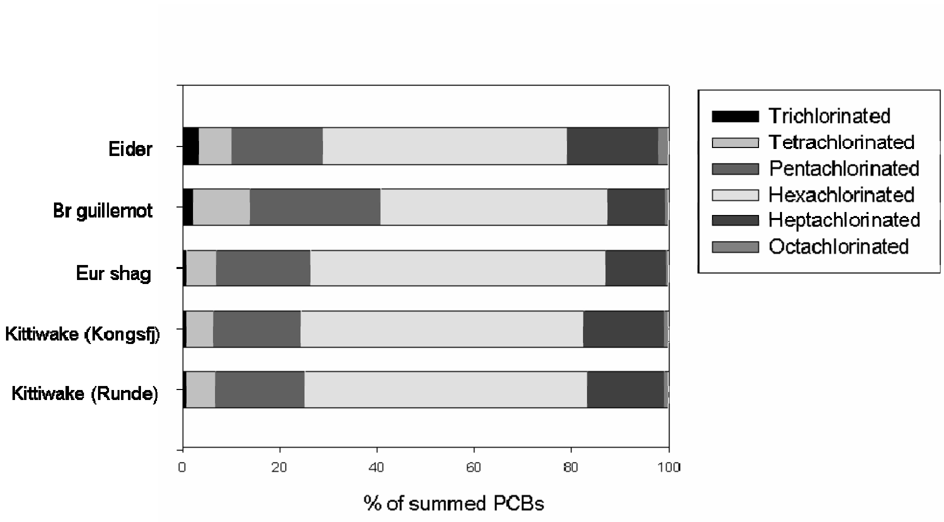


Figure 11: Relative patterns of polychlorinated biphenyls in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14). In shag hatchlings, 12 individuals had not detectable levels of PCB-52. In common eider hatchlings, no individuals had detectable levels of PCB-52, -47 and -114. One common eider hatchling had not detectable levels of PCB-157. The trichlorinated biphenyl was PCB-28, the tetrachlorinated biphenyls were PCB-52, -47, -74 and -66, the pentabrominated biphenyls were PCB-101, -99, -118, -114 and -105, the hexabrominated biphenyls were PCB-149, -153, -137, -138, -128, -156 and -157, the heptabrominated biphenyls were PCB-187, -183, -180, -170 and -189, the octabrominated biphenyl was PCB-194.

As shown in Figure 11, the lower chlorinated biphenyl congeners (tri- to penta-) were more abundant in Brünnich's guillemot and common eider hatchlings compared to kittiwake and shag hatchlings. An increase of higher chlorinated congeners with trophic position of seabirds has also been reported by others (Borgå et al 2001). The penta-, hexa- and heptachlorinated congeners were the dominated PCB congeners in all species. These congeners were also in higher proportion than other congeners in the commercial mixtures of PCBs and have the greatest bioaccumulation potential (McFarland and Clarke 1989). Tri- and tetrachlorinated biphenyl congeners are more readily metabolized, while higher chlorinated congeners are more tightly bound to sediments and particles and therefore less bioavailable. Once accumulated in organisms, however, high-chlorinated congeners of PCBs tend to biomagnify due to their relcalcitrant nature (Philips and Rainbow 1993).

The higher proportions of hexa- to octachlorinated biphenyl congeners in kittiwakes than in Brünnich's guillemots is in accordance with previous studies (Borgå et al 2001). Several of these higher chlorinated congeners are persistent (e.g. PCB-153, -138, -180) towards metabolizing enzymes according to a classification by Boon et al (1997) based on the position of the chlorine atoms on the biphenyl ring. The most persistent congeners are without vicinal hydrogen atoms (e.g. PCB-153, -180) or have vicinal hydrogen atoms exclusively in the *ortho*- and *meta*-positions in combination with two or more *ortho*-chloro substitutions (e.g. (PCB-128, -138, -170) (Boon et al 1997). The higher scores of persistent congeners in kittiwakes suggest that kittiwakes have higher contaminant metabolic activity than Brünnich's guillemots (Borgå et al 2001). From this, data from the present study indicate that shags and kittiwakes have a corresponding metabolic activity towards PCBs, although species-specific differences with regard to elimination of certain congeners most likely exist. The rate of metabolism may also differ between shag and kittiwake hatchlings due to their different classification according to the altricial-precocial spectrum.

Common eiders had a somewhat deviating PCB pattern, as the proportion of hepta- and octachlorinated congeners were somewhat greater than in kittiwakes and shags, at the same time as the lower chlorinated congeners were more abundant relative to what was found in kittiwakes and shags. The special pattern may reflect the lower trophic position of the eiders, as they are benthic feeders (Dahl et al 2003). Levels of lower chlorinated biphenyls are expected to be higher in animals at lower trophic levels as these congeners are more readily metabolized and thus are not biomagnified to the same extent as higher chlorinated congeners through the food-chain (Philips and Rainbow 1993). The somewhat higher proportion of hepta- and octachlorinated congeners could reflect a relatively high metabolic activity, possibly linked to the precocial nature of the common eider hatchlings.

4.2.3.2 OCPs

The patterns of OCPs were fairly similar between the different species included in the present study (Table 2). The patterns seemed to correspond well to earlier reports on OCPs in kittiwakes (Barrett et al 1996, Braune et al 2001), shags (Murvoll 1996), Brünnich's guillemots (Hop et al 2002) and common eiders (Savinova et al 1995). β-HCH is the most biodegradable compound of the OCPs herein, and on this background kittiwake and common eider hatchlings seemed to be more able to metabolize OCPs than shag and Brünnich's guillemot hatchlings. The trophic position, food choice and distances to pollution sources will however also influence the pattern of OCPs. The species' classification according to the altricial-precocial pattern for birds (metabolic activity) possibly also influences OCP pattern.

Table 2: Gross pattern of organochlorine pesticides in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14).

Species	Gross pattern of organochlorine pesticides
Kittiwake Runde	*p,p'*-DDE > HCB > oxychlordane > mirex > β-HCH
Kittiwake Kongsfjorden	*p,p'*-DDE > HCB > oxychlordane > mirex > β-HCH
Eur shag Sklinna	*p,p'*-DDE > HCB > oxychlordane > β-HCH > mirex
Br guillemot Kongsfjorden	*p,p'*-DDE > HCB > oxychlordane > β-HCH > mirex
Eider Kongsfjorden	*p,p'*-DDE > HCB > oxychlordane > mirex > β-HCH

4.2.3.3 PBDEs and HBCD

The patterns of PBDEs were dominated by PBDE-47. This corresponds to previous reports that PBDE-47 is one of the most abundant congeners of PBDEs in wildlife and humans (Darnerud et al 2001, Guvenius and Norén 2001). PBDE-47 is found in high concentrations in biota due to the fact that PBDE-47 together with PBDE-99 constitutes more than 70 % of the commercial mixture Penta-BDE (Sjödin et al 1998), which has been one of the most used commercial products. However, PBDE-47 was not the most abundant compound of the brominated flame retardants in shag and common eider hatchlings (Table 3). In these species HBCD was the most abundant BFR. HBCD is also one of the most used BFRs.

Table 3: Pattern of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14). In shag hatchlings, PBDE-28 was not detected in two individuals. In common eider hatchlings, PBDE-28 was not detected in any individuals, and PBDE-47, -99, -100, -153, -154 were detected in three to eleven hatchlings. HBCD was detected in only on hatchling of common eider.

Species	Pattern of PBDEs and HBCD
Kittiwake Runde	**PBDE-47** > HBCD > PBDE-99 > PBDE-100
Kittiwake Kongsfjorden	**PBDE-47** > HBCD > PBDE-99 > PBDE-100
Eur shag Sklinna	HBCD > PBDE-100 > **PBDE-47** > PBDE-153
Br guillemot Kongsfjorden	**PBDE-47** > HBCD > PBDE-99 > PBDE-100
Eider Kongsfjorden	HBCD > PBDE-153 > **PBDE-47** > PBDE-99

It should be noted that very low levels of all the PBDE congeners were detected in common eider hatchlings, and all the congeners were detected only in some (or none) of the specimens.

Further, HBCD was detected only in one individual. Thus, too much weight should not be attached to the observed pattern of PBDEs in common eider hatchlings.

As shown in Figure 12, the PBDE pattern of shag hatchlings deviated much from the corresponding pattern of kittiwake and Brünnich's guillemot hatchlings. In shags, PBDE-100 (pentabrominated) and PBDE-153 (hexabrominated) constituted relatively more of ∑PBDE. Furthermore, in shags HBCD was more abundant than any of the PBDE congeners. The reasons why the shag hatchlings have a diverging pattern of PBDEs may be due to local discharges or differences in diet and metabolism (**Paper II**).

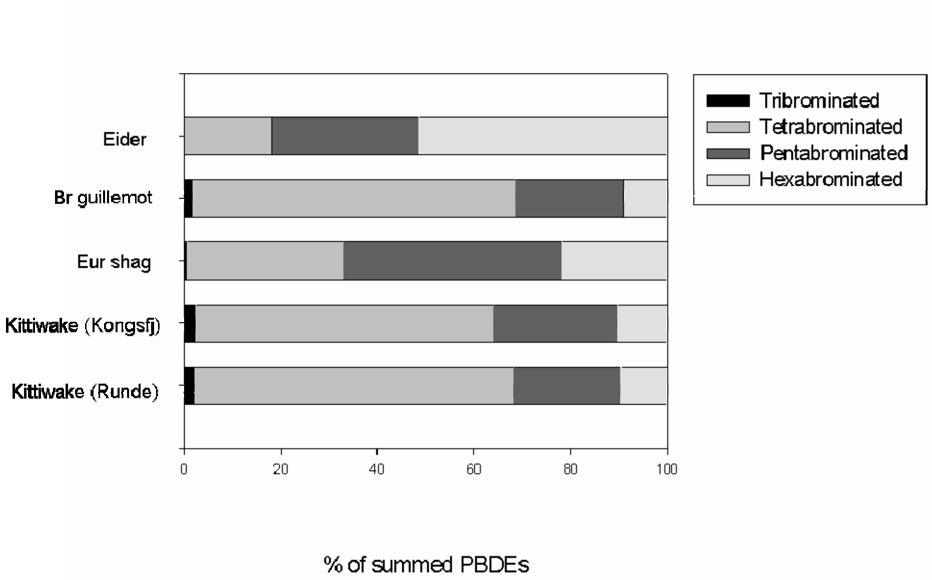


Figure 12: Relative patterns of polybrominated dipenyl ethers in kittiwake (*Rissa tridactyla*) hatchlings from Runde (*n*=19) and Kongsfjorden (*n*=18), in shag (*Phalacrocorax aristotelis*) hatchlings from Sklinna (*n*=30), in Brünnich's guillemot (*Uria lomvia*) hatchlings from Kongsfjorden (*n*=9) and in common eider (*Somateria mollissima*) hatchlings from Kongsfjorden (*n*=14). In shag hatchlings, PBDE-28 was not detected in two individuals. In common eider hatchlings, PBDE-28 was not detected in any individuals, and PBDE-47, -99, -100, -153, -154 were detected in three to eleven hatchlings.

The PBDE pattern in shags seemed to deviate some from what has been reported in cormorant (*Phalacrocorax carbo*) livers from England and Wales (PBDE-47 > PBDE-100 > PBDE-99) (Allchin et al 2000, Law et al 2002), which could indicate different distances from the sources of PBDEs. In addition, *Phalacrocoracidae* species could differ in their species-specific metabolism and in food-choice. The PBDE pattern of kittiwake hatchlings was similar to that reported in herring gull (*Larus argentatus*) eggs from the Great Lakes (Norstrom et al 2002), and this may reflects that gull species have similar capacities to metabolize PBDEs. Brünnich's guillemot hatchlings showed a PBDE pattern deviating somewhat from the pattern reported in guillemot eggs from the Baltic proper (PBDE-47 > PBDE-100 > PBDE-99)

(Lundstedt-Enkel et al 2001), possibly reflecting differences between alcid species with regard to metabolism of brominated compounds, although several other factors also will influence the pattern observed (e.g. local sources, diet).

The pattern of PBDEs was in general dominated by lower (tetra-) brominated compounds in contrast to the pattern of PCBs, dominated by higher (hexa-) chlorinated compounds (Fig. 2). These differences most likely reflect the newer input of PBDEs into the environment due to continuing production and use of these compounds in most of the world, whereas the use of PCBs have been restricted for decades in most countries. Thus, the most persistent congeners of PCBs will be most available in the environment and in biota.

4.3 Responses of morphological variables to exposure to POPs

No relationships were found between POPs and morphological variables in the experimentally exposed domestic duck, the free-living kittiwake and common eider hatchlings (**Paper I, Paper III-IV**). In shag hatchlings, head length and tarsus length correlated negatively to several PCBs, PBDEs, HBCD and some OCPs. However, when the variation explained by yolk lipid content was removed from the statistical model, no correlations between POPs and morphology were found (**Paper II**), and this finding underlines the importance of considering possible confounding impacts of lipid content when studying effects of POPs on morphological variables. Lipids not only accumulate pollutants, but fat reserves are also an important pool of energy, which influences body mass and body condition (O'Connor 1976, Blem 1990). Thus, size parameters, such as hatching mass, head size, tarsus length etc. could be influenced by the amount of lipids allocated into the yolk by the avian mothers.

In Brünnich's guillemot hatchlings, POPs seemed to have a negative influence on morphology (**Paper IV**). In this species, no correlations were found between lipids and morphology, i.e. lipid content was not confounding the relationships between POPs and morphology. Several studies on birds have reported effects of PCBs on morphological parameters related to growth and size (Hoffman et al 1986, van den Berg et al 1994, Champoux et al 2002). The negative relationships between POPs and growth parameters in birds may be related to the relatively well documented effects of these compounds on thyroid hormones and retinol in birds (Spear and Moon 1986, van den Berg et al 1994). Both thyroid hormones and retinol are important for normal growth and development in birds (Spear et al 1986). In the present study no effect on retinol was revealed in Brünnich's guillemot hatchlings. Thyroid hormone status could, however, still be influenced. Dilution of contaminants with growth could also be an explanation to the negative relationships between POPs and morphological variables (Champoux et al 2002), as this would result in lower POP levels in larger animals.

The lack of associations between POPs and morphology in most of the species included in the present study may indicate that levels of chlorinated or brominated contaminants were below those that induce effects associated with growth in the respective species. Brünnich's guillemot might be a more responsive species regarding effects of POPs on morphological variables.

4.4 Responses of vitamins to exposure to POPs

4.4.1 Normal temporal variation in vitamin levels

In order to reduce the risk of confounding from normal temporal variations in vitamin levels in hatchlings, a study on normal temporal variation in domestic duck hatchlings were conducted (**Paper I**). The results showed no significant differences in hepatic tocopherol and retinyl palmitate levels during the first 24 hrs after hatching. However, hepatic retinol levels were significantly reduced between 3 and 24 hrs after hatching (**Paper I**). Nevertheless, the choice of taking samples from the hatchling within 12 hrs after hatching seemed to be a reasonable approach to reduce the risk of confounding from normal temporal variations in vitamin levels. Although similar studies on temporal variation were not conducted in the field species, the results from the laboratory experiment were considered normative. Thus it was assumed that the vitamin levels of hatchlings of wild species also showed no significant differences with respect to vitamin status within the first 12 hrs after hatching.

4.4.2 Vitamin responses

4.4.2.1 Responses of α-tocopherol to POPs

In domestic duck and shag hatchlings, negative relationships were revealed between POPs and liver tocopherol levels (**Paper I, II**). In addition, negative associations between some OCPs and liver tocopherol levels were found in Brünnich's guillemot hatchlings, although the relationships seemed to be influenced by body mass, which was related to POPs and liver tocopherol levels in a negative and positive manner, respectively **(Paper IV).** Reduced levels of hepatic tocopherol levels have also been reported in minks, rats and fish species exposed to PCBs (Palace et al 1996, Käkelä et al 1999, Twaroski et al 2001). However, to my knowledge, the present study is the first to reveal negative relationships between POPs and tocopherol levels in birds. Further, to my knowledge, no previous studies have reported on correlations between OCPs (**Paper IV**), or PBDEs (**Paper I, II**), and liver tocopherol levels. The results thus may indicate that tocopherol is a sensitive response to various POPs in birds. Further, the results emphasize the need of considering possible impact of morphological variables (size) when studying the effect of POPs on vitamins.

Tocopherol levels are believed to be reduced by exposure to PCBs because of the cellular oxidative stress initiated by the substances (Saito 1990), which tocopherol tries to counteract. The oxidative stress is probably linked to the interaction of PCBs with the Ah-receptor and the activation of CYP 1A enzymes (Toborek et al 1995). The CYP 1A enzymes catalyze monooxygenation reactions in which one atom of molecular oxygen is incorporated into a substrate, and if the catalytic cycle is interrupted, oxygen is released as superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2) (Parkinson 2001), which both are reactive oxygen species (ROS) (Pitot and Dragan 2001). Some PBDEs have also been shown to induce CYP 1A enzymes (e.g. PBDE-99, correlating negatively to liver tocopherol levels in domestic duck hatchlings, **Paper I**) (von Meyerinck et al 1990, Chen et al 2001, Pettersson et al 2001). The configuration of PBDE-28, which correlated negatively to liver tocopherol levels in shag hatchlings (**Paper II**), indicates a potential for inducing CYP 1A enzymes (**Paper II**). However, an *in vitro* study using a rat hepatoma cell line indicated that PBDE-28 was not able to activate the Ah-receptor (Meerts et al 1998). The congener was shown to be a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) antagonist (Meerts et al 1998), although the observed antagonism may be due to competition between PBDE-28 and TCDD at the Ah-receptor level (de Wit 2002). Nevertheless, all CYP enzymes catalyze monooxygenation reactions (Parkinson 2001), and thus induction of other CYP enzymes than CYP1A may also result in oxidative stress. In a study on neurotoxicity of PCBs in rats' brain, non-dioxinlike PCBs lead to increase of ROS and induction of cell death (Mariussen et al 2002), which indicates that not only the Ah-receptor (dioxin receptor) is important for generating oxidative stress in organisms. Also OCPs induce CYP 2B enzymes (Parkinson 2001) and hence potentially oxidative stress, which may explain the negative associations between OCPs and liver tocopherol levels in Brünnich's guillemot hatchlings (**Paper IV**). Studies on endosulfan and dieldrin, which are OCPs not included in the present study, have also shown induction of oxidative stress (Stevenson et al 1995, Sohn et al 2004). Furthermore, in Brünnich's guillemot hatchlings, ∑PCBs were not found to affect the levels of tocopherol, despite the potential of several of the congeners included in ∑PCBs (e.g. PCB-118, -114, -105, -156, -157, -189) (Boon et al 1992) to induce CYP 1A enzymes. It is therefore possible that the ability to induce CYP 1A enzymes of Brünnich's guillemots is lower than that of CYP 2B enzymes. Actually, in a study on PCB pattern and biotransformation in seabirds, Brünnich's guillemots showed a limited induction of CYP 1A enzymes in response to PCB exposure (Borgå et al 2005). At the same time, previous studies have suggested elevated biotransformation of OCPs in Brünnich's guillemots compared to that of other alcid species (Fisk et al 2001b), indicating a higher activity of CYP 2B enzymes.

In kittiwake hatchlings, a positive influence of PCBs on plasma and liver tocopherol levels were indicated (**Paper III**). Further, in common eider hatchlings positive relationships between some POPs and tocopherol levels also were revealed (**Paper IV**). Positive relationships between POPs and tocopherol levels contradict the reduced hepatic levels of tocopherol in response to POP exposure in other studies and in the other birds of the present study. However, some studies have also documented positive relationships between POPs and tocopherol levels (Saito 1990, Palace and Brown 1994, Nyman et al 2003). Increased hepatic tocopherol levels have been linked to PCB-induced fatty liver (Saito 1990), which can be a toxicological endpoint from exposure to PCBs (Chu et al 1996; 1998). No certain conclusions regarding fatty liver can, however, be drawn since no histological examination was conducted of the kittiwake and common eider livers (**Paper III-IV**). In common eider hatchlings, the levels of POPs were probably too low to induce fatty liver. It could, however, be that the dose-response relationship connected to the effect of POPs on tocopherol is J-shaped (hormesis) (Calabrese and Baldwin 2003), which means a lower incidence of oxidative stress at low POP doses followed by an increasing incidence of oxidative stress at higher POP levels. A J-shaped dose-response curve should on the other hand lead to more oxidative stress, and depleted tocopherol levels, in kittiwakes. Nevertheless, between-species differences could exist for the dose-response relationships.

In addition to possible different dose-response relationships, a study on rat (Wistor strain) has shown that dietary PCBs can increase tissue tocopherol and the absorption of tocopherol from the intestine (Katayama et al 1991). The increase in tocoherol levels in response to PCB exposure was probably due to a simultaneously increase in the fraction of high density lipoprotein (HDL) cholesterol (Katayama et al 1991). Tocopherol is transported in the blood within plasma lipoproteins and erythrocytes, and thus high correlations exist between tocopherol concentrations and total lipids or cholesterol (Traber et al 1993). Hence, due to a PCB-induced increase in the fraction of HDL cholesterol, increased tocopherol levels may result from PCB exposure. However, since tocopherol is gained from the diet (Sheppard et al 1993), the elevation of tocopherol levels is most likely limited by the tocopherol contents in the diet. In this respect it should be noted that common eiders feed on benthic organisms (Dahl et al 2003). It is therefore possible that diet and nutritional contents (i.e. vitamins) of food items of common eiders influence in a different manner the levels of tocopherol in hatchlings and thereby the relationships between POPs and tocopherol (**Paper IV**).

The (possible) positive influence by PCBs on tocopherol levels in kittiwake and common eider hatchlings could also reflect other mechanisms of toxic action than in the other species. Brouwer (1991) found great variations among fish-eating birds with regard to their ability to induce CYP 1A enzymes and to produce OH-metabolites of PCBs (Ah-responsive and Ah-non-responsive species). Similar species-dependent differences probably exist for other CYP enzymes and possible subsequent toxic actions. According to Brouwer (1991), herring gull was regarded as an Ah-non-responsive species and thereby relatively resistant to some toxic actions of PCBs. In contrast, cormorants seemed to be Ah-responsive, while common eider responded by induction of CYP 1A levels but apparently did not form OH-metabolites (Ah-responsive without formation of some toxic metabolites). On this background, kittiwakes, being a gull species, may be less responsive to some toxic effects by POPs than other species such as cormorants and possibly also shags (*Phalacrocoracidae* species). Borgå et al (2005), however, documented CYP 1A activities in kittiwakes, indicative of Ah-responsiveness, although the CYP 1A activities seemed to be low. Common eiders, forming no OH-metabolites, may also be less responsive compared to other species, or other mechanisms of toxic actions initiate different responses in this species.

It should also be mentioned that tocopherol exists in several oxidized forms (due to the reactions with ROS) (Twaroski et al 2001). Some oxidized forms can be reduced back to tocopherol by other antioxidants, such as ascorbic acid (Katayama et al 1991). Thus, redox reaction may also influence (confound) levels of tocopherol levels, especially when the oxidative stress is low.

The influence on tocopherol levels in the different bird species were caused by different POPs, e.g. PBDE-99 (**Paper I**), PBDE-28 (**Paper II**), PCB-74, -66, -118, -153, -105, -138 (**Paper III**) and OCPs (**Paper IV**). This may be linked to the bird species' different concentrations of POPs, to the disparities in metabolic activity and functionality, and to possible differences in toxic mechanisms and diet.

4.4.2.2 Responses of retinol to POPs

In shag hatchlings, several negative correlations were revealed between POPs and plasma retinol levels (**Paper II**). These negative relationships contradicted the borderline significant positive correlation between PCBs and plasma retinol levels found in shag hatchlings from Sklinna in a previous study (Murvoll et al 1999). The differences between the studies could be caused by between-year variations in dietary levels of retinoids or differences in prey species. It is also possible that recent increase of other pollutants than PCBs, such as PBDEs and HBCD, have influenced on the vitamin homeostasis in a synergistic manner (**Paper II**). Temporal trends of PBDEs in double-crested cormorants from British Colombia (Elliott et al 2005) and in guillemots from the Baltic (Sellström et al 2003) show a peak during 1990-1995, followed by decreasing levels. However, Great Lake herring gulls show a continuing increase in PBDE levels in the period 1981-2000 (Norstrom et al 2002). Thus, an increase of BFRs in shags from Sklinna from 1995 to 2002 could be real, although not documented due to lack of previous data.

Kittiwake hatchlings, having higher levels of POPs in general than shag hatchlings, showed no signs of interrupted retinol levels in connection with POP exposure. It could be linked to possible differences in metabolic ability and functionality. As stated above, Brouwer (1991) found cormorants to be Ah-responsive and to develop effects related to OH-metabolites of PCBs, in contrast to herring gull being Ah-non-responsive. Reduction of retinol levels by PCBs has been associated with the formation of OH-metabolites (Brouwer and van den Berg 1986, Brouwer et al 1990), although other toxic mechanisms for retinol reduction also are proposed (Chen et al 1992). The OH-metabolites have high affinity for TTR, the transport molecule of thyroid hormones in plasma (Brouwer and van den Berg 1986). In addition, retinol-RBP binds to TTR. The RBP-TTR complex is vital to prevent filtration of retinol-RBP through the kidneys (Combs 1992). Binding of OH-metabolites to TTR results in conformational changes and in destabilization of RBP-TTR complex (Brouwer et al 1990). Hence, glomerular filtration of retinol-RBP will increase, leading to reduced levels of retinol. The European shag, being a *Phalacrocoracidae* species and thereby possibly Ah-responsive, may respond to POP exposure by forming OH-metabolites which is linked to reduced levels of retinol. Thus, because of possible differences in the xenobiotic elimination (e.g. Ah-responsiveness), *Phalacrocoracidae* species might show responses in retinol levels to POP exposure whereas gull species may not.

4.4.3 Vitamins as potential biomarkers

In the present study, tocopherol levels seemed to be affected by POPs in all species included, although more studies are needed to clarify the effects of POPs on tocopherol in the different species. Nevertheless, the results indicate a potential of tocopherol as a biomarker for exposure to organic compounds. Retinol levels were only influenced in shag hatchlings, which may imply that higher levels of POPs are needed to elicit this response.

The age of the hatchlings (study objects) was standardized to reduce the risk of confounding due to normal temporal variations in vitamin levels. Possible temporal variations in vitamin levels due to reproductive status etc. of the avian mothers were avoided when using hatchlings. In kittiwakes, being differentiated into males and females, vitamin levels were corrected for sex variation using linear regression residuals. Thus, the present study minimized the number of possible confounding factors. However, still there is a lack of knowledge regarding vitamin levels in the diet and how diet of the avian mothers affects vitamin levels in hatchlings. Also other factors may influence the vitamin concentrations (e.g. the physiology and condition of the avian mothers). Also POP levels could be influenced by the condition of the avian mothers (i.e. age, lipid stores, food choice). Limited resources made it impossible to take samples of the avian mothers and their diet in the present study. Hence, further (and more extensive) studies should be conducted to investigate closer tocopherol and retinol levels as possible biomarkers of POPs in birds.

In addition, the confounding by redox cycling could be diminished by measuring also other forms of tocopherol. Twaroski et al (2001) proposed that tocopheryl quinone, an irreversible oxidized form of tocopherol (Michal 1999), could be a more sensitive marker of oxidative stress than tocopherol. In a study on rats given PCBs, tocopheryl quinone levels were elevated in both short-time and long-time exposure groups and in both gender (due to increased oxidative stress), whereas tocopherol levels were reduced only in short-time exposure groups and in males. Actually, efforts were made to detect tocopheryl quinone in the samples of the present study without succeeding. Due to limited resources these efforts could not be pursued.

4.5 Possible linkages to higher level effects

Reduced plasma retinol levels may indicate that growth and development in hatchlings could be affected, as vitamin A is important for these processes in birds (Spear and Moon 1986). Tocopherol is essential for normal neurological structure and function (Traber et al 1993). As a part of the antioxidant defences, tocopherol is also important in reducing the negative effects of cellular oxidative stress (Saito 1990). Many toxic, carcinogenic and pathological processes are believed to be partly due to oxidative stress occurring through the generation of reactive oxygen species (ROS), which react with lipids, proteins and DNA (Smith et al 1995). Thus, if the cellular antioxidant defences are depleted, this may have consequences such as mutagenic damage and carcinogenicity.

The population of kittiwakes at Runde has in later years declined significantly. Further research is needed to completely assess the cause and effect relationship of this decline, although changes in food access are proposed as possible explanations (Lorentsen 2003). However, if POP levels are linked to the observed reduction in population size, vitamin levels may not to be useful biomarkers to reveal such disturbance. Levels of vitamins in the kittiwake hatchlings seemed to be only minor influenced by POPs, although further studies should be conducted to provide more data on the issue.

The shag population from Sklinna at present shows no signs of detrimental effects at higher biological levels, i.e. reproduction disturbances or population decline (Nils Røv pers comm., Lorentsen 2003). Hence, the negative relationships between POPs and vitamin levels observed in shag hatchlings does not seem to be linked to effects at higher biological levels. The PCB contamination in the shag hatchlings is also much lower than what has been documented to reduce hatching success and reproduction in *Phalacrocoracidae* species (Tillitt et al 1992, Dirksen et al 1995). Still vitamin levels indicate a potential as useful "early warners" of POP exposure by responding to the pollutants, and it could be possible that higher POP levels both relate negatively to retinol and tocopherol levels and show link to effects at higher biological levels. It should be mentioned that the shag population at Runde has declined to one fourth in 2003 compared to 1975, although the size of the population has been relatively stable in the last ten years (Lorentsen 2003). Possible differences between these populations of shags with regard to POP levels and effects could exist. Several environmental factors may also interact (food access variation, habitat destruction, climate changes, pollutant levels), making the situation for each population unique and complex. It could therefore have been interesting to study also other populations of shags with regard to POP levels and vitamins.

The populations of the bird species from Kongsfjorden are reported to be reasonably stable (kittiwake, Brünnich's guillemot, common eider) (Anker-Nilssen et al 2000), despite great between-year variations with regard to the number of breeding birds (www.miljo.npolar.no). Recent data also suggest a negative trend for both kittiwake and Brünnich's guillemot populations from Kongsfjorden (www.miljo.npolar.no/mosj/MOSJ, H. Strøm unpubl results). It is difficult to assess whether the observed responses in vitamin levels could be linked to the negative trends in the development of the sea bird populations. On the other hand, the population of common eider shows a small increase (3-4 %) since the 1980s (www.miljo.npolar.no/mosj/MOSJ, G.W. Gabrielsen pers comm.). However, it should be noted that this species was significantly reduced until the early 1990s due to harvesting of eggs and down, which has been prohibited on Svalbard since 1963 (www.miljo.npolar.no/mosj/MOSJ). Both Brünnich's guillemots and common eiders are believed to be vulnerable species towards anthropogenic disturbance such as over-fishing (Brünnich's guillemot) and increased exploitation of benthic organisms (common eider), and oil spill (Anker-Nilssen et al 2000, www.miljo.npolar.no/mosj/MOSJ). These species also seemed to be responsive to POP exposure with regard to tocopherol levels, although the response in Brünnich's guillemot hatchlings was influenced by body mass. It should also be kept in mind that the levels of POPs in common eiders were very low. The kittiwake seemed to be a less responsive species towards POPs with regard to influence of vitamins, possibly due to between-species differences in the metabolic functionality (i.e. Ah-responsiveness, toxic mechanisms, etc.). Still other physiological variables may be interrupted in kittiwakes. In the Arctic, having low temperatures, extreme seasonal variations in light and lack of nutrients, it is possible that the environmental conditions impose an additional stress to the organisms living there, making them more vulnerable to anthropogenic pollutants such as POPs compared to organisms living in more temperate or tropical biomes. The relatively high POP levels (in an Arctic scale) observed in kittiwake hatchlings from Kongsfjorden could thus be of ecotoxicological interest due to the special environmental conditions and in light of potential additive effects between chemicals of old origin and of new (and still unknown) sources.

Although the responses in vitamin levels to POP exposure in the seabird species herein can not clearly be linked to higher levels effects, the responses could be regarded as "early warners" of effects of POPs in the studied seabird populations, including populations from the remote Arctic. In light of the precautionary principle of the Rio Declaration of 1992 (www.unep.org), the continuing use and release of brominated flame retardants in several parts of the world could be worrying and should be considered seriously by supranational organizations (e.g. United Nations).

4.6 Seabirds as bioindicator species

Seabird bioindicator species represent top predators and are thus relevant in environmental biomonitoring of POP exposure. Hatchlings of seabirds are appropriate bioindicators due to the opportunities of control of important factors such as age and development. On the other hand, sampling from hatchlings requires more resources than sampling of eggs. However, eggs will not give the advantage of several matrices for analysis of both POPs and biological parameters (i.e. biomarkers) as do hatchlings.

The results from the present study indicate that shag is a responsive species with regard to POP exposure, and thereby have a potential as a bioindicator species. Shags breed along the outer part of the whole Norwegian coastline in addition to the coast of several European countries such as Iceland, the Faeroes, Russia, Great Britain, Ireland, France, Spain, Italy and Greece (Snow and Perrins 1998). Thus, shags could be monitored in several countries. In some colonies, shags can be difficult to get because they breed in-between cliffs. However, because they breed on the ground, samples of eggs, hatchlings or adults are relatively easy to collect in most locations. Brünnich's guillemots also seemed to be responsive to POP exposure, although the relationships between OCPs and liver tocopherol levels were less strong when the variation explained by body mass was accounted for. The species could be useful as an indicator species of the Arctic region, as Brünnich's guillemots breed only in the

Arctic region (Snow and Perrins 1998). It is also regarded as a potential indicator of the productivity of the Arctic ecosystem by The Norwegian Polar Institute (www.miljo.npolar.no/mosj/MOSJ). Common eider hatchlings responded to POP exposure by elevated levels of liver tocopherol. Common eiders breed in the Arctic region and along the coast of the Nordic countries, Russia, Great Britain, Ireland, the Netherlands, and Germany (Snow and Perrins 1998). Still common eiders accumulate very low levels of POPs and are probably influenced by most POPs to a minor degree.

Kittiwakes accumulate rather high levels of POPs and thus show a potential as an indicator species. However, vitamin levels seem not to be useful biomarkers of POP exposure and effects in kittiwakes, although further studies need to be conducted before certain conclusions are drawn. Other physiological variables may be influenced and could be possible biomarkers. Kittiwakes breed mainly on low- and high-arctic coasts (i.e. Spitsbergen, the coast of Norway, Iceland, the Faeroes, Russia), but also to a minor extent in several other countries (Great Britain, Ireland, Germany, France, Spain) (Snow and Perrins 1998), allowing widespread monitoring. The kittiwakes breed in steep cliffs and mountains, and sampling may be challenging. Due to migration kittiwakes will not reflect POP levels from breeding regions only, and hence the species is not as useful as more resident species regarding monitoring of regional pollutant levels. Nevertheless, possible responses of kittiwakes to POP exposure could be of importance in the assessment of effects at higher biological levels, including ecosystems.

The Norwegian and Svalbard coast are regarded relatively clean due to the pristine environment with low population density and industrial activity. In a broader sense, POP levels and biomarker responses in Norwegian seabirds could contribute with data from the lower exposure range in the environment. However, in a European scale, PBDE and HBCD levels were relatively high in some of the species included in the present study, implying the difficulties in making general conclusions. The present study thus implies the importance of follow-up surveys, especially with respect to BFRs.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This study provided up-to-date data on POP concentrations and vitamin levels in seabirds from the coast of Norway and from Kongsfjorden, Svalbard. In short, levels of OC compounds in the hatchlings seemed to be higher than reported in sea bird eggs from the Canadian Arctic but lower than reported in eggs of other seabirds from the Netherlands, the Baltic, the Great Lakes and Japan. In contrast to this, the levels of PBDEs and HBCD seemed to be high in some of the species (kittiwakes, shags) relative to a European scale. With regard to national temporal trends, the study gave no indications on a further decline in OC levels compared to the 1990s (Barrett et al 1996), probably due to fluctuations around relatively low levels following regulatory restrictions on the use of OC compounds decades ago. Due to the lack of temporal data regarding BFRs, and the relatively high levels documented herein, it would be recommended to be attentive in this respect in years to come.

There were significant differences in POP levels between the sea bird species included in the study. In general, kittiwake hatchlings had higher levels of POPs than the other species, followed by shag, Brünnich's guillemot and common eider hatchlings. The differences could be explained by trophic position, diet and rate of food intake (i.e. metabolic activity), in addition to migration pattern, to breeding sites (Norway vs. Svalbard) and possibly also to the species' different classification in accordance to the altricial-precocial spectrum for birds.

Negative relationships between morphological variables and POP levels were revealed in Brünnich's guillemot hatchlings. This species is probably more responsive with regard to the effects of POPs on morphological variables than the other species included in the present study. In shag hatchlings, the importance of considering possible confounding impacts of lipid content when studying effects of POPs on morphological variables, was emphasized.

The study revealed interesting relationships between POPs and liver tocopherol levels (**Paper I-IV**). In domestic duck, a negative relationship between PBDE-99 and liver tocopherol levels was revealed under controlled laboratory conditions. Further, in free-living shag hatchlings, negative relationships between POPs and liver tocopherol levels were found. In addition, liver tocopherol levels were negatively associated with POPs in Brünnich's guillemot hatchlings, although the relationships seemed to be influenced by variation in body mass. However, in kittiwake hatchlings, there seemed to be a possible positive influence by POPs on tocopherol levels. In common eider hatchlings, positive relationships between POPs and tocopherol levels also were revealed. Despite the apparently opposite effects of POPs on tocopherol levels in different bird species, an influence of liver tocopherol was a consistent trend in these species, representing different trophic positions and types of birds according to the precocial-altricial spectrum. The species' responses probably reflect differences with regard to dose-response relationships, toxic mechanisms and trophic position (diet). The results should encourage further research on the effects of POPs on tocopherol levels.

In shag hatchlings, a negative relationship between POPs and plasma retinol levels were observed (**Paper II**), in line with several previous studies on birds (Greichus and Hannon 1973, Spear and Moon 1986, Champoux et al 2002). Since the same correlation was not found in any other species included in the study, tocopherol levels might be more responsive than retinol levels to POP exposure. Further studies should, however, be conducted before firm conclusions are drawn.

Concerning the work needed for further development of vitamins as biomarkers of POP, it should be kept in mind that still there is a lack of knowledge with regard to the natural physiological events that can alter vitamin concentrations. Hence, efforts should be made to characterize confounding factors, such as diet and condition of the avian mothers, for the further validation of retinol and tocopherol as biomarkers. In addition to tocopherol levels, measurements of the oxidized form tocopheryl quinone could be useful.

Although the observed responses of vitamins to POP exposure were difficult to link to effects at higher biological levels, the relevance of vitamins as potential biomarkers of POP exposure should not be repelled. Further studies should be conducted on birds to investigate closer the vitamins as possible biomarkers. Other possible physiological variables may also have potential as biomarkers. To ease the work and limit the need of resources, a method for analyzing vitamins in yolks of eggs (in addition to POPs) should be encouraged.

Relatively high levels of BFRs, possibly increasing, were documented in the present study. The knowledge of the distribution and effects of other chemicals than POPs, such as perfluorooctane sulfonate (PFOS), is however scarce. PFOS has also been shown to increase oxidative damage in fish (Oakes et al 2005), and due to the potential synergistic action of chemicals, severe oxidative damage could occur earlier than expected from the concentrations of one group of chemicals only. Associations between vitamin levels and POPs could thus be early warners of negative impact on biological systems (i.e. populations or ecosystems), although not yet demonstrated.

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